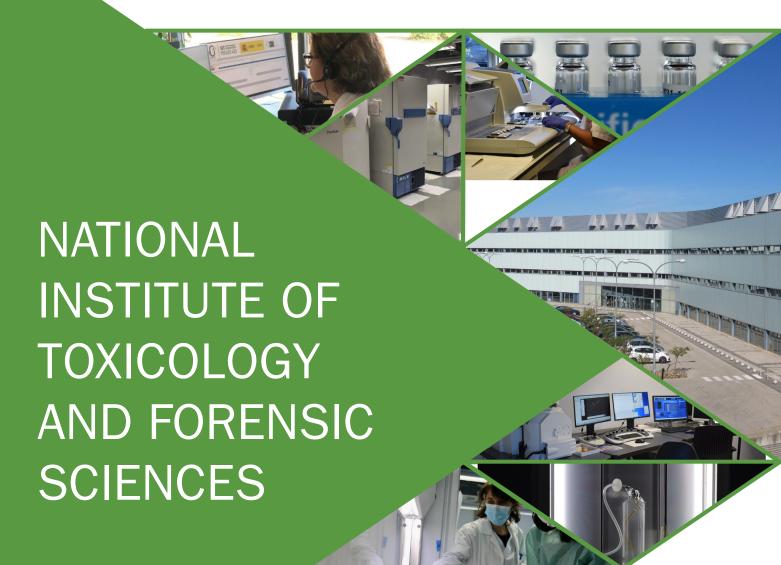
NATIONAL INSTITUTE OF TOXICOLOGY AND FORENSIC SCIENCES















Report 2021

National Institute of Toxicology and Forensic Sciences

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National Institute of Toxicology and Forensic Sciences

Report 2021



Report presented by Antonio Alonso Alonso, Director of the National Institute of Toxicology and Forensic Sciences

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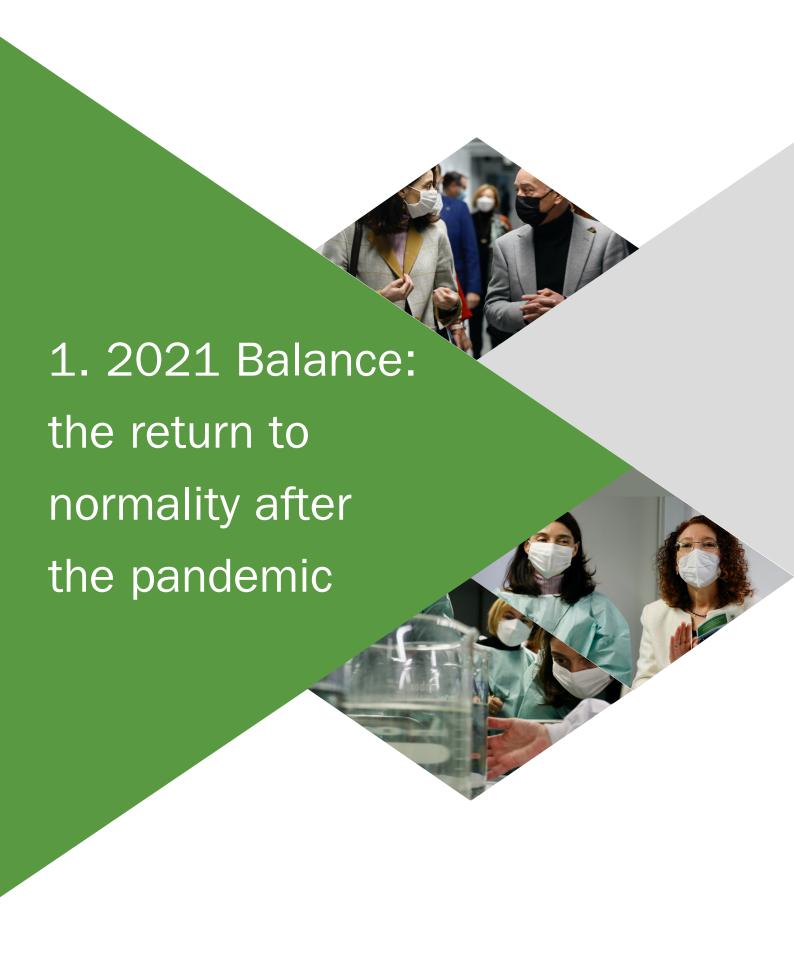
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Annex II. Regulations applicable to the National Institute of Toxicology and Forensic Sciences (chronological order)



Possibly one of the biggest challenges faced in 2021 was the recovery of the scientific and expert activity at the National Institute of Toxicology and Forensic Sciences (INTCF), with all its sites having been seriously affected during the SARS-COV-2 pandemic, to pre-pandemic levels. This recovery has resulted in a considerable increase both in the number of requests from judicial bodies (27%) and in the number of samples (42%) and analyses (33.6%) performed, as well as the number of reports issued (13%) compared to 2020.

Undoubtedly, the high degree of commitment and the unwavering dedication of all INTCF professionals, of the special bodies of the INTCF (facultatives, specialist technicians and laboratory assistants) and of the general authorities of the Justice Administration assigned to the INTCF has played a critical role in this recovery.

The digital transformation process, launched in 2020, aims to address the challenge of the lack of mobility arising during the pandemic in addition to other factors, and has been a key tool in this recovery. In 2021, the digital transformation was a fundamental axis of development for the Ministry of Justice and also for the INTCF, where total investment (€1.3 million) increased by 47% compared to 2020. The digital transformation projects implemented in 2021 include: the European Harmonisation Project of the European Chemicals Agency (ECHA), the implementation of Agilent's Openlab Software Suite system for interoperability between INTCF analytical instruments, the network of the Ministry of Justice and the Laboratory Information Management System (LIMS), the Digital Signature project for expert reports generated using the LIMS system and the project for the processing and use of forensic data via the Datalab platform.

The digital transformation process has also been a key factor in the continuous training of INTCF professionals. In 2021, the institute developed 18 online courses with more than 790 places as part of the continuous training plan of the Centre for Legal Studies (CEJ). For the second year in a row, this has made it possible to include not only facultative career civil servants, but also interim staff and personnel from the State Security Forces and Corps and the regional police departments in training processes.

The need for laboratory facilities and environmental conditions suited to the analytical tasks to be performed by the INTCF and that do not compromise the quality of the results of the expert activity are an essential factor of the ISO 17025 standard to which many INTCF analytical procedures are subject. The significant growth in the expert activity of the different analytical services at the INTCF over the past twenty years has generated serious problems in relation to space at the obsolete older INTCF facilities. In the case of the institutes in Seville, Barcelona and La Laguna, the construction of new facilities is essential. With regard to the new facilities, the following initiatives have been carried out in 2021 in Seville and Barcelona. The contract for the design of the new INTCF headquarters in Seville was entered into with Jovino Martínez Sierra Arquitectos, who submitted the final proposal to the Subdirectorate General for Works and Heritage at the Ministry. The tender dossier for the performance of the work will be launched

in 2022, with the new headquarters expected to be completed in 2025. Contacts and informative meetings have also been held with the Autonomous University of Barcelona to consider the existing possibilities for a new site for the Barcelona Department at the existing land on the Bellaterra campus.

In relation to equipping the laboratories with state-of-the-art equipment, eight tender dossiers were organised in 2021 with an investment of more than 5 million euros, ensuring that the different sites will be equipped with the most modern technology for the chemical-toxicological, biological, genetic, forensic and environmental analysis of the forensic samples received by the INTCF. Take, for example, the implementation in 2021 of high-performance liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) at the Chemistry and Drugs Services or the acquisition of several analytical devices and an expert software system for the Biology Services.

In 2021, a first draft of the new INTCF job descriptions (RPT) was prepared, including the staffing needs and new requirements of the different INTCF specialities, as well as the creation of new specific jobs facilitating the management of research tasks, the economic administration of the INTCF's supply unit and the administration of the INTCF's Library.

Furthermore, and with a view to alleviating the excessive workload suffered by the INTCF, under the Resolution of 21 May 2021, issued by the Directorate General for the Public Justice Service, a reinforcement plan was approved for certain projects at the Institutes of Legal Medicine and Forensic Sciences (IMLCF) and to support the pendency time of the National Institute of Toxicology and Forensic Sciences (INTCF) until December 2021.

The development of standardised working procedures, the validation of analytical methods, the participation of laboratories in proficiency/intercomparison exercises and, where appropriate, the accreditation of tests under ISO standards by the National Accreditation Body (ENAC) are, inter alia, fundamental elements as part of the INTCF quality system that have undoubtedly led to a significant improvement in the quality of the expert analyses performed by the different INTCF analytical services. During 2021, progress was made with the validation of 26 analytical methods and the INTCF has participated in three intercomparison exercises: the Study of DNA Polymorphisms in Bloodstains and other Forensic Samples (INTCF-Madrid), the Interlaboratory Exercise for the Determination of Ethyl Alcohol in Blood (EIAS, INTCF-Seville) and the Interlaboratory Exercise on Commonly Abused Drugs in Additions (DAHA, INTCF-Barcelona).

During 2021, as well as the recovery of the INTCF's expert activity, two studies on causes of death were carried out, leading to the publication of two new case study reports. For the first time in Spain, a national report was presented on *Toxicological Findings in Traffic Accident Fatalities*. This collaboration between the INTCF and the IMLCFs of Catalonia, the Basque Country, Aragon and Murcia generated much more comprehensive statistical data than had been obtained in previous years, making it possible to collect

toxicological information on a very representative proportion of the total number of cases of drivers and pedestrians killed in traffic accidents during 2020. Furthermore, a report was published on *Epidemiology and Toxicology of Suicide Deaths in Spain*, addressing general epidemiological data to assess the influence of age, gender, date of the event and autonomous community on deaths by suicide. A population-based study of the different mechanisms of suicide (hanging, intoxication, jumping from a height, drowning, firearm, knife and others) and the influence of the different epidemiological variables mentioned above was also undertaken. This included a case study chapter containing a detailed toxicological study of suicide victims, highlighting, among other aspects, the high prevalence of multi-drug consumption in this type of death.

About other data of the 2021 balance, we refer to the reader the document *Activity Balance prepared by the National Institute of Toxicology and Forensic Sciences during 2021 pursuant to the 2020-2022 Action and Investigation Plan*, where they can find the initiatives in detail developed during 2021 concerning the fundamental axes of the INTCF activity: expert activity, quality assurance, digital transformation projects, collaboration agreements, training and education programmes. Furthermore, the Annex II of the report collects the legal regulations applicable to the INTCF, where they further define the structure and the functions of the INTCF.

In this edition of the INTCF 2021 report, we maintain the structure first used in 2019 to give greater prominence to the seven scientific services at the INTCF which present their statistical data classified by type of investigation and describe various forensic cases of interest with a view to disseminating the wide range of interdisciplinary scientific areas that make up the forensic sciences. Apart from the expert activity, the INTCF also investigates and collaborates with other institutions. It also presents the teaching and training activities carried out during 2021. This year it also included a section describing the work carried out by different INTCF units that perform various support functions for the INTCF's Forensic Services and whose work is essential to the functioning of the different INTCF Departments.

I would like to end this introduction by thanking all the INTCF staff for their scientific and human efforts and qualities, extending this also to the staff from the Ministry of Justice units that collaborate closely with the INTCF in regulatory development and administrative management. I greatly appreciate, and specifically, the dedication and compromise effort of the Directors of the Department and from the INTCF Delegation, the dedication and professionalism of the Heads of Service and all the specialist teams as (facultatives, specialist technicians, and laboratory assistants), and of the bodies of the Administration of Justice assigned to the INTCF.

I would also like to take this opportunity to thank all institutions, universities, national and international bodies, which in 2021 have collaborated with the INTCF, with special mention going to the Legal Medicine and Forensic Sciences Institutes. The INTCF

maintains a very close collaboration in our common task of giving technical-scientific advice at the service to courts and the Public Prosecutor's Office.

Antonio Alonso Alonso Director of the National Institute of Toxicology and Forensic Sciences



The following graphs show the INTCF chart organisation, the territorial scope of action of the four headquarters, the staff distribution by gender and according to the different professional bodies, the budget expenditure, the overall statistical data of the expert activity, the telephone inquiries attended by the SIT, the distribution of the registered cases, and the distribution of the cases registered by each region of the country during 2021.

2.1. INTCF Chart

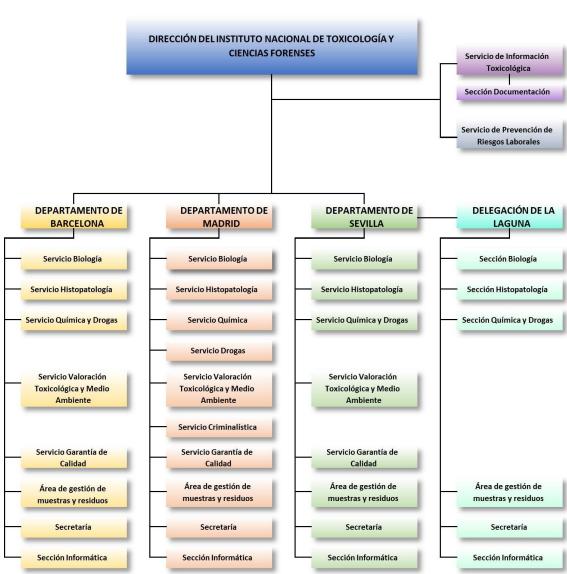


Figure 2.1. INTCF Chart

2.2. Scope of action of the various INTCF sites



Figure 2.2. Scope of action of the various INTCF sites

2.3. INTCF staff

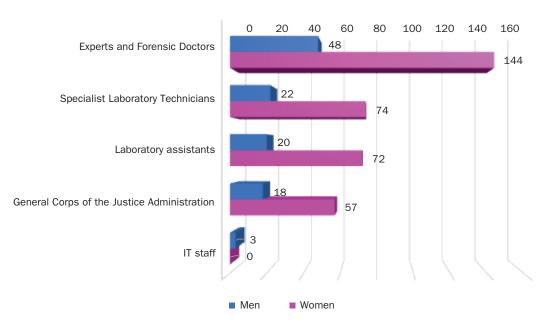


Figure 2.3. INTCF staff in 2021 classified by gender

2.4. Expenses incurred by the INTCF in the financial year 2021

Figure 2.4. Expenses incurred by the INTCF in the financial year 2021



2.5. Summary of the Scientific-Expert Activity

Figure 2.5. Summary of the Scientific-Expert Activity

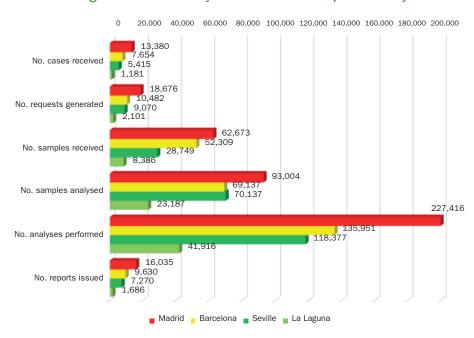


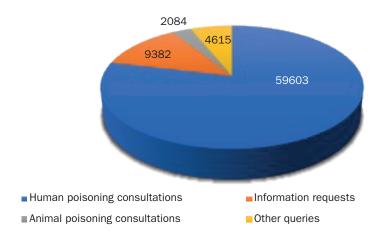
Table 2.1: Statistical global data by department

2021	No. cases received	No. requests generated	No. samples received	No. samples analysed	No. analyses performed	No. reports issued
Madrid	13,380	18,676	62,673	93,004	227,416	16,035
Barcelona	7,654	10,482	52,309	69,137	135,951	9,630
Seville	5,415	9,070	28,749	70,137	118,377	7,270
La Laguna	1,181	2,101	8,386	23,187	41,916	1,686
TOTAL	27,630	40,329	152,177	255,465	523,660	34,621

2.6. Telephone enquiries handled by the Toxicology Information Service in 2021

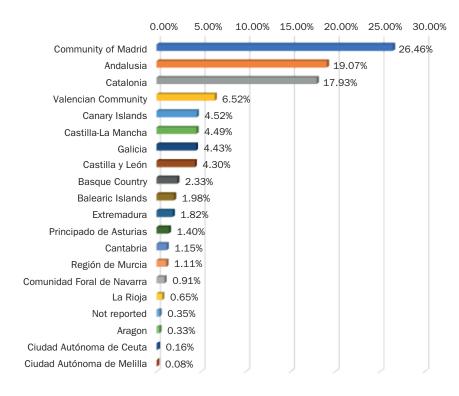
Figure 2.6. Telephone enquiries handled by the Toxicology Information Service in 2021

Distribution by type of enquiry



2.7. Distribution of registered cases by autonomous communities

Figure 2.7. Percentage of registered cases by autonomous community in 2021





Each department counts with a Chemistry and Drugs Service except for the Madrid Department, which has a Chemistry Service and a Drugs Service. Finally, the Delegation of La Laguna counts with a section of Chemistry and Drugs.

The Chemistry and Drugs Services carries out mainly expert activities in compliance with the functions entrusted to them, but also carries out teaching and research functions. Within its expert work, the following types of investigation are included:

Post-mortem toxicological information

- · Deaths by homicide
- Deaths by suicide
- · Deaths by an adverse reaction to psychoactive substances
- Deaths by car accidents
- · Deaths by a work accident
- · Deaths related to sport
- Deaths by drowning
- · Deaths by fire
- · Deaths by malpractice
- Death in custody
- Deaths of unknown aetiology suspected of being of a criminal nature
- Death data (from ions in vitreous humor)
- Deaths of unclear aetiology (sudden adult death, sudden infant death, sudden infant death, sudden death associated with sport, and others)

Toxicological Investigation in Live Subjects:

- Offences against traffic safety
- · Crimes against sexual liberty and chemical submission
- Crimes against public health
- · Other types of offences
- · Recent use of alcohol, drugs and psychotropic drugs
- Chronic use of alcohol, drugs and psychotropic drugs
- Clinical samples
- Suspect of poisoning

Chemistry toxicological analysis of non-biological samples from drug seizures (caches)

The staff of the Chemistry and Drugs Services who have carried out this type of research during 2021 are shown in Table 3.1. During the year, additional staff were recruited to the Staff Reinforcement Services with a view to improving the INTCF's performance. Table 3.2 details the professional profile of the staff recruited.

Table 3.1: Staff of the Chemistry and Drugs Services of the different Departments

	INTCF-MADRID (Chemistry Service)	INTCF-MADRID (Drug Service)	INTCF- BARCELONA	INTCF- SEVILLE	INTCF-LA LAGUNA
Head of the Department	1	1	1	1	0*
Facultatives	14	13	17	17	4
Specialist technicians	11	5	9	7	3
Laboratory assistants	6	12	5	6	1
Clerical staff	1	3	-	2	-

^{*} There is no head of the department and instead there is a coordinator who rotates among the experts who make up the section

Table 3.2: Reinforcement staff recruited during 2021 at the Chemistry and Drugs Services of the different Departments.

	INTCF-MADRID (Chemistry Service)	INTCF-MADRID (Drug Service)	INTCF-BARCELONA	INTCF-SEVILLE	INTCF-LA LAGUNA
Facultatives	2	2	1	1	-
Specialist technicians	1	1	1	1	1

In 2021, the daily activities of the Chemical and Drug Services gradually returned to normal. As a result, the number of cases registered during this period reached figures similar to those recorded before the pandemic. During 2021, a total of 22,553 expert cases were received, 24,139 requests were generated and a total of 94,095 samples were recorded. The number of analyses carried out during 2021 by the different Services came to 206,888. In terms of expert reports, 22,685 were issued. All these figures are reflected in Figure 3.1.

Since 1996, the INTCF has been drafting a monographic report *Toxicological Findings in Road Traffic Fatalities* analysing in detail the influence of ethyl alcohol consumption, drugs, and psychopharmaceuticals has resulted in this type of death. In 2021, apart from the annual report of fatal victims in traffic accidents, a monographic report on suicide deaths in 2019 was elaborated (*Epidemiology and Toxicology of Suicide Deaths in Spain*). This report, to be continued in the coming years, has the main objective to alert about the problem of suicide in Spain through the INTCF casework. Furthermore, at the initiative of the INTCF, this year, updated instructions were made available for cases of suspected chemical submission through the preparation of a *Guide of good practices for forensic action when dealing with the victim of a crime facilitated by psychoactive substances: intervention in cases of suspected chemical submission*.

As part of the digital transformation projects to be undertaken at the INTCF, during 2021, the implementation of Agilent's Openlab Software Suite system for interoperability between the INTCF's analytical instruments, the network of the Ministry of Justice and the

LIMS system was launched. During an initial pilot phase, different liquid and gas chromatography devices were connected to the Openlab system. These preliminary tests will continue during 2022, covering an increasing number of chromatographic devices. In the future, the System will also encompass chromatography devices coupled to mass spectrometry detectors. Training in the use of the Compound Discoverer software in high-performance liquid chromatography (UPLC) coupled to high-resolution mass spectrometry (Q-Exactive Orbitrap) devices also began in 2021. One of the main contributions of this software is the identification of unknown compounds in a biological matrix, thus extending and improving the quality of our expert work.

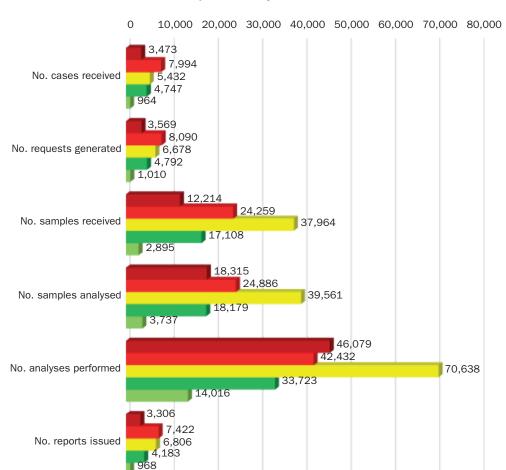


Figure 3.1. Overall data of the INTCF Chemistry and Drugs Services' Expert Activity in 2021

Seville

La Laguna

■ Madrid - Chemistry ■ Madrid - Drugs ■ Barcelona

Table 3.3: Overall data of the INTCF Chemistry and Drugs Services' Expert Activity in 2021

2021	No. cases received	No. requests generated	No. samples received	No. samples analysed	No. analyses performed	No. reports issued
Madrid - Chemistry	3,473	3,569	12,214	18,315	46,079	3,306
Madrid - Drugs	7,994	8,090	24,259	24,886	42,432	7,422
Barcelona	5,432	6,678	37,964	39,561	70,638	6,806
Seville	4,747	4,792	17,108	18,179	33,723	4,183
La Laguna	964	1,010	2,895	3,737	14,016	968
TOTAL	22,553	24,139	94,095	104,678	206,888	22,685

The Chemistry and Drugs Services has also acted as a reference centre in matters within its field of activity. Apart from the expert activity, it has participated in teaching and training activities collaborating with legal medicine institutes and university centres in protocols for action with medical-legal repercussions, making validation studies and technological evaluations. It acted as a reference centre of the Society of Hair Testing to analyse drugs in the hair.

Hereunder, the Chemistry and Drugs Services from the different Departments collect the expert and scientific activity and the teaching activities during 2021. Each Service has included a forensic interesting case description to publish the expert labour.

3.1. Madrid Department Chemistry Service

During 2021, the Chemistry Service expert activity received 3,569 requests and 18,315 samples were analysed with a total of 46,079 analyses, issuing a total of 3,306 expert reports.

As is reflected in Figure 3.1.1, most requests for analysis involved a general toxicological study (2,237 requests with 11,532 samples analysed), as part of which it is necessary to rule out the presence of any toxins that may have contributed to the events under investigation. Analytical systematics was applied to this group aimed at the identification and quantification, as applicable, of samples received to clarify the cause of death employing different technology to cover the highest number of substances investigated (> 400 substances) in different matrices.

The second largest group of requests for analysis corresponds to crimes of a sexual nature (495 requests with 3,045 samples analysed); in these cases, an analytical system is applied with a view to identifying the possible use of substances capable of leading to chemical submission. This entails the use of analytical techniques and maximum

resolution detectors to investigate the large number of substances at very low detection thresholds.

In third place, based on number of requests received, were toxicological investigations in sudden deaths (adult, children, and infant) (364 requests with 1,671 samples analysed). The toxicological investigation will be aimed at determining the presence of any substance that could cause or contribute to the death.

Another group accounting for a significant number of requests are drowning deaths (170 requests with 919 samples analysed); in these cases, in addition to a general toxicological study, a study of hydremia (strontium and manganese levels in blood samples from the ventricles) is performed.

Other research carried out in 2021 by the Chemistry Service at the Madrid Department, accounting for a smaller number of requests, included: toxicological research in fires, toxicological research in cases of deaths due to malpractice, study of toxins in hair, study of metal poisoning or studies of a chemical-environmental nature.

The Madrid Department Chemistry Service analyses to answer the requests received, but to determine alcohol and other volatiles (3,266 analyses). It also carries out the screening through enzyme immunoassay (39,287 analyses) of the requests received by the Drug Service.

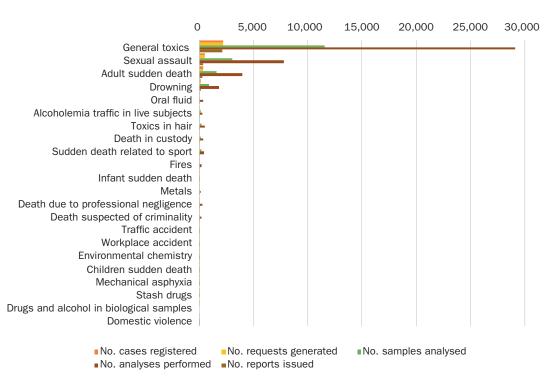


Figure 3.1.1. Casework of the Madrid Department Chemistry Service during 2021 according to the type of report

Table 3.1.1. Casework of the Madrid Department Chemistry Service during 2021 according to the type of report

Type of report	No. cases registered	No. requests generated	No. samples analysed	No. analyses performed	No. reports issued
General toxics	2,212	2,237	11,532	29,124	2,135
Sexual assault	492	495	3,045	7,832	377
Adult sudden death	342	343	1,600	3,991	298
Drowning	155	170	919	1,851	168
Oral fluid	68	68	86	405	74
Alcoholemia traffic in live subjects	59	59	135	271	71
Toxics in hair	38	40	186	553	33
Death in custody	25	25	121	344	26
Sudden death related to sport	21	23	204	455	28
Fires	21	21	77	230	21
Infant sudden death	15	18	54	99	20
Metals	14	16	73	173	11
Death due to professional negligence	13	13	85	286	12
Death suspected of criminality	12	12	96	237	11
Traffic accident	9	10	32	68	7
Workplace accident	5	5	8	20	4
Environmental chemistry	5	7	21	38	5
Children sudden death	3	3	17	66	3
Mechanical asphyxia	1	1	6	16	1
Stash drugs	1	1	16	16	0
Drugs and alcohol in biological samples	1	1	0	0	0
Domestic violence	1	1	2	4	1
TOTAL	3,473	3,569	18,315	46,079	3,306

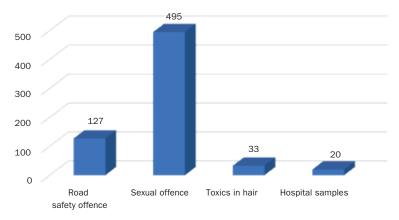
In Figure 3.1.2, the expert investigations carried out are focused on *post-mortem* cases (80%).

The investigations in living subjects are grouped in four fields (Figure 3.1.3); the majority (73.3%) is the investigation of substances in crimes against sexual liberty followed by the confirmation of alcohol or drugs in biological samples in road safety offences (18.9%). To a lesser extent and with similar percentages are investigations of substances in hair samples (4.9%) and substance investigations causing a clinical condition or intoxication that the hospital cannot investigate (2.9%).

19% 19% 80% 80% ■ Investigation post-mortem ■ Investigation live subjects ■ Others

Figure 3.1.2. Classification according to type of investigation





As we have already explained before, 80% of the investigations are based on samples of deceased persons. When analysing the type of aetiology in the requests received, natural aetiology represents about 35% of the cases received (Figure 3.1.4), a similar volume to violent aetiology (34%). Although, in 23% of the cases, the expert reflects neither the aetiology nor any information concerning the cause of death.

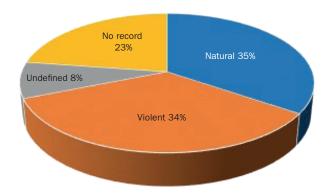


Figure 3.1.4. Type of aetiology of post-mortem investigations carried out

Inside the group of violent aetiology, it is worth noting that the predominant group (61.6%) was suicide (757 cases; Figure 3.1.5). Cases of violent suicidal deaths represent 21.2% of the total number of cases received. There has been an increase in this type of caseload year on year. In 2019, 570 cases of suicide violent aetiology were received, accounting for 16.2% of cases. In 2020, this type of casework increased to 19% of the total number of the received cases.

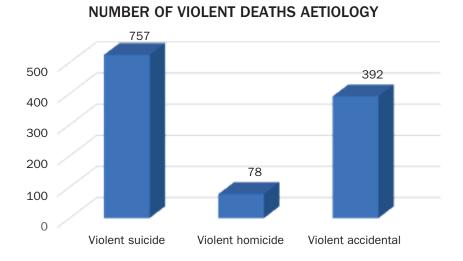


Figure 3.1.5. Classification of cases according to the type of violent aetiology

In terms of violent suicide aetiology, the most frequent suicide mechanism used was hanging (37.6%), followed by poisoning (21%) and falling from a height (19.9%), which coincides with the study published by the INTCF on *Epidemiology and toxicology of suicide deaths in Spain in 2019*. (Figure 3.1.6).

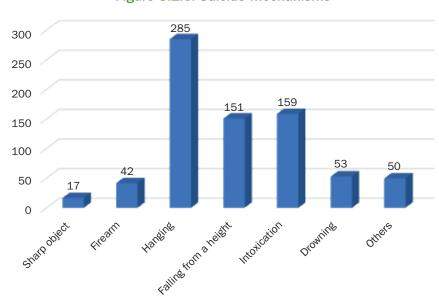


Figure 3.1.6. Suicide mechanisms

3.1.2. Forensic case of interest: Toxicological investigation of skeletal remains

Hereafter, a case received in the Chemistry Service during 2021 is exposed. The interest of the case lies in the fact that the toxicological investigation was carried out on skeletal remains, an alternative option when no other option is available (Figure 3.1.2.1).

Data

62-year-old man who died alone at home. When the coroner arrived, the body was found mummified and in the stage of dry decay (the final stage of the putrefaction process, leaving only the skeletal remains). The coroner estimated that the death had taken place seven months prior. Worth particular note, the coroner's report showed: carcinoma and fracture of the greater trochanter of the left hip.

Expert investigation

The coroner requested a toxicological study from the INTCF.



Figure 3.1.2.1. Sample of skeletal remains analysed

A general investigation of organic toxins was performed on the bone sample received, with a view to detecting drug abuse and the most frequently used drugs: barbiturates, benzodiazepines, pyrazolones, antipsychotics, antidepressants, antiepileptics, analgesics (paracetamol, tramadol, metamizol, etc.), phenothiazines and similar, oral antidiabetics, non-steroidal anti-inflammatory drugs, diuretics, xanthine bases, antiparkinsonians, antihistamines, antihypertensives, antiplatelet agents, benzamides, opiates derived from morphine, cocaine and metabolites, methadone, amphetamine and related drugs.

To this end, the bone sample was first treated and purified by solid phase extraction (SPE) and then analysed by gas chromatography with mass spectrometry detector (GC-MS) and high performance liquid chromatography with coupled mass spectrometry (UPLC-QTRAP) and (UPLC-QTOF).

Results

Benzoilecgonina	Positive		
Cocaine	Positive		
EDDP	Positive		
mCPP	Positive		
Methadone	Positive		
Naproxen	Positive		
Trazodone	Positive		

Evaluation of the results

The results obtained were consistent with the deceased's use of or exposure to cocaine, methadone, trazodone and naproxen. EDDP is the main metabolite of methadone and mCPP of trazodone.

A positive result in terms of these substances may be due to their incorporation into the bone matrix (marrow), or from skeletal remains (blood and tissues) or artefacts of mummification on the surface of the bone itself (which may have contained the substances close to death).

Considerations

The bone sample is an alternative option that may be useful in skeletonised remains where no other option is available.

In 2021, four cases were analysed at the Madrid Department (including this case) where the sample received consisted of bones from different anatomical areas (head, ribs, etc.), and in three cases, positive results were returned. The substances detected were drugs (cocaine) and pharmaceuticals (benzodiazepines, hypnotics, antidepressants, synthetic opioids and anti-inflammatory drugs).

In the absence of other options, the analysis of bone tissue may provide the only source of toxicological information, as it is the most resistant tissue in the human body.

Limited information is available about the detectability and incorporation of drugs into bone. Several factors influence the deposition of drugs in this matrix, including acute versus chronic exposure, distribution at the time of death, bone harvest site and type of bone, as well as the physical and chemical characteristics of the drug.

This type of analysis is also of interest to forensic anthropology, as the toxicological results from bone can provide important information to add to the biological profile, confirming the role of forensic toxicology as an aid in the field of identification and study of human skeletal remains.

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3.1.3. Teaching and scientific activity

3.1.3.1. Participation in investigation projects

Bravo Serrano B. Collaborator in the project "Service-learning on the problem of chemical submission: interdisciplinary collaborative action with horizontal and vertical coordination in several grades" University of Alcalá. 2020/21; 2021/22 Academic year.

Bravo Serrano B. Short-term expert participating in the European project "Cooperation in criminal investigation in Central America to combat crime and drug trafficking at an international level". ICRIME-LA/2017/39066.

Bravo Serrano B., Quintela Jorge O. Research project "Evaluation and educational intervention to prevent drug use and sexual violence in youth leisure contexts Epidemiological area". Department of Health, Social Services, and Equality. University of Alcalá de Henares and National Institute of Toxicology and Forensic Sciences. From 11/12/2018 to 31/12/2021.

Bravo Serrano B. Participation in university activity "Transfer and dissemination of knowledge: co-educating and building international support and networks against sexual aggression facilitated by alcohol and other drugs in youth leisure contexts". UAH/EV1282. Ministry of Equality.

Quintela Jorge O. Participation in the project "Learning model for the study of arrhythmias associated with local anaesthetic intoxication and its application to improve teaching in clinical toxicology". Vice-Chancellor for Quality Innovation Projects 2020-2021 Innova-Docencia. Complutense University of Madrid. Duration: 2020/2021 Academic year.

Quintela Jorge O. Participation in the project "Learning model for the study of alterations in the QT interval, Tpeak-Tend interval and ventricular repolarisation dispersion due to toxic substances and its application to improve teaching in clinical toxicology". Vice-Chancellor for Quality Innovation Projects 2020-2021 Innova-Docencia. Complutense University of Madrid. Duration: 2021/2022 Academic year.

Bravo Serrano B. Participation in the PNSD grant project "Transdisciplinary approach to investigate and prevent drug-facilitated sexual aggression through a prospective approach". 2021-2024.

Bravo Serrano B. Participation in the project related to the implementation and dissemination of the 2030 agenda in the university community "Youth in action overcoming global challenges". UAH.

3.1.3.2. Contribution in scientific congresses

Poster: Carlos García Caballero, Óscar Quintela Jorge. A case of alprazolam-facilitated sedation in children and its analytical confirmation by hair analysis. 25th Annual Scientific Meeting of the Society of Hair Testing (2021). 16 and 17 September 2021. Santiago de Compostela (Spain).

3.1.3.3. Scientific publications

Lázaro del Nogal M, Fernández Alonso C, Serrano Cuesta P, García Briñón M A, Merino Díaz T, Quintela Jorge O, Santiago Sáez A. Probable chemical submission in elderly patients seen at a hospital emergency department: first case series. 2nd Virtual Congress of the Spanish Society of Geriatrics and Gerontology: new challenges for ageing. 2-4 June 2021 (virtual format).

Martínez P, Quintela O, Valle E del, et al. Genetic identification and subsequent LC-QTOF MS analysis of plant remains (*Oenanthe spp.*) could prove the cause of an undetermined sudden death. *Int J Legal Med.* 2021;135:1407-11.

Rodríguez L, Fernández I, Varela O, Urcelay A, Ramos C, Navarro P, Quintela O, Velázquez S, Melone A, García S, Anadón Baselga MJ, Zaballos M. Value of the QRS interval duration in predicting ropivacaine toxicity. Implications for the practice of regional anaesthesia in major outpatient surgery. *Revista de Cirugía Mayor Ambulatoria*. 2021;26(3):164-70.

3.1.3.4. Teaching and training activities

Bravo Serrano B. Associate Professor of the Degree in Forensic Sciences. University of Alcalá. 2020/21; 2021/22 Academic year.

Burgueño Arjona MJ. Associate Professor of the Department of Analytical Chemistry, Faculty of Chemical Sciences, Complutense University of Madrid. 2020/21 Academic year.

Valle Pérez ME del. Honorary teacher of the degree in Forensic Sciences. University of Alcalá. 2020/21; 2021/22 Academic year.

López Uceda EM. Honorary teacher of the degree in Forensic Sciences. University of Alcalá. 2020/21; 2021/22 Academic year.

Quintela Jorge O. Associated teacher in the Department of Toxicology and Health Legislation at the Complutense University of Madrid. Lecturer of the Degree in Medicine,

Degree in Criminology and Official Master's Degree in Health Expertise. 2020/2021; 2021/22 Academic year.

Quintela Jorge O. Tutoring of the Final Degree Project of the Degree in Criminology of the Department of Toxicology and Health Legislation of the Faculty of Medicine of the Complutense University of Madrid, entitled "Analysis of hair in cases of chemical submission" to the student Inmaculada del Carmen Monje. 2020/2021 Academic year.

Quintela Jorge O. Tutoring of the Final Degree Project for the degree in Forensic Sciences: Forensic and Technologies Sciences at the UAH, "Determination of benzodiazepines, antidepressants and antipsychotics in hair samples by LC-MS: interpretation of results in forensic toxicology". Student: Ana Moreno Navarro. 2020/2021 Academic year.

Bravo Serrano B. Lecturer in the subject "Fundamentals of Criminal Investigation" for the university master's degree in Police Sciences. 2020/2021 Academic year. UAH.

Bravo Serrano B. Lecturer in the subject "Forensic Chemistry". 2020/21 Academic year. Autonomous University of Madrid.

Bravo Serrano B. Tutoring of the Final Degree Project for the degree in Forensic Sciences: Forensic Technologies and Sciences at the UAH, "Screening of drugs in hair by UHPLC/ MS-MS Orbitrap™ for forensic purposes". Student: Beatriz Ávila Barrio. 2020/2021 Academic year.

Bravo Serrano B. Lecturer in the Judiciary Police Course on Drug Trafficking and Detection. Regional Government of Castilla y León. Civil Protection Agency. From 26 April to 18 May 2021.

Bravo Serrano B. Director of the course "Interpretation of toxicological results and their influence on the expert context in which the analysis is requested". Organised by the Centre for Legal Studies. From 10 to 17 May 2021.

Bravo Serrano B. Speaker as part of the training activity "Interpretation of toxicological results and their influence according to the expert context in which the analysis is requested", with the paper "The contribution of toxicological results and their interpretation in road safety offences". Centre for Legal Studies. From 10 to 17 May 2021.

Quintela Jorge O. Speaker as part of the training activity "Interpretation of toxicological results and their influence according to the expert context in which the analysis is requested" with the paper "Interpretation of results in suspected cases of chemical submission: How far can we go? Centre for Legal Studies. From 10 to 17 May 2021.

Quintela Jorge O. Speaker on the course: "Protocol of the Community of Madrid. Chemical submission". Organised by the Directorate General for Research, Teaching and Documentation in collaboration with the Technical Commission for Health Actions against Gender Violence. 19 May 2021.

Quintela Jorge O. Tutoring of the doctoral thesis at the University of Alcalá, entitled "A new ecological working framework for overcoming drug-facilitated sexual assault", by the student Pablo Prego Meleiro. September 2021.

Velázquez Romanos S. Lecture on "Rapid detection systems: screening methods", as part of the multidisciplinary course on drugs: review of toxicity, updating of analysis methods, judicial and social value of analytical expertise. Organised by the Ministry of Justice. From 18 to 26 October 2021.

Quintela Jorge O. Participant in the delivery of the "Toxicology" subject on the Degree in Pharmacy, giving a lecture entitled "Applications of Forensic Toxicology: chemical submission and drugs in road traffic", on 4 November 2021. Faculty of Pharmacy, University of Salamanca.

Bravo Serrano B. Tutoring of the student Miguel Ángel Merino Sierra, as part of the curricular work placement on the Bachelor's Degree in Chemistry. Complutense University of Madrid. 2021/2022 Academic year.

Velázquez Romanos S. Delivery of the conference "Multidisciplinary introduction to the expert activity of the INTCF" for students of Vocational Training of the Health Branch in the following educational centres of the Community of Madrid: Escuela Técnica de Enseñanzas Especializadas, IES Las Musas, Instituto de FP Claudio Galeno, IES Benjamín Rúa, Instituto Técnico de Estudios Profesionales (ITEP). 2020/21 Academic year.

Experts from the Chemistry Department. Treatment of offences against sexual freedom and integrity in the forensic laboratory. Organised by the Centre for Legal Studies and held online from 14 to 21 June 2021.

Experts from the Chemistry Department. Practical workshop on validation of forensic toxicology methods. Organised by the Centre for Legal Studies and held online from 4 to 8 October 2021.

Experts from the Chemistry Department. Multidisciplinary forensic intervention in multivictim incidents. Organised by the Centre for Legal Studies and held online from 15 to 22 November 2021.

3.2. Madrid Department Drug Service

Concerning the expert activity of the Madrid Department Drugs Service, a total of 8,093 requests were received, analyzing 24,886 samples through a total of 42,432 analyzes. This brought the total number of expert reports issued to 7,423.

As can be seen in Figure 3.2.1 and in the following table, most requests for analyses received corresponded to chemical-toxicological analyses with a view to detecting alcohol, drugs of abuse and psychotropic drugs in biological samples requested by the courts from both living subjects and *post-mortem* studies in deaths where adverse reactions to substance abuse was suspected (3,079 requests). Next, there were 2,795 requests for

chemical analyses of non-biological samples requested by the courts in relation to drug seizures. Special interest deserves the investigation on emergent drugs, the New Psychoactive Substances (NPS). Unfortunately, many are still unmonitored and remain illegal but constitute a serious health hazard for society. Most of them are sold on the internet, with a false appearance that isn't safe. It is important to highlight the increase of these new drugs (NPS), long overdue to appear on the illegal market, hence the importance of the laboratory role in analysing these new structures and alerting the Spanish Early Warning System (SEAT) to their existence.

Ranked third was the study of chronic drug use in hair, with 1,737 requests received during 2021. The investigation of chronic drug abuse as well as alcohol abuse by means of hair analysis is used in criminal investigations both to study drug dependence in relation to criminal liability cases and for the enforcement of court sentences. Worth particular mention is the study of substance abuse in the hair of young children who may be affected in environments where a family member uses illegal substances deserves. In these cases, hair analysis contributes to the medico-legal investigation of a possible child abuse offence. Also in relation to child custody and guardianship, although in civil cases, come requests for hair analysis in contentious divorce proceedings.

Furthermore, a total of 328 requests were received for chemical-toxicological studies on biological samples from victims of traffic accidents. The global data of these toxicological studies have been published before in case study form in the Report 2021 from the INTCF about *Toxicological Findings in Road Traffic Fatalities*.

Throughout 2021, the blood, vitreous humour and urine sample collection procedures have been modernised, helping to streamline the laboratory's work. The roll-out of Captiva EMR cartridges for ultrafiltration and lipid removal for blood and vitreous humour samples in the analysis of substance abuse and psychotropic drugs by high performance liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS), as well as the dilution and injection method for urine samples and their subsequent analysis also by LC-MS/MS, has saved time and resources without compromising the sensitivity of the analysis and, therefore, the quality of toxicological expertise. Thus, the use of the traditional Chemelut columns has been relegated to the extraction of urine samples that require prior hydrolysis for the determination of benzodiazepines. However, we have launched the process to perform one-step hydrolysis, followed by dilution and injection into LC-MS/MS.

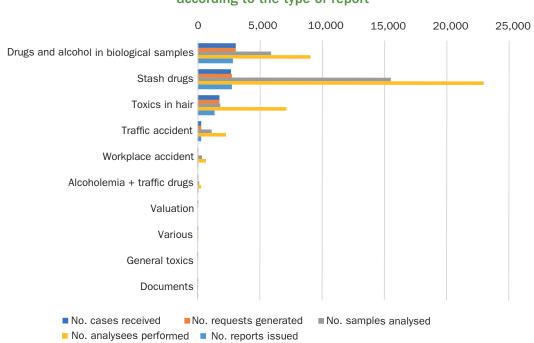


Figure 3.2.1. Casework of the Madrid Department Drug Service during 2021 according to the type of report

Table 3.2.1 Casework of the Madrid Department Drug Service during 2021 according to the type of report

Type of reports	No. cases received	No. requests generated	No. samples analysed	No. analyses performed	No. reports issued
Drugs and alcohol in biological samples	3,069	3,079	5,867	9,087	2,837
Stash drugs	2,719	2,795	15,515	22,906	2,742
Toxics in hair	1,738	1,737	1,821	7,112	1,417
Traffic accident	328	328	1,152	2,288	292
Workplace accident	104	104	351	693	93
Alcoholemia + traffic drugs	41	41	174	338	40
Valuation	3	3	0	0	0
Various	1	1	4	4	0
General toxics	1	1	0	0	0
Documents	1	1	2	4	1
TOTAL	7,994	8,093	24,886	42,432	7,423

In relation to hair drug analysis, in 2021, we embarked upon the migration of this type of analysis from gas chromatography coupled to mass spectrometry (GC-MS) to LC-MS/MS, in line with international trends. This migration, on which work remains ongoing, will in turn lead to a change in the current system of drug extraction in hair, discarding the

traditional liquid-liquid and solid-phase extractions, switching to a single methanolic incubation step. This change will undoubtedly mean that a higher volume of cases can be dealt with in less time, in addition to the savings in resources such as organic solvents (for example, hexane or ethyl acetate), in line with the international trend in analytical laboratories towards a progressive reduction in the use of non-polar solvents that pose a risk to health and the environment.

3.2.1. Forensic case of interest: Report of the death of an adolescent from poly-substance abuse, with a special focus on morphine.

Brief introduction and history of the case

As an example of the expert activity undertaken by the Drugs Service, the case of the death of a fifteen year old adolescent has been selected, in which the involvement of both substance abuse and psychotropic drugs was determined, with a predominant role of morphine obtained from pharmaceutical tablets.

The teenager was found lifeless on his bed and, following the investigations carried out by the forensic doctor during the recovery of the body, a bottle of marijuana and a blister pack with two tablets of morphine (30 mg) were found in the room, both of which belonged to his mother, who had died of cancer the previous year.

According to the testimony given by friends of the deceased to a relative, the teenager had taken morphine tablets days before, apparently for recreational purposes. The autopsy did not include a full examination of the body, as it tested positive for SARS-CoV-2. Histopathological examination of heart and lung samples did not reveal any significant findings.

Samples of central blood with NaF as a preservative and vitreous humour were submitted by the coroner to the Drugs Service.

Analytical aspects

Following the Toxicological Analytical System established by the Drugs Service for biological samples, the determination of ethyl alcohol and other volatiles (methanol, acetone, etc.) in blood and vitreous humour samples was undertaken by gas chromatography with the "headspace" method and flame ionisation detector (HS-GC-FID).

The blood sample was subject to an anion exchange solid phase extraction (Oasis MAX) for subsequent analysis by gas chromatography coupled to tandem mass spectrometry (GC-MS/MS) for the detection of cannabinoids. Blood and vitreous humour samples were also analysed by LC-MS/MS *target screening* after filtration and lipid removal using Captiva EMR cartridges.

Given the likely involvement of morphine, and for the purpose of detecting conjugated morphine (morphine-6-glucuronide), the blood sample was also analysed by high-performance liquid chromatography coupled to high-performance mass spectrometry (LC-HRMS Orbitrap).

Toxicological aspects

The following table shows the toxicological findings obtained from the different analyses performed under both options.

Table 3.2.1.1. Toxicological results according by matrices and techniques used

	Determination of alcohol and volatiles (HS-GC-FID)	Determination of cannabinoids (GC-MS/MS)	Determination of other abused drugs and psychotropic drugs (LC-MS/MS)	Determination of morphine conjugated metabolites (LC-HRMS Orbitrap)
Blood	Negative	THC < 1 ng/mL 11-OH-THC < 1 ng/ml THC-COOH: 6.3 ng/ml CBD < 0.5 ng/ml CBD < 0.5 ng/ml	Morphine (free): 184.5 ng/ ml (Figure 3.2.1.2) Alprazolam: 32.8 ng/ml №OH-alprazolam: 10.0 ng/ml	Morphine-6-glucuronide: 500 ng/ml
Vitreous humour	Negative	_	Morphine (free): 84.7 ng/ml (Figure 3.2.1.3) Alprazolam: 10.0 ng/ml &OH-alprazolam: 4.0 ng/ml	_

Figure 3.2.1.1. Chemical structure of morphine

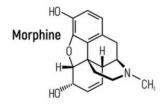
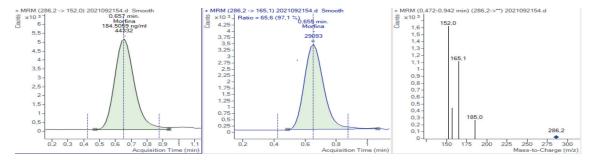


Figure 3.2.1.2. Chromatogram of morphine in blood (LC-MS/MS)



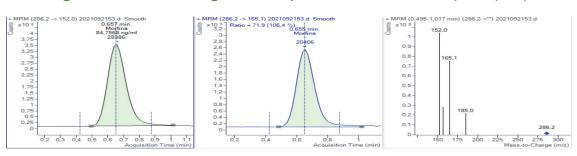


Figure 3.2.1.3. Chromatogram of morphine in vitreous humour (LC-MS/MS)

The toxicological conclusions that could be drawn based on the above were firstly, the absence of heroin metabolites such as 6-monoacetylmorphine, as well as alkaloids present in both opium and heroin stashes as impurities (codeine, noscapine or papaverine), ruled out that the morphine found was partly or wholly derived from heroin or opium consumption.

Furthermore, 6-monoacetylmorphine, a pharmacologically active metabolite of heroin, with an even more potent central nervous system depressant effect than morphine itself, was not detected. The ratio of morphine-6-glucuronide/morphine blood concentrations (2.7) was consistent with oral morphine consumption or administration, according to the literature consulted.

The central nervous system depressant action of morphine was reinforced by the action of a benzodiazepine (alprazolam) and an abused substance (cannabis). Therefore, we concluded that this was a violent death, the aetiology of which could have been accidental or suicidal, due to poly-substance abuse, with morphine playing a predominant toxic role.

It would have been desirable, however, to have taken a hair sample from the deceased, to prove the possible repeated consumption of morphine.

3.2.2. Teaching and scientific activity

3.2.2.1. Participation in investigation projects

Matey JM. Participation as a teacher in the short-term trainer-expert in activity 1.37: "Trace material analysis through Q-TOF and Q-ORBITRAP", as part of the project "TWINNING: Forensic Trainings Towards Advanced Examination Methods", held in Turkey from 13 to 17 December 2021.

3.2.2.2. Contribution in scientific congresses

Matey JM, López-Fernández A, García-Ruiz C, Montalvo G, Zapata F, González del Campo V, Martínez MA. "Hair Analysis of New Psychoactive Substances by High Resolution Mass Spectrometry (Q-Orbitrap). Analytical strategy using different data acquisition modes".

[Poster presentation] 26th Annual Scientific Meeting of the Society of Hair Testing (2021), held in Santiago de Compostela from 16 to 18 September 2021.

Matey JM, García-Ruiz C, Montalvo G, Zapata F, González del Campo V, Martínez MA. "Reimagining the future of pharmacy and toxicology. Non-target analysis of new psychoactive substances using the chemical strategy in high resolution mass spectrometry". [Online oral communication] KSAPT 2021. Remaining the future of pharmacy and toxicology, held in Saudi Arabia from 27 November to 2 December 2021.

García-Caballero C, Quintela O. "A case of alprazolam-facilitated sedation in children and its analytical confirmation by hair analysis". [Poster communication] 26th Annual Society of Hair Testing Meeting (2021), held in Santiago de Compostela from 16 to 18 September 2021.

Martínez MA (*keynote speaker*). "Great Toxic Catastrophes. A Review from Past to Present" (1 hour). KSAPT 2021. Remaining the future of pharmacy and toxicology. Online participation, held in Saudi Arabia from 27 November to 2 December 2021.

Martínez MA (*keynote speaker*). "Herbal Highs" (1 hour). KSAPT 2021. Remaining the future of pharmacy and toxicology. Online participation, held in Saudi Arabia from 27 November to 2 December 2021.

3.2.2.3. Scientific publications

Matey JM, Montalvo G, García-Ruiz C, Zapata F, López-Fernández A, Martínez MA. Prevalence study of drugs and new psychoactive substances in hair of ketamine consumers using a methanolic direct extraction prior to high-resolution mass spectrometry. *Forensic Sci Int.* 2021;(329):111080. https://doi.org/10.1016/j.forsciint.2021.111080

Matey JM, López-Fernández A, García-Ruiz C, Montalvo G, Zapata F, Martínez MA. Identification of 2C-B in hair by UHPLC-HRMS/MS. A real forensic case. *Toxics*. 9(7):170. https://doi.org/10.3390/toxics9070170

Zapata F, Matey JM, Montalvo G, García-Ruiz C. Chemical classification of new psychoactive substances (NPS). *Microchemical Journal*. 2021 April;(163):105877. https://doi.org/10.1016/j.microc.2020.105877

3.2.2.4. Relation of teaching and formation activities

Matey JM. Teacher on the online course at the Centre for Legal Studies (CEJ) "Practical workshop on the validation of forensic toxicology methods" (16 hours), lecturer at the conference "Modernisation of the Toxicological Analytical Systematics in the Drug Service of the INTCF. Introduction and validation of new methodologies based on GC-MS/MS LC-MS/MS and LC-HRMS/MS. Latest validated method: GC-MS/MS analysis of cannabinoids in blood samples" (1 h). Held on 6 October 2021.

Matey JM. Teacher on the online course at the Centre for Legal Studies (CEJ) "Multidisciplinary course on drugs: review of toxicity, updating of analytical methods, judicial and social value of analytical expertise" (16 hours), lecturer at the conference "Analytical confirmation: notions on different analytical techniques" (1 h). Held on 22 October 2021.

Martínez MA. Director of the Centre for Legal Studies (CEJ), online course "Update in Forensic Chemistry and Toxicology" (10 hours), aimed at forensic doctors and INTCF practitioners, lecturing on "Classical drugs of abuse" (2 hrs), "New psychoactive substances (NPS)" (1 hr), "The epidemic of opioid intoxication" (1 hr), "Herbal highs" (30 mins), "Alcohol, drugs and driving" (30 mins), "The role of samples in toxicological analysis and in the interpretation of results. Round table" (2 hrs), "The interpretation of post mortem toxicological results" (1 h) and "Selection of real cases in forensic toxicology. Round table" (2 hrs). Held from 8 to 16 March 2021.

Martínez MA. Guest lecturer: "Major toxic catastrophes. Review from the last century to the present" (1 hr), conference included in the seminars on "Accidents and NBC Terrorism", organised by the Almirante Juan de Borbón Chair of the Faculty of Chemical Sciences, Complutense University of Madrid. Invited by professor Dr. José Torrecilla. Held on 4 May 2021.

Martínez MA. Tutor in Toxicology and Forensic Chemistry for Cristina Carrasco Santos, from the National University of Distance Learning (UNED), graduate in Chemical Sciences. June-September 2021, during continuous working hours from 7:30 am to 2:30 pm.

Martínez MA. Tutor responsible for the training in Forensic Toxicology of Ms Natalia María García Simón, resident of the Laboratory of Clinical Biochemistry at Hospital Universitario Puerta de Hierro (Madrid), in the specialism of Clinical Biochemistry R4, from 1 September to 30 October 2021, from 7:30 am to 2:30 pm.

Martínez MA. Tutor responsible for the training in Forensic Toxicology of Ms Ana Jambrina Prieto, resident of the Laboratory of Clinical Biochemistry at Hospital Gregorio Marañón (Madrid), in the specialism of Clinical Biochemistry R4, from 12 September to 15 November 2021 during continuous working hours from 7:30 am to 2:30 pm.

Martínez MA. Coordinator and tutor of supervised internships for the 30th graduating class of the National Corps of Forensic Doctors. Practicuum at the National Institute of Toxicology and Forensic Sciences from 6 to 17 September 2021.

Martínez MA. Lecturer of the "Multidisciplinary course on drugs: review of toxicity, updating of analytical methods, judicial and social value of analytical expertise", aimed at technical specialists and laboratory assistants of the National Institute of Toxicology and Forensic Sciences, held from 18 to 26 October 2021.

Martínez MA. Speaker at the Centre for Legal Studies (CEJ) online course "Medicine and Public Health" (10 hrs) with the lecture "Chemical-Toxicological Expertise and their Social and Health Projection" (1 hr). Directed by Dr. José Amador Martínez, director of the

Institute of Legal Medicine of Burgos and aimed at forensic doctors and members of the National Institute of Toxicology and Forensic Sciences. Held on 24 November 2021.

Martínez MA. Organiser and speaker on the online *workshop* "Pesticides in Forensic Toxicology". XVI TIAFT Latin American Regional Meeting (4 hrs). Held on 18 November 2021.

3.2.2.5. Other activities

González del Campo V, Matey JM. Online attendance of the KSAPT 2021 conference "Reimagining the future of pharmacy and toxicology". Held from 27 to 2 December 2021.

García-Caballero C, González del Campo V, Martínez MA, Matey JM. Attendance at the conference "25th Annual Scientific Meeting of the Society of Hair Testing (2021)". Held in Santiago de Compostela from 16 to 18 September 2021.

Pedregosa A. Participation as a student at the Centre for Legal Studies (CEJ) online course on "Quality assurance in the forensic process. A step forward". Held from 2 to 5 November 2021.

Matey JM, Montero A, Pedregosa A. Participation as students at the Centre for Legal Studies (CEJ) online course "Practical workshop on the validation of forensic toxicology methods". Held from 4 to 8 October 2021.

González del Campo V, Montero A, Pedregosa A. Participation as students at the Centre for Legal Studies (CEJ) online course "Practical workshop for the dissemination of the INTCF quality system". Held from 21 to 28 September 2021.

Martínez MA. Participation as a student in the *webinar* of The International Association of Forensic Toxicologists (TIAFT) "2021 Continuing Education" at both sessions: "Therapeutic use of psychodelic drugs" and "Minimum requirements for drug identification" (6 hrs). Held on 13 July 2021.

García-Caballero C, González del Campo V, Matey JM, Montero A, Pedregosa A. Participation as students at the Centre for Legal Studies (CEJ) online course "Pesticides in forensic toxicology". Held from 31 May to 7 June 2021.

Matey JM, Montero A. Participation as students at the Centre for Legal Studies online course (CEJ) "LIMS_Basic._Structure, data organisation and queries". Held from 17 to 24 May 2021.

García-Caballero C, González del Campo V, Matey JM, Pedregosa A. Participation as students at the Centre for Legal Studies (CEJ) online course "Interpretation of toxicological results and their influence according to the expert context in which the analysis is requested". Held from 10 to 17 May 2021.

Pedregosa A. Participation as a student at the Centre for Legal Studies (CEJ) online course "Detection and identification of chemical weapons in forensics. Toxicity and impact on the environment". Held from 4 to 11 May 2021.

Montero A. Online attendance of the specific activity organised by the University of Alcalá de Henares entitled "The phenomenon of chemical submission in Spain: present, difficulties and future challenges". Held on 05 May 2021.

Matey JM. Participation as a student in the *webinar* organised by the Society of Forensic Toxicologists (SOFT) on the analysis of new psychoactive substances (NPS). 23 April 2021 (4 hrs).

González del Campo V, Montero A. Participation as students at the Centre for Legal Studies (CEJ) online course "Update in Forensic Chemistry and Toxicology". Held from 8-16 March 2021.

Martínez MA. Member of the examining board for the INTCF Special Corps of Laboratory Assistants, pursuant to Order JUS/780/2021, of 12 July (Official State Gazette No. 173, of 21 July 2021).

Martínez MA. Reviewer of the doctoral thesis by D. Najmitdinov Saidolim Bohodirovich from the Tashkent Pediatric Medical Institute (Uzbekistan). Submitted for the degree of Doctor of Medical Sciences entitled "Forensic assessment of poisoning with ethanol and its surrogates".

García-Caballero C, Martínez MA, Matey JM and Montero A. Members of The International Association of Forensic Toxicologists (TIAFT), with M. A. Martínez being the Spanish regional representative of the association since 2018.

Martinez MA and Matey JM. Members of the Society of Hair Testing (SoHT).

Matey JM and Montero A. Members of the University Institute for Research in Police Sciences (IUICP). University of Alcalá de Henares.

Martínez MA. Member of the following organisations: Society of Forensic Toxicologists (SOFT), Spanish Association of Toxicology (AETOX), Spanish Registry of Toxicologists of the AET, Registry of the European Association of Toxicologists and European Societies of Toxicologists (EUROTOX).

Martínez MA. Reviewer of the following scientific journals: Forensic Science International, Journal of Forensic Sciences, Journal Analytical Toxicology, Journal of Chromatography B, Journal of Chromatography A, Egyptian Journal of Forensic Sciences (board member), Revista Española de Medicina Legal, Revista de Toxicología and Acta Pediátrica.

Matey JM. Reviewer for Journal of Analytical Toxicology.

3.3. Chemistry and Drugs Service of the Barcelona Department

The Barcelona Department's Chemistry and Drugs Service's expert activity returned to normal in 2021, following the exceptional circumstances seen in 2020. The number of requests has not only returned to the normal figures registered by this Service, but in 2021, the caseload increased compared to 2019, with 5,432 cases registered and

6,679 requests generated. This increase can mainly be attributed to the number of requests for drug testing on stashes, with 3,614 requests generated compared to 2,752 and 2,614 in 2020 and 2019, respectively. The total number of requests generated came to 6,679, with 39,561 samples analysed and 70,638 analyses performed. The number of reports issued was 6,806. The figures for all expert activity through 2021 are reflected in Figure 3.3.1. In this context, it is important to note that the implementation of teleworking has ensured that this increase in caseload has not affected the Service's pendency time (which has even reduced), by optimising the time devoted to laboratory work and the time spent at the computer.

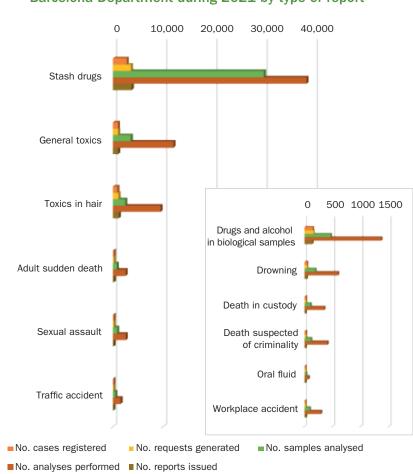


Figure 3.3.1. Casework of the Chemistry and Drugs Service of the Barcelona Department during 2021 by type of report

Table 3.3.1. Casework of the Chemistry and Drugs Service of the Barcelona Department during 2021 by type of report

Type of report	No. cases registered	No. requests generated	No. samples analysed	No. analyses performed	No. reports issued
Stash drugs	2,776	3,614	30,111	38,623	3,783
General toxics	1,035	1,053	3,596	12,103	1,050
Toxics in hair	927	1,144	2,478	9,526	1,197
Adult sudden death	206	206	813	2,549	190
Sexual assault	178	182	872	2,595	140
Drugs and alcohol in biological samples	150	163	465	1,368	143
Traffic accident	110	113	498	1,555	99
Drowning	49	49	194	601	44
Death in custody	28	28	110	351	26
Death suspected of criminality	27	28	120	411	25
Oral fluid	24	24	32	78	23
Workplace accident	21	22	96	296	27
Alcoholemia + traffic drugs	12	12	31	103	15
Sudden death related to sport	9	9	42	132	8
Fires	9	9	38	122	12
Alcoholemia traffic in live subjects	8	8	8	31	8
Infant sudden death	7	7	31	112	8
Children sudden death	5	7	26	82	6
Drugs and/or alcohol chronic consumption	0	0	0	0	2
TOTAL	5,432	6,679	39,561	70,638	6,806

The Chemistry and Drugs Service of the Barcelona Department currently provides a major number of communications to the European Database on New Drugs (EDND). During 2021, 272 cases were reported to the Spanish Early Warning System (SEAT) via the EDND platform for new psychoactive substances included in the monitoring list set out by the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA). This represents almost 23% of all communications made in Europe, five of which were first communications. Against this backdrop, in year 2021, the analysis of drugs in seizures (3,614 requests generated, with 30,111 samples received and 38,623 analyses performed) remained the main type of request for analysis by this Service (54.1% of the total number of requests received). This is reflected in Figure 3.3.2, which shows the distribution of the Service's caseload. As can be seen in this figure, in addition to requests resulting from seizures, the other major part of this Service's expert activity focuses on the analysis of samples from deceased subjects (22.8% post mortem) and requests related to living persons

(23.1% living). Figure 3.3.3 details, in relation to *post mortem* cases, those that correspond to a violent aetiology and those that are classified as non-violent.

Figure 3.3.2. Distribution of the casework of the Barcelona Department Chemistry and Drugs Service during 2021

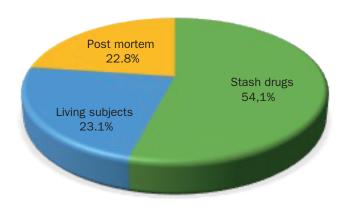
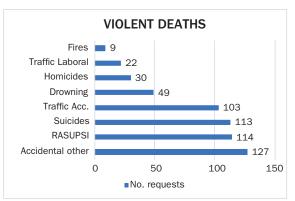
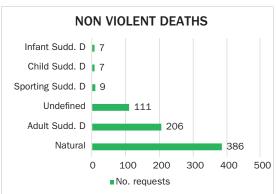
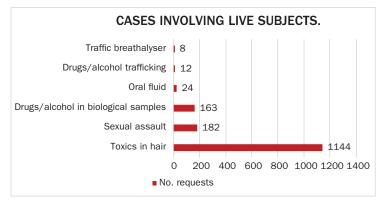


Figure 3.3.3. Types of *post mortem* cases: violent and non-violent deaths.

Type of living persons cases







In relation to biological samples from deceased/living subjects, the largest group of requests for analysis carried out corresponds to the general study of toxics (1,053 requests with 3,596 samples analysed). This group includes deaths of a natural aetiology (35.7%), violent suicidal deaths (29.3%), accidental deaths (11.7%), and deaths due to an adverse reaction to psychoactive substances (10.4%), among others. An analytical system is applied to this group aimed at the identification, confirmation and quantification, if applicable, of substances present in the samples received in order to help establish the cause of death. Figure 3.3.4 provides a breakdown of the reporting sub-types included in the overall study of toxic substances.

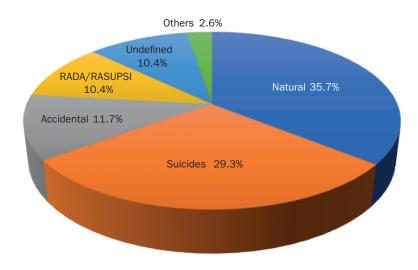


Figure 3.3.4. Reporting sub-types under General Toxic Substances

The analysis of toxic substances in hair also accounts for a large volume of work (1,144 requests generated with 2,478 samples analysed), as part of which the chronological study of chronic substance use is performed, mainly within a framework of criminal liability. The increase in 2021 in the number of requests received in cases of sexual assault (182 requests generated with 872 samples analysed) is worth particular note; in these cases, analyses are performed using highly sensitive techniques with a view to detecting substances at minute concentrations, and which may be involved in a possible chemical submission. The number of traffic accident cases also increased during 2021 (113 requests generated with 498 samples analysed), following the lifting of Covid restrictions originally implemented in 2020.

At the same time as the expert activity carried out by the Service, during 2021, the methods were fine tuned for the new equipment received. This year, high-performance liquid chromatography equipment coupled to a tandem mass spectrometer (UPLC-MS/MS) has been included in the routine work system. Efforts have also been made to validate the new headspace-gas chromatography equipment with flame ionisation detector

(HS-GC-FID) for the performance of breathalysers. For these devices, a Python program has also been developed for the automatic validation of the daily breathalyser sequences. Against this backdrop, work has been undertaken to make improvements to streamline the Service's operations. An Excel file has been developed with macros and dynamic graphics for the management and control of the flow of analysis requests for the different devices. An Excel database has also been implemented for the control of the different methods and calibration curves of each of the devices. Furthermore, macros have been created to maintain the connection of network drives to ensure these connections are not affected by changes in the passwords of Ministry of Justice users. Finally, the Masshunter software has been modified using macros to directly quantify substances in hair samples. With a view to boosting quality, the following validations have been launched at the Service during 2021: determination of carboxyhaemoglobin in blood by UV-visible spectrophotometry, determination of GHB (g-hydroxybutyrate) in urine by gas chromatography coupled to mass spectrometry (GC/MS) and analysis of drugs of abuse in hair by GC/MS/MS. In addition, the required analyses have been performed to extend the scope of the accreditation to include the quantification of ketamine in samples of seizures.

3.3.1. Forensic case of interest: Screening for new psychoactive substances in the context of death with an undetermined aetiology

The Chemistry and Drugs Service of the Barcelona Department received an urgent case involving a subject (due to the nature of the case, information is omitted), with a history of drug use and psychiatric pathology, with an undetermined medico-legal aetiology of death. The body was found with a significant amount of blood on the ground and an open fracture in one of his limbs. There were also multiple contusions on the thorax and upper and lower limbs, leading to suspicions of criminal involvement. The forensic doctor in charge of the case informed us that the injury to one of the limbs and the resulting blood loss were not sufficiently extensive to conclude that this was the cause of death. Similarly, bruising is not a life-threatening injury. Samples of peripheral blood, vitreous humour, urine and hair were sent to us for toxicological investigation.

The following analyses were carried out by the Service:

- Determination of ethyl alcohol in blood and vitreous humour by HS-GC-FID.
- Investigation of the presence of opiates, monoacetylmorphine, cocaine, benzodiazepines, barbiturates, methadone, amphetamines, cannabis, tricyclic antidepressants, propoxyphene, and buprenorphine in blood and urine by homogeneous enzyme immunoassay Cedia® /DRI®.
- General investigation of abuse and psychotropic drugs in blood and urine by solid and liquid-liquid phase extraction, respectively, and analysis by GC-MS and LC-Q Exactive Orbitrap high-performance liquid chromatography.

 General investigation abuse and psychotropic drugs in hair by washing, trituration and incubation in organic solvent and subsequent analysis by UHPLC-Q Exactive Orbitrap.

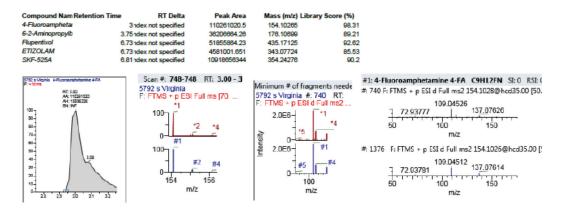
The blood sample showed an ethyl alcohol concentration of 0.19 g/l and the active substance flupenthixol, an antipsychotic indicated for the treatment of chronic schizophrenia and paranoid psychosis, which had been prescribed to the victim.

Given the deceased's history of multi-drug abuse, it was to be expected that different substances would be detected in the samples analysed. However, of the so-called "classic" drugs, only delta-9-tetrahydrocannabinol (THC) acid metabolite, 11-nor-carboxy-THC, was found in urine, indicating relatively recent cannabis use. The remaining substances detected were all new emerging drugs or new psychoactive substances.

Figure 3.3.1.1. Chemical structure of etizolam and benzodiazepine structure of MDA and 6-APB and amphetamine and 4-fluoroamphetamine.

The following were identified in the blood, urine and hair: 4-fluoroamphetamine, 6-APB (6-(2-aminopropyl)benzofuran) and etizolam. In hair, the presence of sildenafil, a drug used for the treatment of erectile dysfunction, was also detected. The presence of flupenthixol was not detected in this sample.

Figure 3.3.1.2. Identification of 4-fluoroamphetamine, 6-APB, etizolam and flupenthixol in the blood sample by LC-Orbitrap Exactive



This is not the first time that this Service has detected the presence of the thiodiazepine etizolam, although until now it has only found this designer benzodiazepine in investigations of living persons: sexual assaults and hair. This was the first case of a deceased person in which the Service reported this benzodiazepine. It is worth mentioning that this is the third case of biological samples that the Chemistry and Drugs Service of the Barcelona Department reported to SEAT in 2021.

Of the 30 new benzodiazepines monitored by the EMCDDA, the market in Europe is dominated by etizolam and flualprazolam, which have already been included in list IV of the UN Convention on Psychotropic Substances of 21 February 1971. The use of etizolam as an anxiolytic drug is licensed in countries like Japan, Italy and India, but it is not marketed in Spain [1].

Prescription benzodiazepines are relatively safe drugs, and fatalities from their use are generally attributed to toxicity resulting from co-administration with alcohol and opiates/opioids. In the case of etizolam, new designer benzodiazepines and new psychoactive substances in general, the effects of their use are unknown. Thus, in 2019, recreational use of etizolam in Scotland was involved in 752 deaths due to an adverse reaction to psychoactive substances [2].

As regards 6-APB and 4-fluoramphetamine, this is the first time that these substances have been detected in biological samples at this Facility. 6-APB is a phenylethylamine with stimulant and psychoactive effects, which has a structure similar to that of the Schedule IV drug methylenedioxyamphetamine (MDA). 4-fluoroamphetamine is a halogenated derivative of amphetamines. Its use has increased dramatically in countries such as the Netherlands due to a false sense of safety in its use; in fact, 4-fluoroamphetamine is known as 'ecstasy light'. Contrary to this perception, 4-fluoramphetamine and the new benzofurans, including 6-APB, are substances that pose major health risks, with fatalities associated with these new psychoactive substances having been reported in Europe [3] [4].

Our results indicate that the deceased was under the influence of three newly emerging drugs at the time of his death: etizolam, 6-APB and 4-fluoroamphetamine. Hair analysis reveals chronic use of these substances. Given the lack of knowledge of the pharmacology and toxicology of these new psychoactive substances, as well as the risks associated with their use (which are increased because there is no guarantee of the concentration of an unregulated substance and, in this case, because of the concomitant consumption of these substances), it cannot be ruled out, in this case, that these substances may have contributed in part to the cause of death.

Bibliographic references

- [1] New benzodiazepines in Europe a review. European Monitoring Centre for Drugs and Drug Addiction June 2021.
- [2] McAuley *et al*. From the clinic to the street: the changing role of benzodiazepines in the Scottish overdose epidemic. *Int J Drug Policy*. 2002;100:103512.

[3] Hondebrink L *et al.* Fatalities, cerebral hemorrhage, and severe cardiovascular toxicity after exposure to the new psychoactive substance 4-fluoroamphetamine: a prospective cohort study. *Ann Emerg Med.* 2018;71(3):294-305.

[4] Nugteren-van Lonkhuyzen JJ et al. Pharmacokinetics, pharmacodynamics and toxicology of new psychoactive substances (NPS): 2C-B, 4-fluoroamphetamine and benzofurans. *Drug Alcohol Depend*. 2015; 157:18-27.

3.3.2. Teaching and scientific activity

3.3.2.1. Participation in investigation projects

Agustí Mora Font. Participation in the FIAPP (Fundación Internacional y para Iberoamérica de Administración y Políticas Públicas) project in Belize. Strengthening of investigation units, forensic institutes, criminal investigation networks and procedures in the Central American Integration System. Activity at the Chemistry Department and in the Quality System. As part of the Project for Cooperation in Criminal Investigation in Central America to combat crime and drug trafficking at the international level ICRIME. February 2021.

3.3.2.2. Attendance at scientific congresses

Marta Sánchez Fité and Elena Hernández Marían. Attendance at the conference "25th Annual Scientific Meeting of the Society of Hair Testing (2021)". Held in Santiago de Compostela from 16 to 18 September 2021.

3.3.2.3. Scientific publications

Muñoz-Quirós Caballero JM, Mira E, Moyano S, Abad R, García García E, Fernández-Rodríguez A. Multidisciplinary medico-legal investigation of death due to SARS-CoV-2 (COVID-19): review of the literature in relation to a case. *Gaceta Internacional de Ciencias Forenses*, 2021: 41:6-16.

3.3.2.4. Teaching and training activities

Nuria Sanvicens Diez. Coordination of the "Multidisciplinary course on drugs: review of toxicity, updating of analytical methods, judicial and social value of analytical expertise", aimed at laboratory assistants and technicians from the Barcelona Department of the INTCF. Streamed from 15 to 23 November 2021.

As part of this course:

Cristina Marín Hernández. Lectures

- "Classic and emerging drugs: physico-chemical properties, origin, toxic effects, national and international legislation and control".
- "What is sampling? Notions on sampling in seizures. Applicable regulations: rules for the preparation and submission of samples for analysis by the INTCF and Framework agreement".
- "Samples of drug seizures. Methods of concealment".
- "Accuracy and precision of measurements. Uncertainty. Reference materials.
 Traceability. Expression of results".

Nuria Sanvicens Diez. Lectures

- "Analytical techniques for identification and confirmation".
- "Exhibition of cases analysed at the INTCF in Barcelona in relation to drugs".
- "Drugs in society: deaths due to acute reaction to drugs of abuse".
- "Drugs in society: chemical submission".

Ramón Rey Aguilar. Lectures

- "Biological samples. Types, treatments and usefulness of information from biological samples analysed in these cases".
- "Hair sample in the toxicological analysis. Chronic consumption. Applications".
- "Rapid detection systems: screening methods. Urine and Saliva. Traffic offences (DGT)".
- "Exhibition of cases analysed at the INTCF in Barcelona in relation to drugs".

Ramón Rey Aguilar. Lecture "Chemical analysis in crimes against public health", as part of the Master's Degree in Criminal Law at Pompeu Fabra University. 06 April 2021.

Nerea Picazas Márquez. Director and coordinator of the course organised by the Centre for Legal Studies: "Detection and identification of chemical weapons in forensics. Toxicity and impact on the environment". Lecture as part of this course: "Methods of analysis of chemical weapons and related compounds in biological and environmental samples" and development of a case study. *Streamed* from 4 to 11 May 2021.

Ramón Rey Aguilar. Lecture "The chemical-toxicological expert report I. Detection and quantification of toxins in biological samples and the resulting consequences". As part of the course "Interpretation of reports issued by the National Institute of Toxicology and Forensic Sciences", organised by the Centre for Legal Studies. 21 April 2021.

Ramón Rey Aguilar. Lecture "The chemical-toxicological expert report I. Interpretation of the expert reports issued by the Drugs Section". As part of the course "Interpretation of reports issued by the National Institute of Toxicology and Forensic Sciences", organised by the Centre for Legal Studies. 22 April 2021.

Cristina Marín Hernando. Lecture "Advancing systematics: validation of a calibration line obtained by artificial intelligence and its applicability in the validation of forensic toxicology methods". As part of the course "Practical workshop on validation of forensic toxicology methods", organised by the Centre for Legal Studies. 07 October 2021.

Cristina Hernando Torrecilla. Lecture "The Chemistry Service. Chemical Submission and Vulnerability: Theoretical and Practical Aspects of Substance Detection in Cases of Alleged Sexual Assault". As part of the course "Treatment of offences against sexual freedom and integrity in the forensic laboratory", organised by the Centre for Legal Studies. 14 June 2021.

Cristina Hernando Torrecilla. Lecture "Update on substances that facilitate sexual aggression-submission and chemical vulnerability". As part of the course "Update on the expert assessment of sexual violence", organised by the Centre for Legal Studies. 12 November 2021.

Experts from the Chemistry Department. Action in Forensic Chemistry and Toxicology. From the laboratory to the courts. Organised by the Centre for Legal Studies and *streamed* from 8 to 15 March 2021.

Experts from the Chemistry Department. The INTCF Forensic Sciences Service: Fields of action, analytical possibilities. Organised by the Centre for Legal Studies and *streamed* from 22 to 26 March 2021.

Experts from the Chemistry Department. Interpretation of expert opinions in the field of forensic medicine. Organised by the Centre for Legal Studies and *streamed* from 19 to 26 April 2021.

Experts from the Chemistry Department. Forensic detection and identification of chemical weapons. Toxicity and impact on the environment. Organised by the Centre for Legal Studies and *streamed* from 4 to 11 May 2021.

Experts from the Chemistry Department. Interpretation of toxicological results and their influence on the expert context in which the analysis is requested. Organised by the Centre for Legal Studies and *streamed* from 10 to 17 May 2021.

Experts from the Chemistry Department. Basic LIMS: structure, data organisation and queries. Organised by the Centre for Legal Studies and *streamed* from 17 to 24 May 2021.

Experts from the Chemistry Department. Pesticides in forensic toxicology. Organised by the Centre for Legal Studies and streamed from 31 May to 7 June 2021.

Experts from the Chemistry Department. Forensic toxicology. Cause of death pathology. Organised by the Centre for Legal Studies and streamed from 7 to 14 June 2021.

Experts from the Chemistry Department. Treatment of offences against sexual freedom and integrity in the forensic laboratory. Organised by the Centre for Legal Studies and streamed from 14 to 21 June 2021.

Experts from the Chemistry Department. Practical workshop on the dissemination of the INTCF quality system. Organised by the Centre for Legal Studies and *streamed* from 21 to 28 September 2021.

Experts from the Chemistry Department. Practical workshop on validation of forensic toxicology methods. Organised by the Centre for Legal Studies and *streamed* from 4 to 08 October 2021.

Experts from the Chemistry Department. Quality assurance in the forensic process. A step forward. Organised by the Centre for Legal Studies and *streamed* from 2 to 5 November 2021.

Experts from the Chemistry Department. Update on the expert assessment of sexual violence. Organised by the Centre for Legal Studies and *streamed* from 11 to 18 November 2021.

Experts from the Chemistry Department. Multidisciplinary forensic intervention in multivictim incidents. Organised by the Centre for Legal Studies and *streamed* from 15 to 22 November 2021.

Virginia Lostao Abadía. Training at the Department of Chemistry and Drugs of Seville in solid-phase extraction techniques applied to analytical toxicological systematics and application of high-performance liquid chromatography with diode array detector (HPLC-DAD) in the detection of substances of toxicological interest in biological samples.

3.3.2.5. Other activities

Nuria Sanvicens Diez. Interview for the radio programme "Por fin no es lunes" on Onda Cero. "Fentanyl: the drug that is revolutionising drug trafficking". April 2021.

Nuria Sanvicens Diez. Article published in the digital newspaper *El Taquígrafo*. Title of the article: "Cannabinoides sintéticos: la droga camuflada de efectos devastadores". April 2021.

Cristina Marín Hernández. Working Group of the Spanish Network of Official Forensic Laboratories (RFLOE).

Nuria Sanvicens Diez. Member of the Society of Hair Testing (SoHT).

3.4. Chemistry and Drugs Service of the Seville Department

Expert activity is the basis of the Seville Department Chemistry and Drug Service. During 2021, 4,747 expert cases were received, 4,792 requests were generated and 18,179 samples were analysed. In terms of expert reports, 4,183 were issued.

Figure 3.4.1 and Table 3.4.1 reflect the work carried out by the Chemistry and Drugs Service of the Department of Seville during 2021.

Figure 3.4.1. Casework of the Seville Department Chemistry Service during 2021 by the type of report

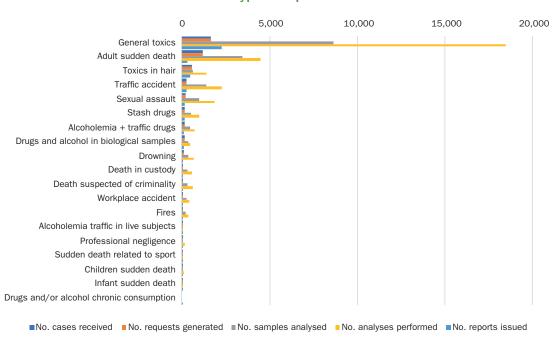


Table 3.4.1. Casework of the Seville Department Chemistry Service during 2021 by the type of report

Type of report	No. cases received	No. requests generated	No. samples analysed	No. analyses performed	No. reports issued
General toxics	1,658	1,669	8,641	18,480	2,285
Adult sudden death	1,204	1,207	3,460	4,470	297
Toxics in hair	571	592	644	1,399	447
Traffic accident	257	257	1,368	2,255	240
Sexual assault	230	230	992	1,846	178
Stash drugs	158	160	501	1,005	153
Alcoholemia + traffic drugs	141	142	477	746	131
Drugs and alcohol in biological samples	140	140	379	451	110

Type of report	No. cases received	No. requests generated	No. samples analysed	No. analyses performed	No. reports issued
Drowning	86	86	376	658	74
Death in custody	76	76	320	586	55
Death suspected of criminality	65	66	312	621	66
Workplace accident	55	55	257	418	51
Fires	47	48	226	370	40
Alcoholemia traffic in live subjects	17	17	46	62	15
Professional negligence	17	17	71	147	18
Sudden death related to sport	10	10	36	61	7
Children sudden death	9	10	39	85	6
Infant sudden death	9	9	34	63	9
Drugs and/or alcohol chronic consumption	0	0	0	0	1
TOTAL	4,747	4,792	18,179	33,723	4,183

The cases handled by the Seville Department, as can be seen in Figure 3.4.2, are very varied. From the 4,792 requests generated, 72.33% corresponded to *post mortem* cases and 23.87% to cases of living subjects, with drug testing in seizures accounting for 3.34%. Violent deaths accounted for 54.70% of the *post mortem* cases.

Sudden adult death is the most common request in *post mortem* samples: 60.35% (1,207 requests). An analytical system is applied to this group aimed at the identification, confirmation and quantification, if applicable, of substances present in the samples received in order to help establish the cause of death. It should be noted that ranking second is death by suicide (765 requests), accounting for 38.25% of violent deaths. This is followed by deaths due to an adverse reaction to psychoactive substances (RASUPSI), on 330 requests, accounting for 16.50% of violent deaths, an increase when compared to 2020 (13.8%).

Toxicological studies in traffic accidents are the second most frequent type of cases in the violent deaths group, accounting for 12.85% of the total, with the number of requests increasing compared to 2020: 257 versus 201. The most commonly requested investigation for living subjects was the determination of toxins in hair, 52.83%, with 592 requests, up on 2020, when 473 requests were generated. The number of requests for deaths in custody remained the same as in the previous year, with a total of 76 cases (33 natural and 43 violent).

Finally, there was an increase in the number of toxicological investigation requests in cases of sexual assault, both in absolute terms and as a percentage compared to 2020, from 16% of the cases involving living subjects in 2020 (170 requests) to 20% in 2021, with 230 requests.

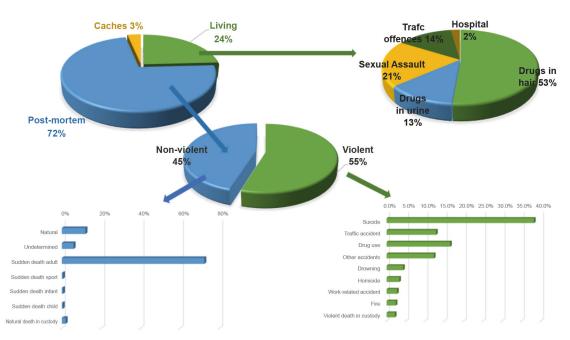


Figure 3.4.2. Casework of the Chemistry and Drugs Service of the Department of Seville

The Chemistry and Drugs Service of the Department of Seville, in addition to carrying out the analyses to respond to the requests it receives, also carries out the determination of the chronic consumption of ethyl alcohol, through the analysis of ethyl-glucuronide in hair samples, in the requests that are received in all departments of the INTCF.

The significant improvement in the instrumentation of the Service, following the receipt of a high performance liquid chromatograph coupled to a triple quadrupole mass spectrometer (UPLC-MS-tQ), which, together with the two high resolution devices, another UPLC-MS-tQ and a high performance liquid chromatograph coupled to a hybrid quadrupole time-of-flight mass spectrometer (UPLC-MS-QTOF), received in 2020, and high performance liquid chromatography coupled to a mass spectrometer-ORBITRAP (UPLC-MS-ORBITRAP), received previously, is facilitating the adaptation of the workflow, simplifying the sample pretreatment processes, which will be reflected in greater efficiency and a reduction in the time for the issuance of reports. Furthermore, this equipment will not only make it possible to update the Service's routines, but, fundamentally, to identify and quantify new substances and/or compounds that can only be analysed with these instruments.

One of the Service objectives is expertise quality. In this sense, the Service is internationally recognised. It is one of the three reference centres of a "Proficiency Test" to analyse drugs in hair, organised by the Society of Hair Testing, which has a worldwide scope. It should be noted that this year, special efforts have been made in training, as reflected in the section on teaching and training activities. This Chemistry and Drugs Service is characterised by the good relationship and dialogue with forensic doctors and the legal medicine institutes of our scope of action that is not limited to judicial cases we share, but also to collaboration in courses and other activities organised.

3.4.1. Forensic case of interest: Importance of complementary samples in the resolution of forensic cases

One case has been selected from those received at the Chemistry and Drugs Service in which the analysis of the hair sample collected led to a change in the classification of death.

Case history: 36-year-old male with 97% degree of disability, bedridden and without mobility. Social Services reported his disappearance and the Guardia Civil began searching for him, arresting his brother, with whom he lived. He reported that he died overnight and that he himself buried him the next day. He was found 35 cm from the surface and the grave measured 120 cm in length \times 60 cm in width, with depths ranging from 45 to 55 cm. Cause of death according to the coroner: "Pneumonia in a Caucasian subject".

Samples of urine, gastric contents and hair obtained from the autopsy of the exhumed corpse were received by the INTCF. The determination of ethyl alcohol was performed using the blood sample with the INTCF usual method (Gas Chromatography FID-HS), obtaining a result of 0.15 g/l. A preliminary test by enzyme immunoassay (CEDIA) specific for amphetamine, barbiturate, benzodiazepine, cannabis, cocaine, opiate, tricyclic antidepressant and methadone compounds was also carried out, obtaining positive results for barbiturate compounds.

Subsequently, in the urine and gastric content samples from the autopsy, the usual toxicological analytical systematic in this laboratory was performed, which consists of solid phase extraction and subsequent instrumental analysis that allows the identification and quantification of the compounds of interest in general toxicology. The instrumental techniques used in this case were: High-Performance Liquid Chromatography-DAD (HPLC-DAD), Gas Chromatography-Mass Spectrometry (GC-MS), and High Performance Liquid Chromatograph coupled to a Triple Quadrupole Mass Spectrometer (UPLC-MS-TOF).

From the hair sample received, the 6 cm closest to the root were analysed. The hair sample, previously washed and cut into small pieces, was subject to direct extraction with methanol. The extract obtained was analysed by UPLC-MS-tQ to determine the opiate, cocaine and amphetamine compounds, using high performance liquid chromatography coupled to an Orbitrap-mass spectrometer (UPLC-MS-Orbitrap) for the general toxicological investigation. The investigation of the presence of cannabis compounds was carried out after basic digestion, followed by liquid-liquid extraction and GC-MS-SIM analysis.

Figures 3.4.1.1, 3.4.1.2 and 3.4.1.3 correspond to the identification of phenobarbital, acetyl codeine and cocaine respectively when analysing the hair sample by UPLC-MS-Orbitrap.

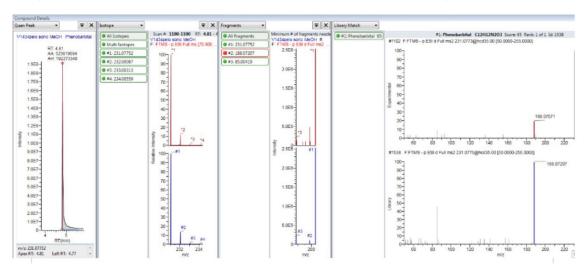
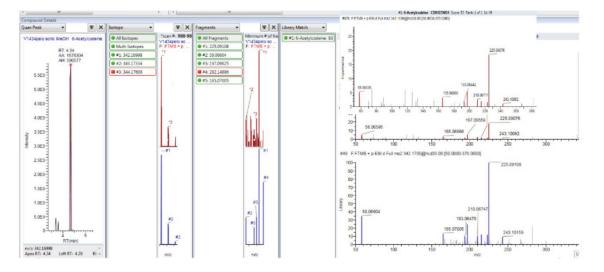


Figure 3.4.1.1. Identification of phenobarbital in the hair sample by UPLC-MS-Orbitrap

Figure 3.4.1.2. Identification of acetyl codeine in the hair sample by UPLC-MS -Orbitrap



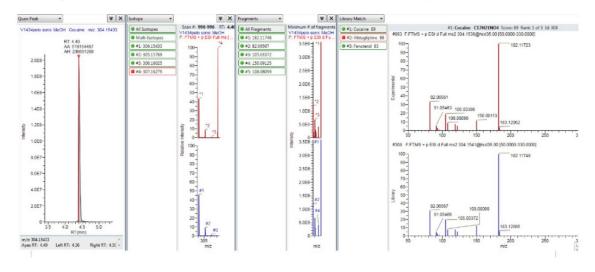


Figure 3.4.1.3. Identification of cocaine in the hair sample by UPLC-MS-Orbitrap

The results obtained for the urine and gastric content samples are reflected in Table 3.4.1.1. Table 3.4.1.2 reflects the results for the hair sample.

Table 3.4.1.1. Results obtained on urine and gastric content samples

	Phenobarbital	Morphine	Methadone			
Urine	POS	POS	POS			
Gastric content	POS	ND:	ND:			
POS: positive; ND: not detected.						

Table 3.4.1.2. Results obtained on the hair sample

Cocaine	1.67	THC	ND:
Benzoilecgonina	0.57	CBD	ND:
Ethylbenzoylecgonine	ND:	CBN	ND:
Morphine	0.19	Amphetamine	ND:
Monoacetylmorphine	0.14	Methamphetamine	ND:
Codeine	Traces	MDMA	ND:
Acetylcodeine	POS	MDA	ND:
Papaverine	POS	Phenobarbital	210
Methadone	ND:	Levetiracetam	ND:
EDDP	ND:	Paracetamol	ND:

MDA: 3,4-Methylenedioxyamphetamine, or 'love pill'; MDMA:

3,3-diphenylpyrrolidine, a metabolite of methadone.

^{3,4-}Methylenedioxymethamphetamine, or 'ecstasy'; EDDP: 2-ethylidene-1,5-dimethyl-

In this case, the analysis of the hair sample meant the conclusion was reached that the morphine detected in the urine could have come from heroin use, as the presence of monoacetylmorphine, acetylcodeine and papaverine was detected in this sample. In addition, poly-drug use is confirmed by the presence of cocaine and benzoylecgonine. The analytical results obtained show the importance of complementary samples in the resolution of forensic cases; in this particular case, the death can no longer be classified as natural, as was originally considered.

3.4.2. Teaching and training activities

3.4.2.1. Participation in investigation projects

Moreno Bernal E and Soriano Ramón T. Members of the Technical Monitoring Committee of the RASUPSI Mortality Indicator.

Soriano Ramón T and Peso Bejarano A del. Working Group of the Spanish Network of Official Forensic Laboratories (RFLOE).

3.4.2.2. Contribution in scientific congresses

Soriano T. International Meeting of the Society of Hair Testing (SoHT) for the Revision of the Consensus of Drug of Abuse in Hair Analysis. Santiago de Compostela, September, 2021.

3.4.2.3. Attendance at scientific congresses

Soriano T and González A. International Meeting of the Society of Hair Testing (SoHT). Santiago de Compostela, September, 2021.

Soriano T. 16th Latin American Regional Congress TIAFT. Held on 23, 25 and 30 November 2021. Virtual event.

3.4.2.4. Teaching and training activities

Teachers

Soriano Ramón T and Moreno Bernal E. Lecturers on the Master's Degree in Criminology and Forensic Sciences organised by the Pablo de Olavide University of Seville, teaching the module "Toxics and intoxications: the chemical toxicological analysis laboratory". 2020/2021 Academic year.

Peso A del. Lecturer on the Master's Degree in Criminology and Forensic Sciences, teaching the class "The Laboratory: Chemical-Toxicological Analysis". Pablo de Olavide University of Seville. December 2021.

Peso A del. Lecturer on the seminar on career opportunities in Criminology and Forensic Sciences. Pablo de Olavide University of Seville. May 2021.

Bueno J. Lecturer in the Forensic Sciences Degree. University of Seville. Seminar on Ethyl Alcohol. March 2021.

Soriano T, Bueno J, Del Peso A, Escobar L, García S, González A and Moreno E. Supervised internships for forensic medicine trainees in Andalusia. At INTCF, Dept. of Seville. October 2021.

Soriano T, Bueno J, Contreras MT, Del Peso A, Escobar L, García S, Moreno E and Pareja C. Multidisciplinary course on drugs: review of toxicity, updating of analytical methods, judicial and social value of analytical expertise (specialist technicians and laboratory assistants). (3-TOX-2021). Included in the Directorate General's Training Plan for the Public Justice Service for 2021, held online from 18 to 26 October 2021.

Soriano T. Practical workshop on validation of forensic toxicology methods. Organised by the Centre for Legal Studies as part of the Continuous Training programme from 04/10/2021 to 08/10/2021, online.

Soriano T. Multidisciplinary forensic intervention in multi-victim incidents. Organised by the Centre for Legal Studies as part of the Continuous Training programme from 15/11/2021 to 22/11/2021, online.

Soriano T. CCV Cycle of Forensic Pathology Seminars. Organised by the Pontifical Catholic University of Argentina. Faculty of Medical Sciences. September 2021, online.

Training

Soriano T. 2021 Continuing Education Webinar. Therapeutic use of Psycodelic Drugs. Minimum Requirements for Drug Identification. 13 July 2021, online.

Chemistry and Drugs Service professionals. Comprehensive forensic perspective of suicide. Organised by the Centre for Legal Studies (CEJ), as part of the Continuing Education Programme. From 05/05/2021 to 12/05/2021, lasting a total of 10 hours online.

Chemistry and Drugs Service professionals. Interpretation of toxicological results and their influence on the expert context in which the analysis is requested. From 10/05/2021 to 17/05/2021, lasting a total of 10 hours online.

Chemistry and Drugs Service professionals. Basic LIMS: structure, data organisation and queries. Organised by the Centre for Legal Studies as part of the Continuous Training Plan. From 17/05/2021 to 24/05/2021, lasting a total of 10 hours online.

Chemistry and Drugs Service professionals. Forensic medicine and public health. Organised by the Centre for Legal Studies from 18 to 25 November 2021, lasting a total of 10 hours online.

Chemistry and Drugs Service professionals. Multidisciplinary forensic intervention in multi-victim incidents. Organised by the Centre for Legal Studies from 15 to 22 November 2021, lasting a total of 10 hours online.

Chemistry and Drugs Service professionals. Update on the expert assessment of sexual violence. Organised by the Centre for Legal Studies from 11 to 18 November 2021, lasting a total of 10 hours online.

Chemistry and Drugs Service professionals. Quality assurance in the forensic process. A step forward. Organised by the Centre for Legal Studies from 2 to 5 November 2021, lasting a total of 10 hours online.

Chemistry and Drugs Service professionals. Practical workshop on validation of forensic toxicology methods. Organised by the Centre for Legal Studies from 4 to 10 October 2021, lasting a total of 10 hours online.

Chemistry and Drugs Service professionals. Practical workshop on the dissemination of the INTCF quality system. Organised by the Centre for Legal Studies from 21 to 28 November 2021, lasting a total of 10 hours online.

Chemistry and Drugs Service professionals. Pesticides in forensic toxicology. Organised by the Centre for Legal Studies from 31 May to 7 June 2021, lasting a total of 10 hours online.

Chemistry and Drugs Service professionals. Forensic detection and identification of chemical weapons. Toxicity and impact on the environment. Organised by the Centre for Legal Studies from 4 to 11 May 2021, lasting a total of 10 hours online.

Chemistry and Drugs Service professionals. The INTCF forensic sciences service: fields of activity, analytical possibilities. Organised by the Centre for Legal Studies from 22 to 26 March 2021, lasting a total of 10 hours online.

Chemistry and Drugs Service professionals. Update in Forensic Chemistry and Toxicology. Organised by the Centre for Legal Studies from 8 to 16 March 2021, lasting a total of 10 hours online.

Chemistry and Drugs Service professionals. Interpretation of Expert Opinions issued by the INTCF. Organised by the Centre for Legal Studies from 19 to 27 April 2021, lasting a total of 10 hours online.

Chemistry and Drugs Service professionals. Forensic Toxicology. Toxic pathology. Organised by the Centre for Legal Studies from 7 to 14 June 2021, with a total duration of 10 hours online.

Chemistry and Drugs Service professionals. Practical workshop on the dissemination of the Quality System. Organised by the Centre for Legal Studies from 21 to 28 September 2021, lasting a total of 10 hours online.

Laboratory technicians and assistants from the Chemistry and Drugs Service. Quality. Study of the UNE-EN ISO/17025:2017 standard. General Requirements for the Competence of Testing and Calibration Laboratories. Organised by the Centre for Legal Studies from 16 to 23 November 2021, lasting a total of 10 hours online.

Laboratory technicians and assistants from the Chemistry and Drugs Service. Quality. Quality Assurance at the INTCF. Quality Manuals. Standard Operating Procedures. Concepts and definitions. Organised by the Centre for Legal Studies from 8 to 15 November 2021, with a total duration of 10 hours online.

Laboratory technicians and assistants from the Chemistry and Drugs Service. Multidisciplinary course on drugs: review of toxicity, updating of analytical methods, judicial and social value of analytical expertise (technical specialists and laboratory assistants). (3-TOX-2021). Included in the Directorate General's Training Plan for the Public Justice Service for 2021, held online from 18 to 26 October 2021.

3.5. Chemistry and Drugs Section at the La Laguna Delegation

During 2021, a total of 1,010 requests corresponding to 964 cases were received at the Chemistry and Drugs Section of the La Laguna Delegation. The number of samples analysed came to 3,737, with a total of 14,016 analyses and 968 expert reports issued. The breakdown of total requests by type of report is reflected in Figure 3.5.1.

The bulk of the Section's work continues to be in response to requests from court authorities. However, support is also provided to the emergency and intensive care services at hospitals in the Canary Islands, with a total of 11 requests received during 2021. Most of these cases involve intoxication with drugs of abuse, psychotropic drugs and pesticides. The result of the analysis is communicated directly to the medical team responsible for the patient as soon as possible after receipt of the samples.

The general toxicity study is the most common type of analysis requested, with 635 requests received (Table 3.5.1). This is a category that mostly includes cases of suicide, death due to an adverse reaction to psychoactive substances, natural deaths, accidental deaths, deaths classified as undetermined and homicides. The analytical system applied to this group is aimed at the identification and quantification, where appropriate, of substances present in the samples to help establish the cause of death. This type of study accounts for 67.12% of the analyses performed and 59.56% of the expert reports issued. Figure 3.5.1 provides a breakdown of the caseload of the reporting sub-types included in the overall study of toxic substances.

Toxicological analyses on samples taken as part of cases for sexual freedom offences, accounting for a total of 79 requests, constitute the second largest group. In these cases, an analytical system is applied to identify and quantify substances that could be used to cause a state of chemical submission in the victim.

The request for drugs and/or alcohol in biological samples ranks third, with a total of 53 requests. These are samples taken from living subjects, mostly urine, with the request involving the determination of alcohol and psychoactive substances for different procedural purposes.

Ranking fourth and fifth in terms of the number of requests were requests for the analysis of toxics in hair, with 47 requests, and requests for toxicological analysis of alcohol and psychoactive substances in the case of deaths caused by traffic accidents, with a total of 39 requests.

During 2021, 44 requests were received for analysis of seizures. Most of these corresponded to pharmaceutical specialities seized at Customs, in particular anabolic steroids and drugs used for the treatment of erectile dysfunction. Two requests for the analysis of nitrous oxide capsules intended for recreational use were also received.

1,000 2,000 3,000 4,000 5,000 6,000 7,000 8,000 9,000 10,000 General toxics Sexual aggression Drugs and/or alcohol in biological samples Stash drugs Traffic accident Toxics in hair Adult sudden death Drowning Alcoholemia + traffic drugs Death in custody Death suspected of criminality Workplace accident Oral fluid Professional negligence Fauna investigation Infant sudden death Alcoholemia traffic in live subjects Children sudden death ■ Number of cases registered ■ Number of requests generated ■ Number of samples analysed ■ No. analyses performed ■ No. reports issued

Figure 3.5.1. Caseload of the Chemistry and Drugs Section at the La Laguna Delegation during 2021 by type of report

BREAKDOWN OF THE "GENERAL TOXICS" REPORT TYPE.

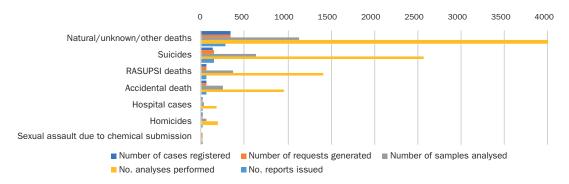


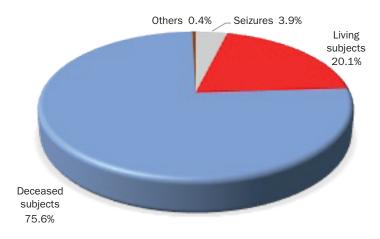
Table 3.5.1. Caseload of the Chemistry and Drugs Section at the La Laguna Delegation during 2021 by type of report

Type of report	No. cases registered	No. requests generated	No. samples analysed	No. analyses performed	No. reports issued
General toxics	624	635	2,479	9,400	576
Natural/unknown/other deaths	343	345	1,132	4,112	278
Suicides	141	142	635	2,562	143
RASUPSI deaths	64	64	369	1,406	65
Accidental deaths	63	64	252	950	68
Hospital cases	11	11	36	174	13
Homicides	8	8	54	194	8
Sexual assault due to chemical submission	0	1	1	2	1
Sexual aggression	79	79	316	1,173	75
Drugs and alcohol in biological samples	52	53	145	474	46
Traffic accident	38	39	88	172	65
Stash drugs	37	44	197	766	49
Toxics in hair	35	47	58	362	42
Adult sudden death	30	30	119	461	34
Drowning	29	29	103	380	23
Alcoholemia + traffic drugs	15	16	60	196	15
Death in custody	9	9	42	150	8
Death suspected of criminality	8	9	40	158	9
Workplace accident	4	4	21	88	5
Oral fluid	4	4	6	28	1
Professional negligence	3	3	16	68	6
Infant sudden death	2	2	13	28	3
Fires	2	3	11	50	4
Fauna investigation	1	1	3	10	1
Traffic breathalyser	1	3	20	52	5
Children sudden death	0	0	0	0	1
TOTAL	964	1,010	3,737	14,016	968

Note: the total number of cases is lower than the sum of the records in the column because there are cases involving more than one type of report.

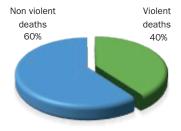
As reflected in Figure 3.5.2, the analysis of samples from deceased subjects - classified as *post-mortem* cases - encompasses the majority of the Section's expert activity, with 75% of the total number of cases registered, while those related to living persons account for 21% of the caseload. The analysis of samples from seizures accounted for 4% of the total number of cases.

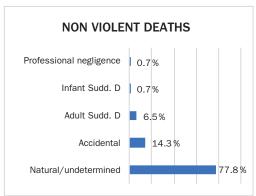
Figure 3.5.2. Classification of the casework of the Chemistry and Drugs Section at the La Laguna Delegation by the origin of the samples.

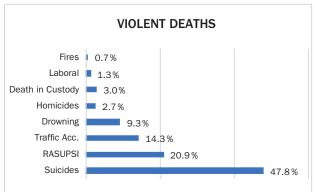


From the total number of *post-mortem* cases, 40% were classified as violent deaths, while the remaining 60% were non-violent (Figure 3.5.3).

Figure 3.5.3. Breakdown of requests associated with *post-mortem* cases at the Chemistry and Drugs Section at the La Laguna Delegation.







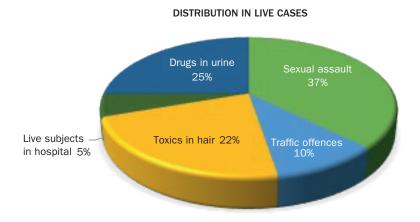
In the case of deaths of a violent nature, suicides account for almost half of this type of case, on 47.7%, followed by deaths due to an adverse reaction to psychotropic substances,

which account for 20.9% of the total. In third place were deaths caused by traffic accidents, accounting for 14.2% of the total, and finally deaths due to drowning, accounting for 9.3%. Homicides, deaths in custody, occupational accidents and fires account for a limited number of deaths compared to the rest (Figure 3.5.3).

In the case of non-violent deaths, the majority of cases relate to deaths from natural causes and deaths for which the reason could not be determined (Figure 3.5.3).

Figure 3.5.4 represents the distribution of the types of toxicological studies requested on biological samples from living subjects. The majority of requests filed relate to sexual freedom offences, accounting for 38% of the total. Requests for the analysis of abuse and psychotropic drugs in urine samples are also worth particular mention, accounting for 25%, followed by requests for the determination of toxins in hair, with 22%.

Figure 3.5.4. Breakdown of the percentage of cases involving living subjects at the Chemistry and Drugs Section at the La Laguna Delegation



To ensure the quality of analytical expertise, the Delegation's Chemistry Section participated in the following proficiency tests during 2021:

- Interlaboratory Exercise in relation to Drugs of Abuse in seizures (DAHA)
- Blood alcohol intercomparison exercise (EIAS)
- Forensic Blood Toxicology Proficiency Testing (Quartz)

3.5.1. Forensic case of interest: Evidence of post mortem ethyl alcohol formation in a corpse recovered from the seabed after 44 days submerged at a great depth.

From all the cases received by the Delegation's Chemistry Service, worth particular note are the investigations performed on the body of a six-year-old girl who was found submerged in the sea, at a depth of 1,000 metres, 44 days after she was reported missing. The scientific interest in this case lies in the determination of the origin of the high concentration of ethyl alcohol found in some of the samples obtained during the autopsy.

Records

On 27 April 2021, two sisters aged one and six years old were reported missing. On 10 June 2021, the body of the six-year-old girl was found submerged in the sea at a depth of 1,000 metres. The body was found inside a sports bag weighted down with an anchor. During the autopsy it was found that the corpse was in the colour stage of putrefaction, although its state of preservation was relatively good due to the low water temperature at that depth. The Institute of Forensic Medicine of Santa Cruz de Tenerife sent several samples to the Chemistry, Biology and Histopathology Sections of the La Laguna Delegation and to the Forensic Sciences Service of the Madrid Department of the INTCF. Based on the results obtained during the autopsy, from the histopathology and forensic sciences study, as well as from the investigations carried out by the National Police and Civil Guard, the forensic doctors concluded that it was a violent death, with a medico-legal homicidal aetiology, establishing the date of death between 7:54 pm and 9:00 pm on 27 April 2021. As part of the search of the home of the main person under investigation for the girls' disappearance, containers of pharmaceutical products containing, among other active ingredients, tramadol and methocarbamol were found. As these are central nervous system depressants, the theory of chemical submission was postulated as a means of facilitating the homicide.

Analyses carried out and results obtained

Samples of extravasated blood, pericardial fluid, gastric contents, liver and muscle tissue were received at the Chemistry Section of the Delegation. First, ethyl alcohol was determined by headspace extraction and gas chromatography analysis with flame ionisation detector (GC-FID/HS). The results obtained were as follows:

Sample	Ethyl alcohol (g/l)
Extravasated blood	2.99
Pericardial fluid	3.19
Gastric content	2.98

The analysis of non-volatile organic toxics was then carried out on all the samples received. To this end, after appropriate pre-treatment in line with the matrix, the samples were subject to solid-phase extraction and ultrafiltration. The extracts obtained were analysed by high performance liquid chromatography with diode array detector (LC-DAD), by liquid chromatography coupled to high performance mass spectrometry (LC-HRMS) and by gas chromatography coupled to mass spectrometry (GC-MS). No other substances that were cause for toxicological concern were detected.

At this point in the investigation, the need arose to determine whether the ethyl alcohol present in the victim's samples originated from *ante mortem* consumption or was formed after death as a result of putrefaction of the corpse. Given the impossibility of obtaining vitreous humour due to the circumstances in which the body was found, the decision was made to approach the problem using three different strategies: a) the determination of volatile compounds that are generated together with ethyl alcohol during the putrefactive process, b) the use of the muscle tissue sample as a contrast matrix and c) the determination of the presence of ethanol metabolites.

Decomposition of a corpse results in the formation of alcohol and a number of low molecular weight volatile compounds such as 1-propanol, 2-propanol, 1-butanol, 2-butanol, acetaldehyde, acetone and ethyl esters (Zijie, 2020; Boumba, 2012). These substances were determined by GC-FID/HS and GC-MS/HS using ter-butanol as internal standard. The results obtained were as follows:

Sample	1-propanol (mg/dl)	2-butanol (mg/dl)	2-butanone (mg/dl)	Acetaldehyde (mg/dl)
Extravasated blood	0.92	1.43	ND:	ND:
Pericardial fluid	0.94	0.53	0.28	1.44
Gastric content	1.15	ND:	ND:	ND:
N.D.: Not detected.				

Muscle tissue offers some resistance to the putrefactive process and is an alternative to the determination of ethanol and other substances of toxicological interest in corpses in an advanced state of decomposition and, in general, in those from which a blood sample cannot be obtained (Kugelberg, 2007; Nanikawa, 1982; Garriot, 1991). The analysis of alcohol in muscle tissue was carried out by incubation in aqueous solution and subsequent GC/FID-HS analysis, with negative results.

Most of the alcohol ingested is eliminated via the oxidative metabolic pathway involving the alcohol dehydrogenase and acetaldehyde dehydrogenase enzymes. However, a small fraction of the ethanol absorbed, less than 0.2%, is metabolised via a non-oxidative mechanism to ethyl glucuronide (EtG) and ethyl sulphate (EtS) (Jones, 2019). The presence of both metabolites in a biological sample is evidence of ethanol consumption. Samples of extravasated blood, pericardial fluid and liver were sent to the Chemistry

Service of the Madrid Department for the determination of EtG and EtS. The test results were negative for both compounds in the three samples tested.

Interpretation of results

The detection of ethyl alcohol in samples from a corpse does not imply that there was any consumption prior to death. The *post-mortem* formation of ethanol due to the putrefaction process that starts after death has been well documented for decades by various authors. Determination of the origin of ethyl alcohol found in a corpse requires analysis in matrices other than blood, such as vitreous humour, urine or muscle tissue.

In the case in hand, it was not possible to collect blood, urine or vitreous humour samples. Extravasated blood obtained from the thoracic cavity, pericardial fluid and gastric contents were sent to the laboratory instead. A high concentration of ethanol was detected in all of them. However, the presence of low molecular weight volatile compounds typical of putrefactive processes, in particular 1-propanol, the result of alcohol determination in muscle tissue and the absence of EtG and EtS are evidence suggesting that the ethanol present in the victim's samples could be traced to the putrefaction of the corpse, including gastric contents.

One important aspect to note in relation to the origin of the alcohol in this case is the conditions in which the body remained from the time immediately after death until the body was retrieved. Considering the depth at which the body was found, it remained at a temperature of around 5°C for the duration of this period. Studies undertaken by several authors indicate that ethyl alcohol formation is minimal at temperatures ranging from below 0°C to 5°C (Cullen, 2005; Pajunen, 2018). Although the formation of ethanol in refrigerated samples in the presence of micro-organisms capable of fermenting glucose has been demonstrated(Vouri, 1983), it is possible that the alcohol detected in the samples analysed was generated between the time at which the body was discovered and the time of the autopsy.

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3.5.2. Teaching and scientific activity

3.5.2.1. Participation in investigation projects

Luis Manuel Menéndez Quintanal. Determination of tacrolimus in the blood of rats and mice. Project title: "Pathways of pancreatic ⊠-cell damage in renal transplantation: the central role of FK506 binding protein 12 (FKBP12)".

3.5.2.2. Contribution in scientific congresses

Luis Manuel Menéndez Quintanal. KSAPT Congress 2021 held in Al Khobar (Saudi Arabia) from 27 November to 2 December. *Guest speaker*.

Luis Manuel Menéndez Quintanal. 25th Annual Scientific Meeting of the Society of Hair Testing (2021), held in Santiago de Compostela from 16 to 17 September 2021.

3.5.2.3. Education activities

María Inmaculada Frías Tejera. Lecturer at the University of La Laguna, Legal and Forensic Medicine Department, Legal and Forensic Medicine and Toxicology subject; Toxicology Department, Drug Addictions subject.

Chemistry and Drugs Section professionals. They lecture under the Training Agreement with the Canary Islands Health Service for the rotation of the laboratory speciality of FIR, MIR, QUIR and BIR at the University Hospital of the Canary Islands and the University Hospital Nuestra Señora de La Candelaria.

3.5.2.4. Training activities

Chemistry and Drugs Section professionals. Forensic studies of injury agents and their effects on soft parts and bones. Organised by the Centre for Legal Studies and held online from 1 to 3 December 2020.

Luis Manuel Menéndez Quintal. Interpretation of forensic toxicology results around the globe and into the future. Organised by the American Association for Clinical Chemistry, Inc (AAVV). Held on 7 December 2021.

Luis Manuel Menéndez Quintanal. Investigation & Certification of Drug Toxicity Deaths in Today's Complex Drug Environment Module 1. Organised by the Center for Forensic Science Research & Education (CFSRE). Held on 23 and 24 September 2021. 8 hours.

Luis Manuel Menéndez Quintanal. Update in Forensic Chemistry and Toxicology. Organised by the Centre for Legal Studies (CEJ). Held from 8 to 16 March 2021.

Luis Manuel Menéndez Quintanal. Forensic detection and identification of chemical weapons. Toxicity and impact on the environment. Organised by the Centre for Legal Studies (CEJ). Held from 4 to 11 May 2021.

Luis Manuel Menéndez Quintal. Investigation & Certification of Drug Toxicity Deaths in Today's Complex Drug Environment Module 2. Organised by the Center for Forensic Science Research & Education (CFSRE). Held on 27, 28 and 29 October 2021. 12 hours.

Luis Manuel Menéndez Quintal. Investigation & Certification of Drug Toxicity Deaths in Today's Complex Drug Environment Module 3. Organised by the Center for Forensic Science Research & Education (CFSRE). Held on 1 and 02 December 2021. 8 hours.

Cristian Martínez Ramírez. Interpretation of expert opinions issued by the National Institute of Toxicology and Forensic Sciences. Organised by the Centre for Legal Studies (CEJ). Held from 19 to 27 April 2021.

Cristian Martínez Ramírez. Forensic detection and identification of chemical weapons. Toxicity and impact on the environment. Organised by the Centre for Legal Studies (CEJ). Held from 4 to 11 May 2021.

Cristian Martínez Ramírez. Interpretation of toxicological results and their influence on the expert context in which the analysis is requested. Organised by the Centre for Legal Studies (CEJ). Held from 10 to 17 May 2021.

Cristian Martínez Ramírez. Basic LIMS: structure, data organisation and queries. Organised by the Centre for Legal Studies (CEJ). Held from 17 to 24 May 2021.

Cristian Martínez Ramírez. Pesticides in forensic toxicology. Organised by the Centre for Legal Studies (CEJ). Held between 31 May and 7 June 2021.

Cristian Martínez Ramírez. Forensic toxicology. Toxic pathology. Organised by the Centre for Legal Studies (CEJ). Held from 7 to 14 June 2021.

Cristian Martínez Ramírez. Practical workshop on the dissemination of the INTCF quality system. Organised by the Centre for Legal Studies (CEJ). Held from 21 to 28 September 2021.

Cristian Martínez Ramírez. Practical workshop on validation of forensic toxicology methods. Organised by the Centre for Legal Studies (CEJ). Held from 4 to 08 October 2021.

M.ª Inmaculada Frías Tejera. Update in Forensic Chemistry and Toxicology. Organised by the Centre for Legal Studies (CEJ). Held from 8 to 16 March 2021.

M.^a Inmaculada Frías Tejera. Forensic detection and identification of chemical weapons. Toxicity and impact on the environment. Organised by the Centre for Legal Studies (CEJ). Held from 4 to 11 May 2021.

M.^a Inmaculada Frías Tejera. Interpretation of toxicological results and their influence on the expert context in which the analysis is requested. Organised by the Centre for Legal Studies (CEJ). Held from 10 to 17 May 2021.

M.ª Inmaculada Frías Tejera. Pesticides in forensic toxicology Organised by the Centre for Legal Studies (CEJ). Held between 31 May and 7 June 2021.

Chemistry and Drugs Section technicians. Quality assurance at the INTCF. Standard operating procedures, concepts and definitions. Organised by the Centre for Legal Studies (CEJ). Held between 8 and 15 November 2021.

Chemistry and Drugs Section technicians. Multidisciplinary course on drugs: review of toxicity, updating of analytical methods, judicial and social value. Organised by the Centre for Legal Studies. Held from 17 to 26 October 2021.

Chemistry and Drugs Section technicians. Quality: study of the UNE-ISO IEC 17025:2017 standard. General requirements for the competence of laboratories. Organised by the Centre for Legal Studies. Held from 16 to 23 November 2021.





Each Department from the INTCF has a Biology Service, and there is a biology section in the La Laguna Delegation. The Biology Services functions are fundamentally forensic but also teaching and investigating activities. Within its expert work, the following types of investigation are included:

- Biological research and genetic identification of biological traces of criminal interest in sexual assault, homicides, and other crimes
- Genetic identificaction of missing persons and corpses
- Genetic investigation of kinship relations in parentage proceedings
- · Genetic identification in irregular adoptions and newborn subtractions
- · Genetic identification of non-human species
- Drowning (diatom studies)
- Sudden death (biochemical and microbiology studies, but only in the Madrid Department)
- Botanical identification (only in the Madrid Department)

Personal staff and the Biology Section that have participated in this kind of investigation during 2021 are shown in Table 4.1 and 4.2.

Table 4.1: Different Departments staffs of the Biology Services

	INTCF-MADRID	INTCF-BARCELONA	INTCF-SEVILLA	INTCF-LA LAGUNA		
Head of the Department	1	1	1	1*		
Facultatives	21	14	10	2		
Specialist technicians	8	5	3	4		
Laboratory assistants	10	4	2	1		
Clerical staff	2	-	2	-		
(*) Expert that also works as the Service coordinator.						

Table 4.2: Reinforcement staff recruited during 2021 at the Biology Services of the different Departments.

	INTCF-MADRID	INTCF-BARCELONA	INTCF-SEVILLA	INTCF-LA LAGUNA
Facultatives	3	1	1	1
Specialist technicians	2	2	1	1

In 2021, the INTCF Biology Services registered a total of 4,641 expert cases and a total of 32,773 evidences to analyse, issuing 5,860 expert reports after the analysis of 40,053 samples on which 175,828 analyses were carried out (Figure 4.1).

These figures reveal an increase of 27.86% in the number of expert cases registered compared to 2020 (3,629 cases registered in 2020, compared to 4,641 cases registered in 2021).

Within the expert work of the INTCF Biology Services, the data and results obtained in the different indices of the INTCF DNA databases during the year 2021 are also presented.

As well as the expert activity, the Biology Service during 2021 also acted as a reference centre for their speciality. It has collaborated with other institutions in different workgroups (National Technical Commission for Multiple Victim Events, National Commission for the Forensic Use of DNA, Spanish and Portuguese-speaking Group of the International Society for Forensic Genetics [Spanish initials, GHEP-ISFG], and Regulation and Coordination Committee of the National Management System of Identifiers obtained from DNA [Spanish initials, COMSIGENI]).

In 2021, the Biology Services collaborated with the Forensic Medical Council in the drafting of the "Protocol for forensic medical action in the face of sexual violence in the Institutes of Legal Medicine and Forensic Sciences". They also developed and updated the kit for the collection of biological evidence from victims of crimes against sexual freedom (KAS), as well as reference samples for the study of DNA, which will help to improve the quality and standardisation at a national level of the collection, preservation, sending and investigation of biological evidence from victims of this type of crime.

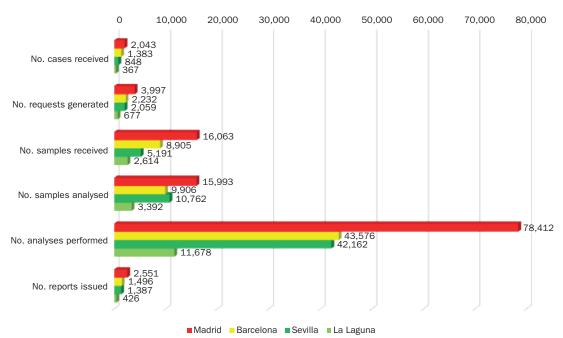


Figure 4.1. Overall data on the INTCF Biology Services expert activity during 2021

Table 4.3: Overall data on the INTCF Biology Services expert activity during 2021

2021	No. cases received	No. requests generated	No. samples received	No. samples analysed	No. analyses performed	No. reports issued
Madrid	2,043	3,997	16,063	15,993	78,412	2,551
Barcelona	1,383	2,232	8,905	9,906	43,576	1,496
Seville	848	2,059	5,191	10,762	42,162	1,387
La Laguna	367	677	2,614	3,392	11,678	426
TOTAL	4,641	8,965	32,773	40,053	175,828	5,860

The Biology Service experts have developed crucial investigative labour validating several application methods in biology and forensic genetics. This has been embodied in a big number of scientific publications and contributions to national and international forensic congresses, collected in the following sections of the report.

We add the teaching activity developed in collaboration with the Institutes of Legal Medicine and Forensic Sciences with different universities and the Centre for Legal Study, apart from the investigative activity.

Hereunder we collect the scientific and expert activities like the teaching and formative activities developed during 2021 for each Biology Services from the different Departments. Each Service includes the description of an interesting forensic case, to publicise the expert activity.

4.1. Madrid Department Biology Service

Concerning the expert activity of the Madrid Department Biology Service, they received 3,997 requests. They analysed 15,993 samples through a total of 78,412 analyses, emitting a total of 2,551 expert reports in 2021.

In terms of the type of investigation requested, as can be seen in Figure 4.1.1, the predominant analysis is the investigation of sexual assault cases (2,406 requests with 10,588 evidences received) in which the biological and genetic studies of semen samples are carried out and the study of DNA profiles being obtained from reference samples of the persons involved in the process (defendants, victims, persons to be ruled out, etc.).

The second most numerous analyses requests are kinship biological studies (696 requests with 991 samples analysed). Followed by studies of deaths suspected of being caused by crime (193 requests with 1,898 samples analysed), the identification of missing persons and corpses (112 requests with 148 samples analysed), and the analysis of biological evidence of criminal interest (104 requests with 510 samples analysed).

The third most numerous requests for analysis correspond to microbiology and biochemical studies in the sudden death in adults, children, and infants (308 requests with

1,203 samples analysed) and the biological analyses of the drowning deaths (87 requests with 564 samples analysed).

Figure 4.1.1. Casework of the Biology Service of the Madrid Department during 2021 by type of report

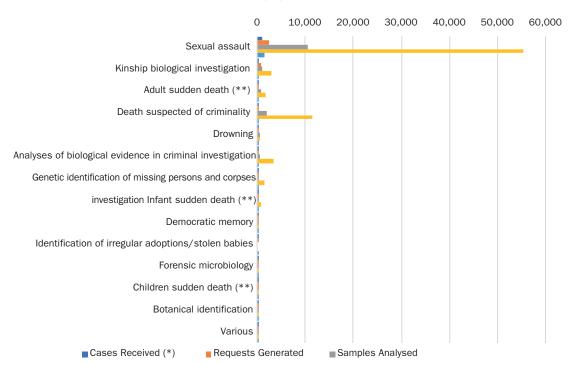


Table 4.1.1. Casework of the Biology Service of the Madrid Department during 2021 by type of report

Type of report	No. cases received*	No. requests generated	No. samples analysed	No. analyses performed	No. reports issued
Sexual assault	1,129	2,406	10,588	55,259	1,570
Kinship biological investigation	355	696	991	3,018	293
Adult sudden death**	216	250	735	1,736	228
Death suspected of criminality	88	193	1,898	11,468	97
Drowning	83	87	564	564	78
Analyses of biological evidence in criminal investigation	76	104	510	3,427	67
Genetic identification of missing persons and corpses	66	112	148	1,521	69
Infant sudden death**	33	39	337	703	29
Democratic memory	30	51	30	85	3
Identification of irregular adoptions/stolen babies	23	25	0	0	84
Forensic microbiology	11	11	42	91	8
Children sudden death**	10	19	131	438	20
Botanical identification	3	1	1	13	1
Various	3	3	18	89	4

Type of report	No. cases received*	No. requests generated		No. analyses performed	No. reports issued
TOTAL	2,043	3,997	15,993	78,412	2,551
* The total number of cases is lower than the sum of the column because there are cases involving more than one type of report. ** Microbiology and biochemical analyses.					

With regard to the type of case, as reflected in Figure 4.1.2, most cases received involved sexual violence (54% of the total number of cases received, of which 65% correspond to adult victims and 35% to minors), followed by cases of kinship and unexplained death (18% each) and, finally, homicides and identification of corpses (4% each).

Figure 4.1.2. Casework of the Biology Service of Madrid Department during 2021 according to the type of case

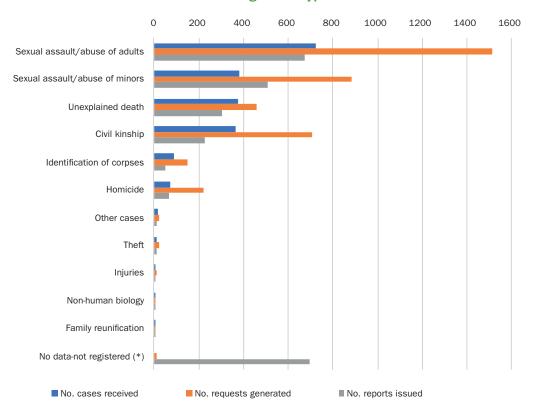


Table 4.1.2. Casework of the Biology Service of Madrid Department during 2021 according to the type of case

Type of case	No. cases received	No. requests generated	No. reports issued
Sexual assault/abuse of adults	721	1511	672
Sexual assault/abuse of minors	381	881	506
Unexplained death	375	459	303
Civil kinship	367	706	226
Identification of corpses	88	152	52
Homicide	72	222	65
Other cases	18	20	14
Theft	12	23	10
Injuries	4	9	4
Non-human biology	3	1	1
Family reunification	2	3	1
No data-not registered*	0	10	697
TOTAL	2,043	3,997	2,551

^{*} These correspond to cases from years prior to 2021 where either new samples or new analyses have been received or the opinion was issued in 2021.

As complementary activities to the expert work related to court cases, professionals from the Service participated in the implementation and validation of different forensic analysis methods, in internal training programmes of 12 professionals, three laboratory technicians and four laboratory assistants in various techniques, as well as in the publication of several scientific articles and in teaching courses organised by the Centre for Legal Studies, among others.

4.1.2. Interesting forensic cases

4.1.2.1. Detection of biological remains in two different cases involving the same defendant: one homicide and one attempted homicide.

A middle-aged man was arrested at a home for the attempted murder of a woman.

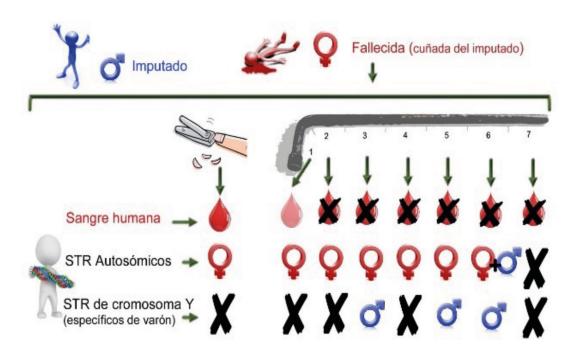
That same day, the body of the detainee's sister-in-law, with whom he lived, was found at a different address. After initial investigations, it was determined that she died a violent death as a result of severe blows to the skull with a blunt object.

Following the investigations and the detainee's statement, he was charged on the same day, in addition to the attempted murder of a woman, with the murder of his sister-in-law.

Doubtful samples related to both events were submitted, as well as the reference sample of the detainee accused in both crimes and the reference sample of the murder victim (sister-in-law of the accused), but not that of the female victim of the attempted murder.

In relation to the homicide of the defendant's sister-in-law, fingernail scrapings from both hands of the deceased victim and a blunt object as a possible murder weapon, a tyre iron, were submitted.

Figure 4.1.2.1.1. Results of blood trace investigation and genetic identification analysis of the homicide samples



Sampling of the L-shaped tyre iron was performed by cleaning its entire surface with sterile swabs moistened with DNA extraction buffer on both the short and long sides of the object. The latter in consecutive portions of about 10 cm each, resulting in a total of 7 samples, the first one corresponding to the short side of the object and the last one to the end of the long side.

Fingernail scrapings from both hands of the deceased victim showed the presence of human blood. After the genetic analysis of autosomal STR markers (hereinafter STRa), a female genetic profile is obtained that matches the profile obtained from the conclusive sample of the deceased victim.

Of all the samples taken from the tyre iron, the presence of human blood was only detected on the short end of the object (sample 1), from which a female STRa genetic profile was obtained that matched that of the deceased victim.

In samples 2 to 5, analysed from the long side of the tyre iron, although the presence of human blood was not detected, a female STRa genetic profile was obtained, which matched that of the deceased victim. Furthermore, in samples 3 and 5, the presence of male DNA was detected in small quantities, sufficient to obtain a male haplotype that matched that of the defendant, as part of the genetic analysis of male-specific Y-chromosome STR markers (hereinafter Y-STR).

In sample number 6, a STRa-mixed genetic profile was detected, in which a majority male contribution was found, matching the genetic profile of the defendant, and a minority female contribution, which matched the genetic profile of the victim. This analysis was complemented by obtaining a Y-STR male haplotype that matched that of the defendant.

At the end of the tyre iron, sample number 7, the amount of human DNA detected was very small and the results of the genetic analysis were inconclusive.

These results would be compatible with the hypothesis that the tyre iron could have been used as a murder weapon against the victim, hitting her with the short side of the wrench and being held by the defendant on the long side, with contact being greater towards the end.

After these events, the detainee went to the home of a woman, where the attempted homicide took place.

In relation to the attempted homicide, blood stains collected from the floor, from the bed in the room where the events took place, from the knife used in the assault and from the leg of the defendant, as well as a bunch of keys consisting of two key rings, two washers and five keys that he was carrying, were submitted.

Human blood was detected on the floor, bed and knife samples, and an STRa genetic analysis of these samples yielded a female genetic profile.

This woman could be the victim of the attempted homicide under investigation, whom we refer to as Woman A, as the STRa genetic profile of the woman obtained does not match that of the deceased victim, the sister-in-law of the defendant. This could not be confirmed, as no reference sample from the victim of the attempted homicide was submitted to us to compare her genetic profile to the genetic profile of Woman A.

The presence of human blood was detected in a blood-like stain on the defendant's leg, and the STRa genetic analysis of this sample yielded the same genetic profile of Female A (possibly the victim of the attempted murder).

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Figure 4.1.2.1.2. Results of blood trace investigation and genetic identification analysis of the attempted homicide samples

The amount of male DNA detected in these samples was small in relation to the total DNA, although it was possible to obtain a Y-STR haplotype that matched that of the defendant in the bed samples, in one of the knife samples and in the stain sample from the defendant's leg indicated above.

This result could be compatible with the hypothesis that the traces of human blood detected belong to the victim of the attempted homicide (possibly Woman A) and minority biological traces of the detainee were detected in these samples.

The second blood-like stain on the defendant's leg confirmed the presence of human blood. From this sample, a mixed STRa genetic profile was obtained that is compatible with a majority female contribution, which matched the genetic profile of Female A, and a minor male contribution, which matched the genetic profile of the defendant. This analysis was complemented by obtaining a Y-STR haplotype that matched that of the defendant.

In the bunch of keys carried by the defendant, the presence of human blood was detected in the sample taken from one of the two key rings. From this sample, a mixed STRa genetic profile was obtained that is compatible with a majority male contribution, which matched the genetic profile of the defendant, and a minority female contribution, which matched the genetic profile of Woman A. This analysis was complemented by obtaining a Y-STR haplotype that matched that of the defendant.

In the other samples taken from the remaining components of the bunch of keys, the presence of human blood was not detected and a STRa and Y-STR male genetic profile was obtained that matched that of the defendant, to whom the bunch of keys belongs.

4.1.2.2. Matching a complex mixture profile in the DNA database in relation to sexual assault

The Biology Service at the Madrid Department of the National Institute of Toxicology and Forensic Sciences received samples (reference blood, genital samples and clothing) from a 55-year-old woman who reported having been sexually assaulted by a room-mate, stating that she had had sexual relations with her regular partner eight hours before the assault. As part of the preliminary forensic trace investigation, traces of semen were detected in all genital samples, on a wet wipe and on the victim's underwear. Genetic analysis of these semen remains revealed a unique genetic profile that was recorded in the national DNA database with no matches detected. However, from the first fraction of the differential lysis (fraction enriched in DNA from any cell type other than spermatozoa) performed on a sample taken from the perineal area of the underwear, a mixture of STRa marker genetic profiles from at least three individuals was detected. The genetic profile of the complainant and the genetic profile detected from the semen remains matched this admixture profile, and the presence of at least one additional male was detected, as the complementary Y-STR marker analysis returned a mixture of at least two different haplotypes. Assuming the contribution of the complainant and the semen donor to this mixture, making use of certain functionalities available in the CODIS software that houses the DNA database and respecting the guidelines set out in the Technical Procedural Manual prepared by COMSIGENI (Regulatory and Coordinating Committee of the National Management System for DNA-based identifiers), this mixed profile was registered, directing the search towards this second male. After comparison in the national node of the DNA database, a match was found between the registered mixture profile and a reference genetic profile registered by the General Commissariat of the Scientific Police (CNP), which turned out to belong to the person under investigation in this case.

This case highlights the importance of not ruling out *a priori* the registration in the DNA database of a mixture containing at least three contributors, since, if we can determine at least one of the contributors, matches and/or compatibilities can be detected that are very useful in clarifying the facts under investigation or other different facts.

4.1.2.3. Identification of the kratom plant in the forensic laboratory: a challenge between therapeutic and lethal potential

In recent years, the kratom plant, *Mitragyna speciosa* (Korth.), native to South-east Asia, has grown in popularity (Warner *et al.*, 2016; Figure 4.1.2.3.1). As a relative of coffee (*Rubiaceae*), knowledge of the plant remains largely unknown. Relatively new to the black market and traditionally used as therapeutic pain relief or mood enhancement, interest in the plant has increased worldwide as a curative and recreational substance, and even as an abuse drug (Prozialeck *et al.*, 2019). However, it has been demonstrated that it contains sufficient toxicity to be lethal, mainly on account of its active components such as mitragynine and 7-hydroxymitragynine, causing adverse cardiovascular and cardiotoxic

effects, tachycardia and hypertension, as well as intrahepatic cholestasis. Thus, it is noteworthy that kratom has been classed a public health hazard, having been banned in some countries and not legislated or known in others (Prozialeck *et al.*, 2019). Emerging drugs include those considered "*legal highs*" (Basiliere, 2020). This concept is used to refer to substances that are not included in the list of prohibited drugs and are sold as "legal" in *smart shops, grow shops* or online shops (Basiliere, 2020; Prozialeck *et al.*, 2019). The growing interest in this plant as an abuse drug therefore requires further evaluation in the forensic laboratory to identify it quickly and report on its toxicity.

Figure 4.1.2.3.1. Images of kratom tree (a), fresh leaf to scale (b), dried kratom leaves (c) and kratom leaf powder sold online for consumption in the form of tea (d) (amended from Wagner et al., 2016).

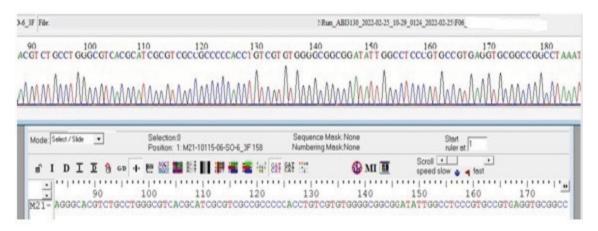


Case history. Envelopes found at the scene or around a corpse, as *vitamin supplements*, were sent to the INTCF by the Ertzaintza to investigate their contents and identify their composition. A study was requested in the context of the sudden death of a 49-year-old man for unknown reasons, found at home with no signs of violence, no history of suicide and a clinical history of eosinophilia. The homogeneous content of possible plant(s) in the form of green powder was observed inside envelopes with different "premium" labelling: Premium Powder Red Riau, Red Borneo, Red Thai Maeng Da, Sumatra Red, Sumatra White, Green Rian, Borneo White and Premium Kratom Green Malay. Species identification and toxin identification analyses were carried out at the Biology and Chemistry Departments, respectively.

DNA extraction of a fraction of the green powder was performed on all envelopes using BioRobot™ EZ1, followed by PCR of the highly conserved intraspecies ITS-2 rDNA region with a high degree of interspecies polymorphism. After quantification of the amplified

products (Agilent microchip), cyclic sequencing and purification of the products, the resulting aligned sequence was compared using BLASTn (Based Local Alignment Search Tool) in the international GenBank database (Figure 4.1.2.3.2). A search of the ITS-2 sequence obtained from the powder contained in all envelopes returned 19 sequences of *Mitragyna speciosa* with a 100% match to the evidence in question (Figure 4.1.2.3.3). The restriction fragment length polymorphism (RFLP) technique has previously been used in large-scale analyses of routinely marketed products to discriminate kratom from other psychoactive plants (Maruyama *et al.*, 2009; Rosenbaum *et al.*, 2012). However, ITS2 sequence analysis as a *barcode* was employed in this study as a rapid species identifier. *Mitragyna* leaves contain compounds with similar effects to opioids and other stimulants, which are not routinely *screened* for toxicity at most police and forensic laboratories (Díaz-Ruiz and Ramos-Campoy, 2017).

Figure 4.1.2.3.2. Gene sequence electropherogram of the ITS-2 fragment obtained from the amplified extract of kratom leaf powder.



After a chemical-toxicological analysis, using liquid chromatography coupled to high performance mass spectrometry (LC-QTOF MS), mitragynine was detected for all sachets containing plant debris (Figure 4.1.2.3.4). The study of the deceased's biological samples (liver, kidney and muscle) also detected mitragynine, as well as citalopram (or its enantiomer escitalopram) and its metabolite, desmethylcitalopram. As part of routine forensic chemistry laboratory methodology, the rapid detection of the drug in biological matrices is, in some cases, not available. Using liquid chromatography coupled to high performance mass spectrometry in this study, it was possible to reliably identify the substances.

Figure 4.1.2.3.3. Example of *internal transcribed spacer 2* (ITS-2) sequence comparison in GeneBank (sequence ID: JF412826.1, corresponding to the *red-veined* variety). Following a genetic analysis, the powder analysed in the envelopes received was found to be a match for the species *Mitragyna speciosa*

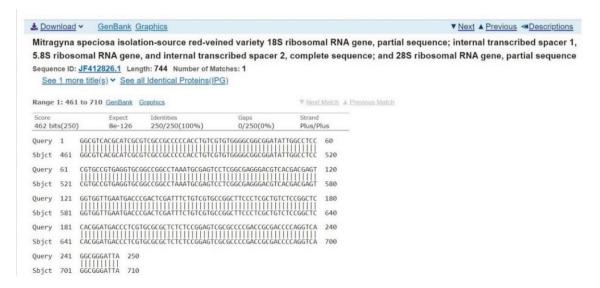
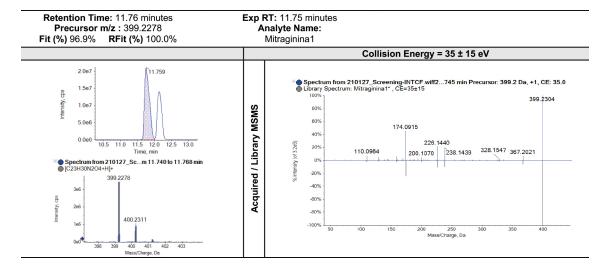


Figure 4.1.2.3.4. Chromatogram, isotopic profile and "exact mass" spectrum of the substance identified as mitragynine in the spectral library of compounds from the sachets containing *Mitragyna speciosa*. The appearance of more than one chromatographic peak is due to the presence of the diastereoisomers of mitragynine: speciogynine and/or speciocilatine.



With this case, the aim is to warn that the species *Mitragyna speciosa*, also *M. hirsuta* (containing mitraphylline), is easily sold as a powdered product for for tea consumption, with the expectation of beneficial effects, legally or not legally listed, depending on the country's legislation (National Institute on Drug Abuse NIH, 2019). Research has shown that there are both stimulant and sedative dose-dependent effects (Warner *et al.*, 2016). Kratom advocates promote its use as a safer and less addictive alternative to opioids for the treatment of pain, as well as for opioid addiction itself. Those against kratom argue that it is a dangerous and addictive drug that should be banned. Given the widespread use of kratom and the significant attention it is receiving in the media, it is important for doctors, scientists and policy makers to be well informed on the subject.

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4.1.2.4. Forensic microbiology in 2021: a case of Children sudden death

2021 continued to be marked by the pandemic that took over Europe in early 2020. SARS-CoV-2 infections can take on a wide range of clinical manifestations, which may be asymptomatic, mild or symptomatic, and can lead to severe pneumonia and multi-organ involvement leading to death.

Occasionally, COVID-19 disease may result in mild or moderate manifestations; however, patients with co-morbidities such as diabetes, hypertension or cardiac disease may require intensive care and mechanical ventilation, which may lead to secondary opportunistic and even nosocomial infections and have a poor prognosis. Soon after the

onset of the pandemic, it became clear that SARS-CoV-2 co-infections could be bacterial, viral or fungal, with different aetiologies depending on the community or nosocomial setting. However, most studies focus on co-infections in patients admitted to hospital with COVID-19. In studies analysing community-acquired pneumonia, it has been shown that the most common pathogenic bacterium accompanying SARS-CoV-2 is *Streptococcus pneumoniae*. While the average age of most co-infections is high, this is the fatal case of co-infection in a minor.

A case of sudden infant death occurred in a one-year-old girl who died suddenly while travelling in a car with her mother after losing consciousness. The mother reported that the family members had COVID-19 and that the girl showed signs of anorexia, fever and petechiae in the previous days. She had lost weight over the preceding three months and had been exhibiting a "change in behaviour" for three weeks. At the Institute of Forensic Medicine, prior to the autopsy, an antigenic test for COVID-19 was carried out and was found to be negative. On examination, significant malnutrition was observed, and the case was suspected to be an infection. During the autopsy, the main findings were cachexia and petechiae in the abdomen, hypochondrium, thighs and back of the torso, and pulmonary oedema with the appearance of jaundice in the left lower lobe. Comprehensive sampling for microbiology and histopathology was performed. Acute respiratory failure due to respiratory infection was indicated as a possible cause of death, and the forensic medical assessment also suggested investigating possible bacterial meningitis.

Therefore, upon receipt of the samples at the Microbiology Laboratory, an antigen test was performed to detect a possible bacteria responsible for meningitis and sepsis, obtaining a positive result in cerebrospinal fluid (CSF) for *S. pneumoniae*, which was reported to the coroner, other pathogens such as *Neisseria meningitidis* were ruled out, for which contact prophylaxis would have been advisable. Furthermore, the bacteriological culture showed the presence of *S. pneumoniae* serotype 3 in lung, pericardial and nasal fluid swabs, tracheal fluid, blood, spleen, liver, CSF and brain (in pure culture in the latter four samples) and accompanied by *S. aureus* in respiratory samples. In addition, real-time PCR also detected *S. pneumoniae* in tracheobronchial swabs, where it had not been isolated in culture. All these findings are compatible with a respiratory infection, probably pneumonia, caused by *S. pneumoniae*.

The minority isolation of *S. aureus* in the respiratory tract, a strain in which no encoding genes were found for virulence factors such as Panton-Valentine-Leukocidin, staphylococcal toxic toxin and exfoliative toxin, could indicate *post-mortem* dissemination from the upper respiratory tract, where it acts as a coloniser.

Although the result of the antigenic test for SARS-CoV-2 performed during the autopsy was negative, as there was a family history of COVID-19 infection, a specific real-time PCR was performed, returning a positive result in lung, tracheobronchial and nasal swabs and tracheal fluid. Furthermore, to assess the possible spread of the virus in the victim, the

decision was taken to investigate this in the other samples received, also returning a positive result in the blood, spleen and liver. The microbiological results obtained are compatible with SARS-CoV-2 infection complicated by pneumococcal pneumonia and are consistent with the clinical symptoms described.

The main histopathological diagnoses (Histopathology Service of the Barcelona Department of the INTCF) detected a severe bilateral bronchopneumonic process with diffuse alveolar damage in the exudative phase and interstitial pneumonia as the main diagnosis. Severe hepatic macrovesicular steatosis was also detected, with no significant morphological alterations in the other samples analysed.

Therefore, the joint interpretation of the microbiological and histopathological results made it possible to conclude that the death had been caused by respiratory co-infection of SARS-CoV-2 and *S. pneumoniae* with the characteristic pulmonary histopathological findings of both viral and bacterial aetiologies. Furthermore, the positive microbiological results for *S. pneumoniae* obtained in CSF and brain suggest that incipient meningitis may have developed from this infection, which for this reason was undetectable in the histopathological study. Finally, real-time PCR detection of SARS-CoV-2 in blood, spleen and liver suggests disseminated SARS-CoV-2 infection.

As take-home messages, it should be considered that, 1) although children are not the main sufferers of SARS-CoV-2, serious infection caused by this virus cannot be ruled out as a cause of death in this population, especially if there are predisposing factors; 2) the detection of SARS-CoV-2 in blood and in different organs, as occurred in this case, suggests a disseminated infection involving this virus; 3) the detection of an infection by a pathogen, be it a virus, bacteria or fungus, does not rule out SARS-CoV-2 infection; 4) the laboratory diagnosis of COVID-19 does not imply that there may not be other pathogens responsible for a co-infection that could contribute to a fatal outcome; and 5) the study of co-infections in suspected cases of SARS-CoV-2 can contribute to a better understanding of this pathology and therefore to its better therapeutic management in order to reduce mortality.

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Figure 4.1.2.4.1. Streptococcus pneumoniae culture in lungs. Microbiology Laboratory, Biology Service. INTCF Madrid Department



Figure 4.1.2.4.2. Microscopic image of bronchopneumonic process with diffuse alveolar damage in exudative phase with hyaline membrane formation. Histopathology Service.

Barcelona Department, INTCF

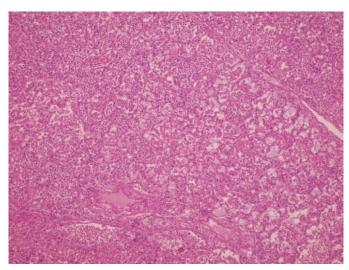
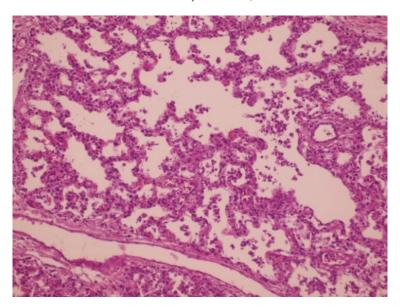


Figure 4.1.2.4.3. Microscopic image of mixed inflammatory infiltrate with interstitial and perivascular lymphocyte predominance. Histopathology Service.

Barcelona Department, INTCF



4.1.3. Teaching and scientific activity

4.1.3.1. Contribution in scientific congresses

Conference on forensic pathology and COVID-19. Catalan Society of Legal Medicine and Toxicology. Conference: Tests for active SARS-CoV-2 infection. Online, 04/03/2021.

ENFSI APST online working group meeting. Netherlands Forensic Institute. Ministry of Justice and Security. 22/04/2021.

HID University. Human Identification Solutions virtual Conference by Thermo Fisher Scientific. 19-20/05/2021.

30th SEAP-IAP National Congress & 6th SEPAF National Congress. Conferences: Forensic SARS-CoV-2 detection. Collection of microbiological samples in forensic pathology. Joint session of the Autopsy Pathology and Infectious Pathology working groups. As part of the SEPAF short course: Impact of the COVID-19 pandemic on forensic institutions. Online, 26-28/05/2021.

24th SEIMC National Congress. Conference: Forensic microbiology: a new perspective. Plenary session 1. Online, 6-11/06/2021.

Justice and Cooperation Seminar. Mobilisation of Experts in International Projects. 16/06/2021.

31st European Congress of Clinical Microbiology & Infectious Disease. Online, July 2021.

12th Meeting of GCLAITH: Latin American Scientific Working Group on Human Identification. Organised by Promega, at the International Symposium on Human Identification ISHI32. Online. 16/09/2021.

"VISAGE. The impact of forensic DNA phenotyping on science and society. Results of the VISAGE EU project and their applications". Organised by the VISAGE Consortium and the University of Santiago de Compostela, online. 24/09/2021.

46th ENFSI DNA working group meeting, online, 28-29/09/2021.

26th GHEP-ISFG Forensic Genetics Conference. Organised by the EC of the GHEP-ISFG. Online. 18-20/10/2021.

2021 Virtual CODIS Conference. Online, 15-18/11/2021.

Oral communication: "The complex recovery and identification of Eloy Campillo: lessons learned". Spanish Association of Anthropology and Forensic Odontology. AEAOF Online Communications Conference, 17-19/11/2021.

Online communications conference of the Spanish Association of Anthropology and Forensic Odontology. Oral communication: "The complex recovery and identification of Eloy Campillo: lessons learned", 17-19/11/2021.

Meeting of the Committee for the Regulation and Coordination of the National Management System for DNA-based Identifiers (COMSIGENI). Online, 29-30/11/2021.

4.1.3.2. Scientific publications

Barrio PA, Fernández-Rodríguez A, Martín P, Fernández C, Fernández L, Alonso A. Forensic evaluation of two nucleic acid extraction systems and validation of a RT-qPCR protocol for identification of SARS-CoV-2 in post-mortem nasopharyngeal swabs. *Forensic Sci Int.* 2021;323:110775. https://doi.org/10.1016/j.forsciint.2021.110775

Crespillo M, Barrio PA, Núñez C. Application of Mitochondrial DNA as a Tool in the Forensic Field: Update and New Perspectives. In: Pilli E, Berti A, editors. Forensic DNA Analysis: Technological Development and Innovative Applications. Palm Bay (Florida): Apple Academic Press; 2021. p. 113-49.

González-Albo MC, Martín P. Monographic study: The recovery and identification of the remains of Eloy Campillo. Democratic memory. Communal graves and exhumations. Coordinated by Fernando Serrulla Rech. The intervention of the genetics laboratory at the National Institute of Toxicology and Forensic Sciences, genetic identification of the remains of Eloy Campillo. 2021, Chapter 5, p. 57-63.

Vullo CM, Catelli L, Ibarra Rodríguez AA, Papaioannou A, Álvarez Merino JC, López-Parra AM, Gaviria A, Baeza-Richer C, Romanini C, González-Moya E, Casals F, Calafel F, Berardi G, Iannacone GC, Vicuña Giraldo GC, Zorba GK, Boschi I, Valdivia Olarte J, Parsons T. Second GHEP-ISFG exercise for DVI: "DNA-led" victims' identification in a simulated air

crash. Forensic Sci Int Genet. 2021;53:102527. https://www.sciencedirect.com/science/article/abs/pii/S187249732100065X#!

Martínez P, Quintela O, Valle E del, Pérez-Gómez B. Genetic identification and subsequent LC-QTOF MS analysis of plant remains (*Oenanthe* spp.) could prove the cause of an undetermined sudden death. *Int J Legal Med.* 2021 Jul;135(4):1407-11. Doi: 10.1007/s00414-020-02488-6.

Muñoz-Quirós JM, Mira E, Moyano S, Abad R, García E, Fernández-Rodríguez A. Multidisciplinary medical-legal investigation of death by SARS-CoV-2 (COVID-19): review of the literature about a case. *Gac Int Cienc Forenses*. 2021; 41:6-16.

Neagu O, Fernandez-Rodriguez A, Callon D, Andreoletti A, Cohen MC. Myocarditis Presenting as Sudden Death in Infants and Children: A Single Centre Analysis by ESGFOR Study Group. *Pediatr Dev Pathol.* 2021 Jul-Aug; 24(4):327-36. Doi: 10.1177/10935266211007262.

Saegeman V, Cohen MC, Burton JL, Martinez MJ, Rakislova N, Offiah AC, Fernandez-Rodriguez A. Microbiology in minimally invasive autopsy: best techniques to detect infection. ESGFOR (ESCMID study group of forensic and post-mortem microbiology) guidelines. *Forensic Science Med Pathol.* 2021 Mar;17(1):87-100. Doi: 10.1007/s12024-020-00337-x.

4.1.3.3. Teaching and training activities

Courses held

Fernández-Rodríguez A. Lecturer on the Master's Degree in Police Sciences at the University of Alcalá de Henares, subject "Fundamentals of Criminal Investigation. Introduction to forensic microbiology and practical applications of forensic microbiology to the resolution of important court cases". 18 January 2021.

Barrio PA. Director and speaker of the training action on "New research tools in the field of forensic genetics", organised as part of the Continuous Training Plan 2021 at the Centre for Legal Studies (CEJ), aimed at INTCF professionals, INTCF interim professionals, the Civil Guard, National Police and regional police. Online, 21/05-02/06/2021.

Vallejo G. Lecturer on the official master's degree in Criminology and Forensic Sciences. Pablo de Olavide University (Seville). "Identification by DNA", at the round table "Actual validity of fingerprinting and DNA in the genetic identification of individuals". Online, 25/05/2021.

Muñoz M. Participation as a teacher in the activity "2.13 Accreditation assistance for PCR and Typing experiment method with YFiler Plus Kit and PCR PowerPlex Y23 system kit", as part of the European project "Forensic Trainings Towards Advanced Examination Methods of the Gendarmerie General Command of Turkey". 31/05-04/06/2021.

Barrio PA. Training action on "Key aspects in the preparation and interpretation of expert forensic genetics reports", of the online training programme for collaboration in activities

to improve Forensic Genetics, within the European project "Cooperation in criminal investigation in Central America to combat crime and drug trafficking at an international level", ICRIME-LA/2017/39066, coordinated by the International and Ibero-American Foundation for Public Administration and Policies F.S.P. (FIIAPP). The lecture imparted was entitled "Requirements, Structure and Communication: General aspects, Reporting 'preliminary analysis' results, 'Biological Parentage Investigation' (PBI) cases, 'Identification of remains' cases, 'New genetic marker' cases". Online, 07/06/2021.

Supervised internship at the INTCF for the 30th promotion of the National Corps of Forensic Doctors organised by the CEJ. Las Rozas, 16-17/09/2021.

- · Albarrán C. Presentation of the INTCF Biology Service.
- · Vallejo G. "Biological drowning studies".
- · Vallejo G. "Biological investigation of paternity and other blood relations".
- Carrillo A. "Genetic identification of biological evidence in forensic cases. A practical overview of the main problems associated with the analysis with a review of some cases".
- · González-Albo MC. "Genetic identification of corpses".
- González-Moya E. "DNA database: applications in criminal and missing persons investigations".
- · Farfan MJ. Visit to the Biology laboratory.

Fernández-Rodríguez A. Lecture on "Biosafety in the laboratory II. Biological agents" on the Adaptation Course to the Higher Level for Pathological Anatomy Technicians, as part of the Training Plan organised by the Directorate General for the Public Justice Service. Valladolid, 27/09/2021-01/10/2021.

Fernández-Rodríguez A, Saegeman V, Abad R. Lecture on "How can post-mortem microbiology support the diagnosis of infectious death causes in the elderly?", on the course "ESCMID postgraduate course co-organised by ESCMID study group ESGIE: Infections in the elderly - from bench to (beyond) bed". 14-15/10/2021.

Farfan MJ. Speaker and tutor on the training activity "Databases of criminal interest: Operational and legal aspects", organised by the CEJ. Subject imparted: "Procedures and management of the DNA database at the INTCF". Online, 14/10/2021.

Farfan MJ. Director and tutor of the training activity "Multidisciplinary forensic intervention in multi-victim incidents", organised by the CEJ. Online, 15-22/11/2021.

Carrillo A. Speaker on the course "DNA identification of victims", organised by the Institute of Legal Medicine and Forensic Sciences of Asturias, aimed at forensic doctors and health officials. Subject imparted: "Genetic investigation and identification of biological

evidence in forensic cases. Sampling, analysis, evaluation of the evidence and expert report". Online, 17-18/11/2021.

González-Moya E. Speaker on the course "DNA identification of victims", organised by the Institute of Legal Medicine and Forensic Sciences of Asturias, aimed at forensic doctors and health officials. Subject imparted: "DNA databases: applications in criminal and missing persons investigations." Online, 17-18/11/2021.

Vallejo G. Speaker on the course "DNA identification of victims", organised by the Institute of Legal Medicine and Forensic Sciences of Asturias, aimed at forensic doctors and health officials. Subject imparted: "Biological investigation of paternity and other blood relations. Sampling, analysis, evaluation of evidence and expert report". Online, 17-18/11/2021.

González-Albo MC. Lecture imparted at the Asturian Public Administration Institute Adolfo Posada: "Genetic identification of corpses Sampling, analysis, evaluation of evidence and expert report". 18/11/2021.

Fernández-Rodríguez A. Speaker on the training course "Forensic Medicine and Public Health", organised by the CEJ. Subject imparted: "Coordination between INTCF and IMLCF in infectious death studies and their projection in Public Health". Online, 24/11/2021.

Fernández-Rodríguez A. Lecturer on the subject "Public Health" of the Master's Degree in Health Biology at the UCM. Subject imparted: "Health contributions of Forensic Biology". Online, 29/11/2021.

Fernández-Rodríguez A. Director and moderator of the joint ESGFOR-SEPAF seminar: Integrated diagnosis in COVID-19 deaths: microbiology and forensic pathology. Online, 14/12/2021.

Courses received

Training actions scheduled as part of the Continuous Training Plan 2021 at the Centre for Legal Studies (CEJ). Online:

- "The INTCF Forensic Sciences Service: fields of action, analytical possibilities", 22-26/03/2021.
- "Treatment of offences against sexual freedom and integrity in the forensic laboratory", 14-21/06/2021.
- "Interpretation of expert opinions issued by the National Institute of Toxicology and Forensic Sciences", 19-27/04/2021.
- "Comprehensive forensic perspective on suicide", 05-12/05/2021.
- "Basic LIMS: structure, data organisation and queries", 17-24/05/2021.
- "Practical workshop on the dissemination of the INTCF quality system", 21-28/05/2021.

- "Update on the expert assessment of sexual violence", 30-31/03/2021.
- "New research tools in the field of Forensic Genetics", 21-28/06/2021.
- "Criminal databases: operational and legal aspects", 13-20/10/2021.
- "Multidisciplinary forensic intervention in multi-victim incidents", 15-22/11/2021.

VISAGE Train-the-Trainers Workshop (Topics covered: Part 1: Theoretical basis of predictive markers; Part 2: Ethical considerations and recommendations; Part 3: MPS technology and laboratory workflow; Part 4: DNA analysis and processing genetic data; Part 5: Forensic phenotyping and VISAGE Software; Part 6: Implementation in a routine forensic DNA service environment; Part 7: Communication of results). Organised by VISAGE Work Package 7, online. 7-9/09/2021.

"VISAGE. The impact of forensic DNA phenotyping on science and society. Results of the VISAGE EU project and its applications", organised by the VISAGE Consortium and the University of Santiago de Compostela. Online, 24/09/2021.

2nd Course on the use of CODIS software. Organised by the Subdirectorate General for Information and Communication Systems for Security. Held at the Security Technology Centre (El Pardo, Madrid), 26-28/10/2021.

Training actions organised by the Subdirectorate General for Access and Promotion of Justice Administration Staff for Special Corps at the INTCF. Online:

- "Multidisciplinary course on drugs: Review of toxicity, updating of analytical methods, judicial and social value of analytical expertise", 18-26/10/21.
- "Quality Study of the UNE-EN ISO/IEC 17025:2017 standard. General Requirements for the competence of testing and calibration laboratories", 02-12/11/2021.
- "Quality assurance in the INTCF, quality manuals, standard operating procedures, concepts and definitions". INTCF Quality Assurance Service and the Subdirectorate General for Access and Promotion of Justice Administration Staff, Training Service, 22-30/11/21.

4.2. Barcelona Department Biology Service

Concerning the expert activity of the Barcelona Department Biology Service, during 2021, a total of 2,232 requests were received with 9,906 samples analysed through a total of 43,576 analyses, emitting a total of 1,496 expert reports.

In terms of the type of investigation requested, as can be seen in Figure 4.2.1, most requests for analysis correspond to the investigation of sexual assault cases (1,563 requests with 7,812 evidences received) in which the biological and genetic studies of semen samples or other biological evidence are carried out and the study of DNA profiles

being obtained from reference samples of the persons involved in the process (defendants, victims, persons to be ruled out, etc.).

The second most numerous group of analysis requests corresponds to kinship biological studies (369 requests with 638 evidences received), followed by analyses of biological evidence of criminal interest (210 requests with 1,255 samples received), and genetic identification of missing persons and corpses (with 80 requests and 168 samples received).

Figure 4.2.1. Casework of the Biology Service of the Barcelona Department during 2021 according to the type of report

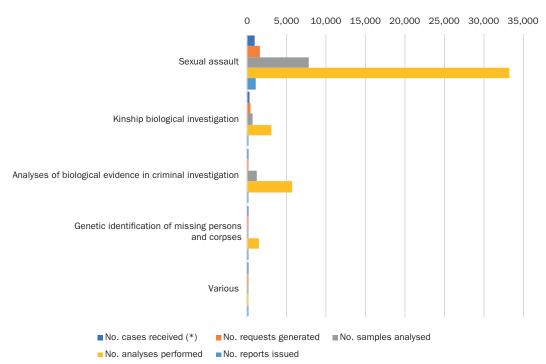


Table 4.2.1. Casework of the Biology Service of the Barcelona Department during 2021 according to the type of report

Type of report	No. cases received*	No. requests generated	No. samples analysed	No. analyses performed	No. reports issued
Sexual assault	1,019	1,563	7,812	33,244	1,111
Kinship biological investigation	237	369	638	3,044	174
Analyses of biological evidence in criminal investigation	87	210	1,255	5,678	148
Genetic identification of missing persons and corpses	50	80	168	1,428	55
Various	6	11	33	182	8
TOTAL	1,383	2,232	9,906	43,576	1,496

^{*} The total number of cases is lower than the sum of the column because there are cases involving more than one type of report.

With regard to the type of case, as can be seen in Figure 4.2.2, most requests for analysis correspond to the investigation of cases of sexual assault (61% of all cases received, 64% of which correspond to adult victims and 36% to minors), followed by cases of kinship (14% of cases), identification of corpses (5%), homicides (4%) and, finally, unexplained deaths (1% of cases).

Figure 4.2.2. Casework of the Biology Service of the Barcelona Department during 2021 by type of case

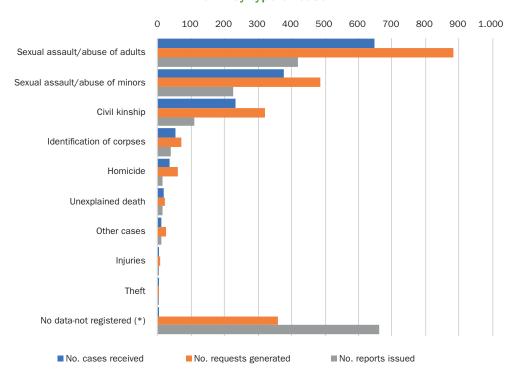


Table 4.2.2. Casework of the Biology Service of the Barcelona Department during 2021 by type of case

Type of case	No. cases received	No. requests generated	No. reports issued
Sexual assault/abuse of adults	646	883	420
Sexual assault/abuse of minors	375	486	224
Civil kinship	232	319	108
Identification of corpses	52	69	39
Homicide	37	60	15
Unexplained death	18	20	13
Other cases	12	25	11
Injuries	4	6	2
Theft	4	5	1
No data-not registered*	3	359	663
TOTAL	1,383	2,232	1,496

^{*} These correspond to cases from years prior to 2021 where either new samples or new analyses have been received or the opinion was issued in 2021.

During 2021, important efforts have been made to update the documentation associated with the standardised working procedures of the Biology Service, to validate methods (sperm visualisation and cytochrome B analysis for non-human genetics) and to accredit new tests for future implementation.

As complementary activities to the expert work related to court cases, professionals from the Service participated in the implementation and validation of different forensic analysis methods, in internal training programmes of seven laboratory technicians and two laboratory assistants in various techniques, as well as in teaching courses organised by the Centre for Legal Studies, among others.

Furthermore, members of the Biology Service have continuously participated training activities aimed to implementing and improving new analysis methods in laboratories at different Turkish institutions (Gendarmerie and Institute of Forensic Medicine). These were developed in the European Project TR 16 IPA JH 03 18 Forensic Training Towards Advanced Examination Methods and the project for Cooperation in Criminal Investigation in Central America to Combat Delinquency and Drug Trafficking at an International Level (ICRIME-CA/2017/39066).

4.2.1. Interesting forensic cases

4.2.1.1. Case to identify of a body found in open water using the Missing Persons DNA database

Records

In mid-November 2021, a body in an advanced state of decomposition was discovered in open waters off the Balearic Islands archipelago. Based on the information provided by the coroner, the body had been in the water for a period of approximately 3 to 8 weeks.

Evidences

A sample consisting of a fragment of femur (femoral cylinder) from the body was received by the Barcelona Department. Furthermore, the court in charge of the investigation was instructed to introduce the results of the genetic analysis in the missing persons DNA database to facilitate the identification of the remains in question.

Results and conclusions

After performing the relevant analyses, complete genetic profiles were obtained for both autosomal markers and Y chromosome markers and these were included in the database. In the first instance, in mid-December, no relevant match or compatibility was found that could help to identify biological family relationships between this genetic profile and those of the other persons included in the database.

Subsequently, the genetic profiles of a possible blood relatives (based on the information available, it could be the blood uncle of the victim, for example) were introduced at the Guardia Civil laboratory. In this particular case, given the genetic distance between the possible relatives and the lack of other possible relatives, a kinship study using autosomal markers would not have been possible. However, in early January 2022, a *match* between the genetic profiles of the Y chromosome markers of the bone remnant and the possible paternal uncle did emerge. The conclusion was, therefore, that it could not be ruled out that the person from whom the bone remains had been taken was the blood nephew of the relative in question.

4.2.1.2. Match in the police database with the profile of a 2012 case.

Records

A 19-year-old woman was sexually assaulted with vaginal penetration by an unknown assailant upon returning home early one morning in early December 2012. The events took place in the city of Barcelona.

Evidences

Samples consisting of an external genital swab, a vaginal swab and a vaginal wash were received at the Biology Service of the Barcelona Department.

Results and conclusions

Traces of semen were detected in vaginal swabbing and washing. Having received a request for information from the investigating court in May 2013 to enter the profiles in the police database, the samples were analysed and a unique genetic profile of autosomal markers was obtained, which was entered in this database. Initially, no match was detected with any genetic profile registered in the database.

In July 2015, a match was found with a genetic profile in the database introduced by the Mossos d'Esquadra following another case of sexual assault, having obtained this profile from the analysis of a piece of underwear. The court was informed of the match, but the alleged perpetrator has failed to be identified.

It was not until January 2021 that a *match* was made through Prüm with France for a genetic profile of a suspect, leading to the identification of the alleged perpetrator.

4.2.2. Teaching and scientific activity

4.2.2.1. Coordinated/taught training activity

Alícia Bofarull. Participation in the project "Cooperation in Criminal Investigation in Central America to Combat International Crime and Drug Trafficking" (ICRIME-CA/2017/39066), with the paper "Use of DNA Databases", online, January 2021.

Coordination of the meeting of the DNA Working Group of the Network of Official Forensic Laboratories of Spain (RLFOE) about adopting ISO 18385. Online, 19 February 2021.

Àlex Pifarré. Lecture on "Genetic Applications in the field of Justice", as part of the master's degree in Criminal Law at Pompeu Fabra University, mixed format (face-to-face and online), 13 April 2021.

Manuel Crespillo, Àlex Pifarré. Participation in the project "Cooperation in Criminal Investigation in Central America to Combat International Crime and Drug Trafficking" (ICRIME-CA/2017/39066), as part of the module on "Validation of Methods", online, 12-23 April 2021.

Araceli Vázquez, Àlex Pifarré. Coordination and participation on the course "Interpretation of expert opinions in the field of Forensic Medicine", organised as part of the Continuing Education Plan at the Centre for Legal Studies (CEJ), online, 19-27 April 2021.

Araceli Vázquez, Àlex Pifarré, Ángel Serrano. Coordination (AV and AP) and participation (AV, AP and AS) on the course "Treatment of offences against sexual freedom and integrity in the forensic laboratory", organised as part of the Continuing Education Plan at the Centre for Legal Studies (CEJ), online, 14-21 June 2021.

Miguel Paredes. Coordination and delivery of the training module "Implementation of the LIMS system in the Histopathology Service at the Barcelona Department of the INTCF". Face-to-face mode, June 2021.

Ángel Serrano, Miguel Ángel Ocaña, Àlex Pifarré. Participation in the activity "Accreditation Assistance for PSA Fast Test Kit and Haemoglobin Fast Test Kit Experiment Instructions", as par of the Twinning Project Tr 16 Ipa Jh 03 18 Forensic Trainings towards Advanced Examination Methods (activity 2.14), online, 7-11 July 2021.

Manuel Crespillo. Coordination and participation in the training activity "Databases of criminal interest: Operational and legal aspects", organised as part of the Continuous Training Plan at the Centre for Legal Studies (CEJ), online, 13-20 October 2021.

Manuel Crespillo. Participation in the project "Cooperation in Criminal Investigation in Central America to Combat International Crime and Drug Trafficking" (ICRIME-CA/2017/39066), face-to-face format, 17-23 October 2021 (El Salvador), and 5-11 December 2021 (Panama).

Àlex Pifarré Rubbel. Presentation entitled "DNA study on samples in offences against sexual freedom. Timeframes for collection and their relationship with the different anatomical regions", as part of the course "Update in the expert assessment of sexual violence", organised as part of the Continuing Education Plan at the Centre for Legal Studies (CEJ), online, 11-18 November 2021.

Manuel Crespillo. Annual plenary session of the National Commission on the Forensic Use of DNA, as the secretary, in person (Madrid), 15 December 2021.

Courses received

Training actions scheduled as part of the Continuous Training Plan 2021 at the Centre for Legal Studies (CEJ), online:

- "The INTCF Forensic Sciences Service: fields of action, analytical possibilities", 22-26/03/2021.
- "Detection and identification of chemical weapons in forensics. Toxicity and impact on the environment", 4-7/05/2021.
- "Treatment of offences against sexual freedom and integrity in the forensic laboratory", 14-21/06/2021.
- "Interpretation of expert opinions issued by the National Institute of Toxicology and Forensic Sciences", 19-27/04/2021.
- "Comprehensive forensic perspective on suicide", 05-12/05/2021.
- "Basic LIMS: structure, data organisation and queries", 17-24/05/2021.
- "Practical workshop on the dissemination of the INTCF quality system", 21-28/05/2021.
- "Update on the expert assessment of sexual violence", 30-31/03/2021.
- "New research tools in the field of Forensic Genetics", 21-28/06/2021.

- "Criminal databases: operational and legal aspects", 13-20/10/2021.
- "Multidisciplinary forensic intervention in multi-victim incidents", 15-22/11/2021.

1st course on the use of CODIS software. Organised by the National DNA Database Administrator, ETSE Technology Centre (El Pardo). Face-to-face mode, 25-26 May 2021.

"VISAGE Train-the-Trainers Workshop. Topics covered: Part 1: Theoretical basis of predictive markers; Part 2: Ethical considerations and recommendations; Part 3: MPS technology and laboratory workflow; Part 4: DNA analysis and processing genetic data; Part 5: Forensic phenotyping and VISAGE Software; Part 6: Implementation in a routine forensic DNA service environment; Part 7: Communication of results. Organised by VISAGE Work Package 7, online, 7-9/09/2021.

International CODIS Users Meeting. Organised by the Federal Bureau of Investigation (FBI), online, November 2021.

Training actions organised by the Subdirectorate General for Access and Promotion of Justice Administration Staff for Special Corps at the INTCF, online:

- "Multidisciplinary course on drugs: Review of toxicity, updating of analytical methods, judicial and social value of analytical expertise", 18-26/10/21
- "Quality Study of the UNE-EN ISO/IEC 17025:2017 standard. General Requirements for the competence of testing and calibration laboratories", 02-12/11/2021
- "Quality assurance in the INTCF, quality manuals, standard operating procedures, concepts and definitions", 22-30/11/2021.

4.3. Seville Department Biology Service

Concerning the expert activity of the Seville Department Biology Service, during 2021, a total of 2,059 requests were received with 10,762 samples analysed through a total of 42,162 analyses, emitting a total of 1,387 expert reports.

In terms of the type of investigation requested, as can be seen in Figure 4.3.1, the predominant analysis is the investigation of sexual assault cases (1,191 requests with 8,908 evidences received) in which the biological and genetic studies of semen samples are carried out and the study of DNA profiles being obtained from reference samples of the persons involved in the process (defendants, victims, persons to be ruled out, etc.).

The second most numerous analyses requests are kinship biological studies (559 requests with 430 samples analysed). Followed by studies of deaths suspected of being caused by crime (104 requests with 680 samples analysed), the identification of missing persons and corpses (103 requests with 254 samples analysed), drowning (67 requests with 404 samples analysed), and the analysis of biological evidence of criminal interest (24 requests with 58 samples analysed).

Figure 4.3.1. Casework of the Biology Service of the Department of Seville during 2021 according to the type of report

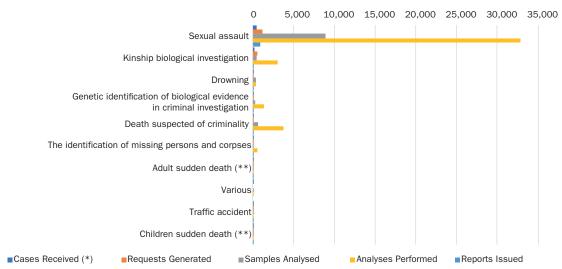


Table 4.3.1. Casework of the Biology Service of the Department of Seville during 2021 according to the type of report

Type of report	No. cases received*	No. requests generated	No. samples analysed	No. analyses performed	No. reports issued
Sexual assault	491	1,191	8,908	32,875	947
Kinship biological investigation	177	559	430	3,082	127
Drowning	66	67	404	413	108
Genetic identification of biological evidence in criminal investigation	65	103	254	1,400	62
Death suspected of criminality	54	104	680	3,718	93
The identification of missing persons and corpses	9	24	58	575	29
Adult sudden death**	9	9	13	48	19
Various	2	0	3	4	0
Traffic accident	1	1	11	41	1
Children sudden death**	1	1	2	6	1
TOTAL	848	2,059	10,762	42,162	1,387

^{*} The total number of cases is lower than the sum of the column because there are cases involving more than one type of report.

^{**} Biochemical analyses

With regard to the type of case, as reflected in Figure 4.3.2, most cases received involved sexual violence (58% of the total number of cases received, of which 69% correspond to adult victims and 31% to minors), followed by cases of kinship (21%) and unexplained death (9%) and, finally, homicides and identification of corpses (9% each).

0 700 800 100 200 300 400 500 600 Sexual assault/abuse of adults Civil kinship Sexual assault/abuse of minors Unexplained death Identification of corpses Homicide Injuries Theft Other cases No data-not registered (*)

■No. requests generated

■ No. reports issued

■ No. cases received

Figure 4.3.2. Casework of the Biology Service of the Department of Seville during 2021 by type of case

Table 4.3.2. Casework of the Biology Service of the Department of Seville during 2021 by type of case

Type of case	No. cases received	No. requests generated	No. reports issued
Sexual assault/abuse of adults	335	755	495
Civil kinship	175	530	77
Sexual assault/abuse of minors	151	291	160
Unexplained death	75	97	68
Identification of corpses	54	69	25
Homicide	53	105	42
Injuries	2	2	1
Theft	2	4	1
Other cases	1	1	0
No data-not registered*	0	205	518
TOTAL	848	2,059	1,387

^{*} These correspond to cases from years prior to 2021 where either new samples or new analyses have been received or the opinion was issued in 2021.

Additional activities in addition to the resolution of court cases, professionals from the Service have participated in the publication of various scientific articles and have imparted teachings in courses organised by the Centre for Legal Studies or in degrees and master's degrees at the Universidad Pablo de Olavide, among others.

4.3.1. Interesting forensic case

4.3.1.1. A case of identifying a man who disappeared several years ago and died as a result of a presumed homicide

Records

Bone remains found inside a well without water were received by the Department of Seville. They are covered by more than a metre of rubble, plant debris and soil. A number of fractures were observed in the skull, and the court in question requested a vitality study of the injuries. This study was inconclusive due to the deterioration of the bones. Furthermore, clothing and a range of objects were found, all in a severe state of deterioration, dirty and covered in dirt.

Evidences

The samples received by the Biology Service at the Seville Department consisted of the skull, clothing and various objects collected from the scene (underwear, slippers, trousers, shirt, watch, metal spoon, etc.), and swabs with biological remains collected from the neck of a bottle.

Results and conclusions

From the genetic study of the skeletal remains, a male genetic profile is returned with autosomal and Y-chromosome markers, generating a match in the CODIS database. The skeletal remains corresponded to a man who had disappeared six years earlier.

Based on the genetic study of the clothing and other objects found next to the skeletal remains, two different genetic profiles were detected: one with a high rate of degradation, but complete, obtained from the study of several swabs taken from the necks of several bottles; and the other from a swab taken from a very deteriorated, soiled garment. In the latter, a complete genetic profile with no signs of degradation was detected. Both profiles were registered in the CODIS database, with the first genetic profile taken from the bottles showing a match with the main person investigated, while the other, obtained from the garment, shows no match. Given the nature of the second profile, it was not possible to rule out contamination unrelated to the events and recently deposited at the site.

4.3.2 Teaching and scientific activity

Additional activities in addition to the resolution of court cases, professionals from the Service have participated in the publication of one scientific article and have imparted teachings in courses organised by the Centre for Legal Studies or in degrees and master's degrees at the Universidad Pablo de Olavide, among others.

4.3.2.1. Scientific publications

Vullo CM, Catelli L, Ibarra Rodríguez AA, Papaioannou A, Álvarez Merino JC, López-Parra AM, Gaviria A, Baeza-Richer C, Romanini C, González-Moya E, Casals F, Calafel F, Berardi G, Iannacone GC, Vicuña Giraldo GC, Zorba GK, Boschi I, Valdivia Olarte J, Parsons T. Second GHEP-ISFG exercise for DVI: "DNA-led" victims' identification in a simulated air crash. *Forensic Sci Int Genet.* 2021;53:102527. https://www.sciencedirect.com/science/article/abs/pii/S187249732100065X#!

4.3.2.2. Teaching and training activities

- Speaker at Activity 3.12 of the Twinning Project Tr 16 Ipa Jh 03 18, Forensic Trainings
 Towards Advanced Examination Methods. Enhancement of the Quality in Kinship
 Reports. Turkey, online, 15-19 March 2021.
- Speaker at the course "Basic LIMS: structure, data organisation and queries", 17-24
 May 2021. Centre for Legal Studies (online).
- University Master's Degree in Criminology and Forensic Sciences organised by the Universidad Pablo de Olavide. 2020/2021 Academic year.

Courses received

- Course: New research tools in the field of Forensic Genetics, from 21 to 28 June 2021. Centre for Legal Studies (online).
- 27th Annual CODIS Conference. Organised by the FBI (online) from 15 to 19 November 2021.
- 2nd Course on the use of CODIS software, from 26 to 28 October 2021 (15 hrs).
 CETSE (Centro Tecnológico de Seguridad), El Pardo, Madrid.
- Course "Criminal databases: operational and legal aspects", organised by the Centre for Legal Studies, as part of the Continuing Education Plan, from 13 to 20 October 2021.
- Symposium "The impact of forensic DNA phenotyping on science and society. Results of the Visage project", held in Santiago de Compostela on 24 September 2021.

4.4. La Laguna Biology Section

Concerning the expert activity from La Laguna Delegation Biology Section, during 2021 a total of 677 requests were received with 3,392 samples analysed through a total of 11,678 analyses, emitting a total of 426 expert reports.

It must be noted that in 2021, the Delegation observed an increase compared to the previous year in the number of cases. This represents a 29.94% increase in the number of requests received compared to 2020 (521 requests received), continuing with the upward trend, up by 65.12% on 2019 (410 requests received).

As can be seen in Figure 4.4.1, the predominant analysis is the investigation of sexual assault cases (303 requests with 1,816 evidences received) in which the biological and genetic studies of semen samples or other biological fluids are carried out and the study of DNA profiles being obtained from reference samples of the persons involved in the process (defendants, victims, persons to be ruled out, etc.).

The second most numerous analyses requests are the identification of missing persons and corpses (130 requests with 219 samples analysed). Followed by the analysis of biological evidence of criminal interest (103 requests with 600 samples analysed), kinship biological studies (76 requests with 205 samples analysed), and studies of deaths suspected of being caused by crime (55 requests with 535 samples analysed).

Special mention must also be made of adult sudden death with biochemical analyses (8 requests with 16 samples analysed).

These data suppose an increase of 91.18% in the number of requests about the genetic identification of missing persons and corpses compared to 2020 (68 requests received in 2020). In the waters off the Canary Islands, 2021 is estimated to have seen the

highest number of migrants dying or going missing since the UN International Organisation for Migration (IOM) began compiling these records back in 2014. The reason is that 2021 was the third year of most immigrants coming in small boats or canoes in the Canary Islands in history. The islands received 22,316 irregular migrants by sea, in a total of 542 vessels (source: Ministry of the Interior and the Government Office in the Canary Islands.

Figure 4.4.1. Casework of the La Laguna Biology Section during 2021 by type of report

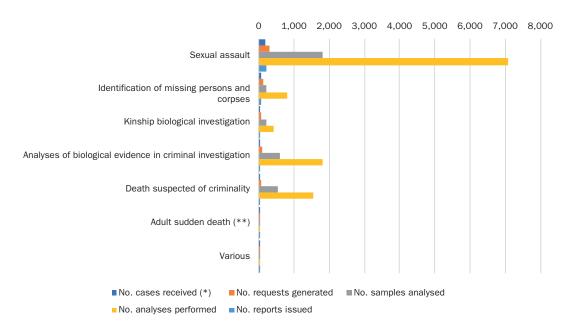


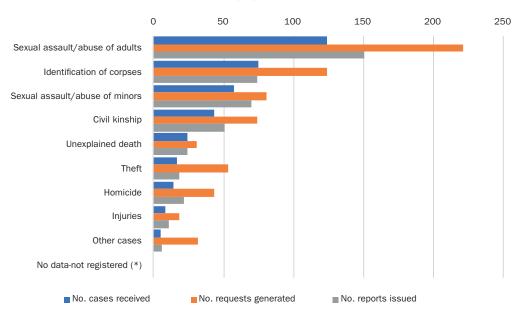
Table 4.4.1. Casework of the La Laguna Biology Section during 2021 by type of report

Type of report	No. cases received*	No. requests generated	No. samples analysed	No. analyses performed	No. reports issued
Sexual assault	182	303	1,816	7,079	221
Identification of missing persons and corpses	79	130	219	807	79
Kinship biological investigation	44	76	205	416	51
Analyses of biological evidence in criminal investigation	30	103	600	1,822	36
Death suspected of criminality	25	55	535	1,534	29
Adult sudden death**	8	8	16	19	9
Various	2	2	1	1	1
TOTAL	367	677	3,392	11,678	426

^{*} The total number of cases is lower than the sum of the column because there are cases involving more than one type of report.

With regard to the type of case, as reflected in Figure 4.4.2, most cases received involved sexual violence (49.59% of the total number of cases received, of which 68.13% correspond to adult victims and 31.86% to minors), followed by cases of identification of missing persons and corpses and kinship (20.44% and 11.72%, respectively).

Figure 4.4.2. Casework of the La Laguna Biology Section during 2021 by type of report



^{**} Biochemical analyses

Table 4.4.2. Casework of the La Laguna Biology Section during 2021 by type of report

Type of case	No. cases received	No. requests generated	No. reports issued
Sexual assault/abuse of adults	124	221	150
Identification of corpses	75	124	74
Sexual assault/abuse of minors	57	81	70
Civil kinship	43	74	51
Unexplained death	24	31	24
Theft	17	53	18
Homicide	14	43	22
Injuries	8	18	11
Other cases	5	32	6
No data-not registered*	0	0	0
TOTAL	367	677	426

^{*} These correspond to cases from years prior to 2021 where either new samples or new analyses have been received or the opinion was issued in 2021.

In addition to the resolution of court cases, in 2021, major efforts were dedicated to the documentation associated with the standard operating procedures, as well as to the promotion of the accreditation of new tests for their forthcoming implementation at the Biology Department.

4.4.1. Interesting forensic case

Amongst the cases analysed by the Canary Islands Delegation of the INTCF, particular mention should be made that during 2021, a significant number of investigations were requested for the identification of migrant corpses from Africa, who unfortunately died on their sea crossing. In 2021, it is estimated that more than 1,153 people died on the route from north-west Africa to the Canary Islands [1]; these figures are up on previous years. During their journey, people suffer a wide variety of threats [2], including alleged sexual assaults, as reflected in the requests for analysis received by the centre.

To illustrate this tragedy, below, details are provided about the identification of 24 male corpses found in a cayuco adrift at sea in April 2021, with hyperosmolar dehydration as a possible cause of death. Laboratory work allowed DNA to be extracted and genetic profiles to be obtained from each corpse for STRa and Y-chromosome markers, which were included in the National DNA Database; however, no match has been obtained to date that would allow the deceased to be identified. In relation to this case, only one presumed sibling on the mother's side was received for comparison with the 24 deceased, and the comparison offered very low statistical significance, with inconclusive likelihood ratio (LR) values, due to the type of relationship between the individuals for comparison (half-siblings). Therefore, regions of mitochondrial DNA were analysed in collaboration

with the Seville Department, resulting in a maternally compatible haplotype and, finally, a slightly higher LR value.

Within the framework of the conventions established in the field of international cooperation, and despite the difficulties faced[2], ideally, conclusive samples or genetic profiles would be obtained from the presumed next of kin to proceed with the identification process. However, privacy concerns and international logistics pose considerable problems that mean it is difficult to take conclusive samples necessary for matching, unless a representative or legal guardian is appointed to represent these families for each proceeding opened in the Canary Islands, which in this case is problematic, increasing the sense of despair amongst the presumed relatives in the country of origin. Therefore, although the laboratory work is complete, unfortunately, it has been impossible to resolve the cases, because, as indicated [2], beyond the scientific steps, measures must also be taken at an international level between the different institutions to guarantee the right of relatives to identify the deceased.

The genetic profiles of the deceased remain in the DNA Database of Genetic Profiles of Human Remains and Unidentified Corpses, meaning that they can be subsequently matched with the genetic profiles registered in the database for identification purposes.

Bibliographic references

[1] Protection, saving lives & solutions for refugees in dangerous journeys. Routes towards the central & west Mediterranean sea and the Atlantic. UNHCR's 2022-2023. Updated Risk Mitigation Strategy and 2022 Appeal. April 2022.

[2] Death on the Border. Summary and conclusions of the 12th Conference of the Spanish Association of Anthropology and Forensic Odontology (AEAOF). University of Granada. 2020.

4.4.2 Teaching and scientific activity

4.4.2.1. Participation in investigation projects

Collaboration agreement with the Canary Islands Institute of Bioanthropology, of the Autonomous Body of Museums and Centres of the Island Council of Tenerife, in the field of research and teaching.

4.4.2.2. Teaching and training activities

Training actions scheduled as part of the Continuous Training Plan 2021 at the Centre for Legal Studies (CEJ), online:

- "Basic LIMS: structure, data organisation and queries", 17-24/05/2021.
- "Practical workshop on the dissemination of the INTCF quality system", 21-28/05/2021.

- "New research tools in the field of Forensic Genetics", 21-28/06/2021.
- "Criminal databases: operational and legal aspects", 13-20/10/2021.
- "Multidisciplinary forensic intervention in multi-victim incidents", 15-22/11/2021.

Training actions organised by the Subdirectorate General for Access and Promotion of Justice Administration Staff for Special Corps at the INTCF, online:

- "Multidisciplinary course on drugs: Review of toxicity, updating of analytical methods, judicial and social value of analytical expertise", 18-26/10/21
- "Quality Study of the UNE-EN ISO/IEC 17025:2017 standard. General Requirements for the competence of testing and calibration laboratories", 02-12/11/2021
- "Quality assurance in the INTCF, quality manuals, standard operating procedures, concepts and definitions", 22-30/11/2021.

Hernández Luis, A. Training action provided: "DNA analysis in criminal investigation", at the Viera y Clavijo Institute, April 2021.

Training action provided to medical interns at the two hospitals in Tenerife.

Online participation at the meeting of the DNA Working Group of the Network of Official Forensic Laboratories of Spain (RLFOE), 19 February 2021.

Training action received in a face-to-face format: "HID 3500 AG Instrument & GMIDX Software training", organised by Life Technologies, INTCF La Laguna Delegation, February 2021.

Training action received online: Workshop 12th GCLAITH Meeting. ISHI (International Symposium on Human Identification). Organised by the Latin American Scientific Working Group on Human Identification, 16 September 2021.

Training action received online: Symposium: "The impact of forensic DNA phenotyping on science and society. Results of the VISAGE EU project and their applications". Organised by the University of Santiago de Compostela, 24 September 2021.

Online participation in the 26th GHEP-ISFG Forensic Genetics Conference, 18-19 October 2021.

Training action received online: "Webinar Interpol DNA Database and I-Family Database", Interpol DNA Unit, 26 October 2021.

Training action received in a face-to-face format: "2nd Course on the use of CODIS software". Subdirectorate General of Information Systems (Ministry of the Interior), El Pardo, Madrid, 25-28 October 2021.

Online participation at the "27th Annual CODIS Conference", FBI, November 2021.

Online participation at the COMSIGENI meeting, November 2021.

Online participation at the 2nd AFORE WP3:2 Webinar on The Validation of Analytical Methods in Forensic Science. Organised by ENFSI, from 1 to 2 December 2021.

Training action received online: Kinship statistics using Families workshop. Organised by ENFSI DNA EWG. Netherlands Forensic Institute, 14-15 December 2021.

4.5. The INTCF DNA DATABASES

In the present report, we analyse the results obtained in the different DNA databases of the INTCF during 2021.

In 2021, a total of 836 genetic profiles were registered in different DNA files of the INTCF. In cases of criminal investigation (708) and identification of missing persons (101), there were a total of 809 DNA profiles, which were in turn registered as well in the national DNA database managed by the State Secretariat for Security at the Ministry of Interior. In the case of genetic profiles obtained in relation to Irregular Adoptions and Abducted Newborns, which are managed by the Ministry of Justice, a total of 27 DNA profiles were registered.

4.5.1. Criminal investigation and identification of missing persons

The INTCF has been responsible for two DNA profile files since 2010 (Order JUS/2267/2010, of 30 July 2010):

- The INTCF-ADNIC file (Criminal Investigation), whose purpose is the genetic comparison of biological traces of unknown origin with each other and with reference samples of individuals investigated in a criminal procedure, to identify coincidences between the DNA profiles, and to provide information in the investigation of offences without a known perpetrator.
- The INTCF-ADNID file (missing personal investigation), to genetic identification of missing persons and corpses without identification in court proceeding through the comparison of genetic profiles obtained from an unknown human with the DNA profiles obtained from the family samples or of DNA profiles obtained from ante-mortem samples of the missing persons.

These DNA profiles are systematically compared using the *software* known as CODIS (Combined DNA Index System, Federal Bureau of Investigation [FBI], Dept. of Justice, USA) in the local node at the Ministry of Justice and in the national node of the DNA Database, which is managed by the Secretary of State for Security at the Ministry of the Interior pursuant to the provisions of Organic Law 10/2007 of 8 October 2007 regulating the police database on DNA identifiers. Also, the DNA profiles registered at the national node are regularly compared against the DNA databases of 23 European nations by the Prüm treaty (Instrument of ratification by Spain of the Convention on the stepping up of

cross-border cooperation, particularly in combating terrorism, cross-border crime, and illegal immigration, entered into in Prüm on 27 May 2005).

In 2021, a total of 1,715 requests for judicial pronouncements to register genetic profiles in the DNA databases were sent from the INTCF to court offices (1,089 [64%] requests to the register in relation to unknown genetic profiles, 512 [30%] in relation to known genetic profiles, 111 [6%] in relation to genetic profiles of unidentified human remains and 3 in relation to genetic profiles of relatives of missing persons).

During 2021, a total of 904 profiles were found to be eligible for registration in the DNA database (either by direct request, by positive judicial pronouncements in response to a previous request from this Institute or because there was no legal impediment), compared to 355 profiles in which registration in the DNA database was considered inappropriate (either by express judicial rejection, by request for direct comparison of the samples with the suspect's reference sample or because the perpetration of the offence was not duly justified). It can be seen that, compared to previous years, there was a slight increase in the proportion of approvals (72%) compared to refusals (28%). By profile type, it can be seen that for dubious genetic profiles, 67% of the cases were eligible for registration, compared to 33% where, for the reasons given above, the registration of profiles in the DNA database was considered inappropriate. For reference genetic profiles, the percentage of positive rulings came to 81%, compared to 19% of cases in which their registration was rejected; and for genetic profiles from unidentified corpses, in 77% of cases, registration was considered appropriate, compared to 23% in which it was not considered appropriate, as the corpse had already been identified by another institution or by other methods.

From the aforementioned data, it is clear that, by the different Departments, a follow-up is carried out on the requests sent to obtain a judicial ruling on whether, depending on the current state of the procedure, the type of crime, etc., genetic profiles are susceptible to being registered in the DNA database, since, following the pattern of previous years, in more than a third of cases, such registration is ultimately not applicable. This monitoring is aimed at optimising the material and personal resources used in the Institute's criminal investigations and ensures that all legal requirements for the registration of each genetic profile in the DNA database are complied with.

Approvals Refusals

Figure 4.5.1.1. Judicial pronouncements for the registration of profiles in the INTCF's DNA database

In 2021, 809 genetic profiles were registered in the national node of the DNA database at the INTCF, of which 708 profiles (87.5%) were registered in the INTCF-ADNIC file and 101 profiles (12.5%) were registered in the INTCF-ADNID file.

Since 2010, the first year in which INTCF had access to the DNA database, there has been a gradual increase in the number of profiles registered each year, both in the criminal index and in the social interest index (identification of missing persons). Specifically, in the case of the latter, in 2021, there was a very considerable increase in the genetic profiles of unidentified corpses compared to previous years, mainly due to the increase in the number of migrants who died in their attempt to reach the Canary Islands.

The following charts show the evolution in the registration of genetic profiles in both indexes since the start of the DNA database at the INTCF.

800 700 600 Reference samples from 500 400 investigated persons 300 Unknown mixture 200 Unique unknown 100 2010 2011 2012 2013 2014 2015 2016 2017 2018 2019 2020 2021 (*) (*)

Figure 4.5.1.2. Evolution of the number of profiles registered in INTCF-ADNIC

(*) During the first two years (2010 and 2011) only the Madrid Department used the DNA database.

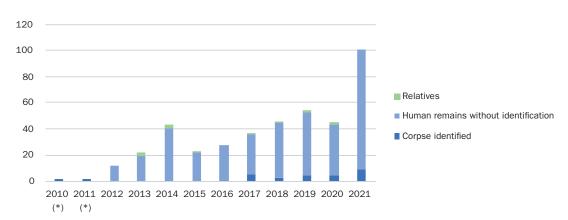


Figure 4.5.1.3. Evolution of the number of profiles registered in INTCF-ADNID

(*) During the first two years (2010 and 2011) only the Madrid Department used the DNA database.

In the field of criminal investigation (INTCF-ADNIC file), 419 (59%) unknown DNA profiles were registered (either individual or a mixture of two or three contributors) coming from forensic samples obtained in the crime scene, the body, or clothes from the victim or the condemned, and 289 (41%) DNA reference profiles of persons investigated in a court procedure. Most DNA profiles in the file correspond to investigations of sexual freedom offences involving adults (64%), followed to sexual freedom offences against minors (19%), then homicides (8%), burglaries (3%), and others (6%) (robbery with violence or intimidation, injuries, gender violence, terrorism and others).

The distribution of DNA profiles registered by INTCF in 2021 in the database by sample type and case type is shown below.

9 92 ■ Investigated reference

Figure 4.5.1.4. Profiles registered in the INTCF DNA database in 2021

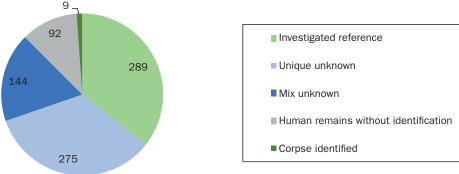
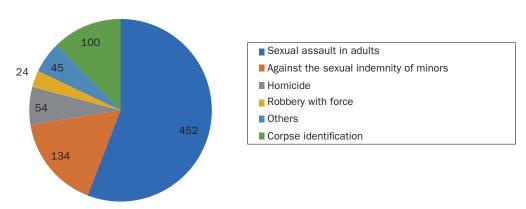
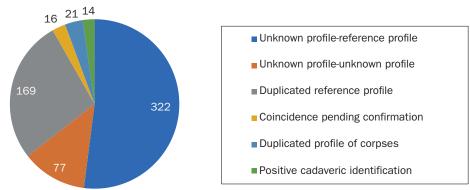


Figure 4.5.1.5. No. of profiles registered in the INTCF DNA Database by type of case in 2021



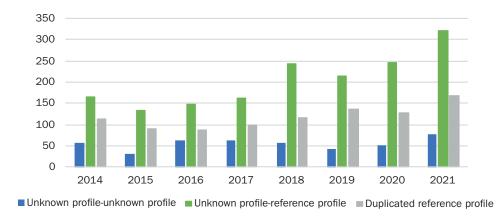
As regards the total number of matches detected during 2021 (619), both in the local node and in the national node, for the genetic profiles registered in the INTCF, in relation to criminal investigation 568 matches were registered, of which 322 (57%) involved profiles of a reference sample of an investigated person and an unknown sample, which has contributed to the resolution of multiple investigations, and 77 correspond to matches of profiles considered unknown with one another (14%). There were 169 matches (30% of the total) among reference DNA profiles involving the same defendant as a result of duplications of the same person in the national database, having been registered by other institutions in addition to the INTCF, either as part of the same or a different case.

Figure 4.5.1.6. Number of matches detected in the DNA database with profiles registered in the INTCF in 2021



The following graph shows the evolution in recent years of the types of match detected (sum of those detected in both the national and local nodes) for the genetic profiles registered in the INTCF's DNA of criminal interest file. The progressive increase in the effectiveness of the database is striking, reflected in the fact that the number of matches between reference and unknown profiles detected in the last eight years has more than doubled.

Figure 4.5.1.7. Evolution of the type of matches with profiles registered in INTCF-ADNIC



Furthermore, in relation to the identification of corpses, there were 14 matches detected in 2021 yielding a positive identification, all of them in the national node. It should be noted that 60% of the total number of matches detected in this file (21 out of 35) correspond to matches between profiles from unidentified corpses that have been analysed as a duplicate at another institution in addition to the INTCF.

The following graph shows the evolution in recent years of the types of match detected for the genetic profiles registered in the INTCF's DNA of social interest file. Although there was a slight increase in the number of positive identifications over the years, it should be noted that this increase is much more pronounced in the number of matches detected between genetic profiles from the same corpse analysed as a duplicate between the INTCF and another official institution.

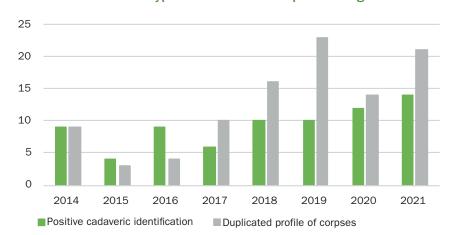


Figure 4.5.1.8. Evolution of the type of matches with profiles registered in INTCF-ADNID

4.5.2. Irregular adoptions and newborn abductions

Order JUS/2146/2012, 1 October established the creation of the file "DNA profiles from people affected by the newborn abductions" managed by the INTCF to identify possible kinship genetic relations between persons affected by the possible newborn abductions always with their consent.

The file intends to avoid the current dispersion of all the DNA data through the genetic profile centralisation (both the generated in private laboratories and the generated in the INTCF in the course of the investigations ordered by magistrates and judges) in a single DNA database to ensure that all cross-referencing between family members of the different indexes is performed and to ensure the highest degree of success in the search. Currently, most of the profiles registered pertain to individuals who have applied for registration through the Information Service for Persons Affected by the Possible Abduction of Newborns.

In 2021, 27 DNA profiles were included in the register of "DNA profiling of persons affected by the abduction of newborns" (obtained by different private DNA laboratories) associated with requests from people affected through the Information Office for Persons Affected by the Possible Abduction of Newborns.

Asuntos Servicio de Información SRN Asuntos judiciales

Figure 4.5.2.1. No. of profiles of persons affected by the possible abduction of newborns registered in the INTCF DNA database

The total number of DNA profiles present in that file at the end of 2021 was 647, with the following distribution according to the type of family member:

Table 4.5.2.1. Type of profiles of persons affected by the possible abduction of newborns registered in the INTCF DNA database

FAMILIAR	No. of profiles	%	
Biological mothers searching for their children	411	74.0	
Biological fathers searching for their children	50	71.2	
Sisters searching for their biological brothers/sisters	48		
Brothers searching for their biological brothers/sisters	33	12.5	
Adopted daughters searching for their biological parents	70		
Adopted sons searching for their biological parents	34	16.1	
Others	1	0.2	
TOTAL	647	100.0	

In the searches carried out during 2021 in this file, all the possible compatibility detected (with none, one or even two genetic inconsistencies) between biological parents who are looking for children and adopted children who are looking for their biological parents, as well as between siblings looking for their biological siblings, turned out to be fortuitous matches after joint evaluation of all the available data.

The degree of success in the parentage relation identifications between the affected registered in this DNA file managed by the INTCF will be determined primarily by the degree of involvement of those concerned in this project and only be guaranteed if they consent to the recording of their DNA profile.

REPORT CODIS 2020

			INTCF Dep	artment		TOTAL
		Madrid	Barcelona	Seville	La Laguna	INTCF
Re	quests of judicial pronouncement to regist	ter in CODIS	remitted in	2021		
	For reference profiles	205	193	53	61	512
	For unknown vestiges	361	486	175	67	1089
For hum	an remains profiles without identification	15	19	27	50	111
	For relative reference profiles	0	0	3	0	3
	Total requests sent	581	698	258	178	1715
	Rulings about the register with CO		1			T
Registration in CODIS	Reference profiles	166	79	26	24	295
(by express judicial	Unknown profiles	148	218	138	17	521
request or approval, or by omission - in case	Human remains without identification	18	21	12	36	87
of unknown profiles)	Relatives	0	0	1	0	1
, , , ,	Total	332	318	177	77	904
No registration in CODIS	Reference profiles	24	17	26	4	71
(due to express	Unknown profiles	153	66	37	0	256
rejection by the court, direct comparison or	Human remains without identification	14	0	12	0	26
dismissal as no offence	Relatives	0	0	2	0	2
was justified).	Total	191	83	77	4	355
	Profiles registered in COD	IS in 2021				
	Unique unknown	96	84	77	18	275
	Unknown mixture	46	42	50	6	144
	Corpse identified	6	0	3	0	9
By profile type	Reference from investigated persons	172	60	42	15	289
	Human remains without identification	13	20	19	40	92
	Relatives	0	0	0	0	0
	Total profiles registered	333	206	191	79	809
_	Sexual assault	183	138	115	16	452
	Against the sexual indemnity of minors	79	27	28	0	134
	Domestic violence	5	1	2	0	8
	Homicide	25	8	15	6	54
	Robbery with force	16	2	1	5	24
	Robbery with intimidation	1	3	1	9	14
By case type	Injuries	4	1	0	3	8
	Corpse identification	19	20	21	40	100
	Against public health	0	0	1	0	1
	Terrorism	0	5	0	0	5
-	Others	1	1	7	0	9
	Total profiles registered	333	206	191	79	809
	Coincidences detected in CO		1			1
	Unknown profile-unknown profile	21	37	15	4	77
	Unknown profile-reference profile	107	97	99	19	322
	Duplicated reference profile	92	35	29	13	169
	Coincidence pending confirmation	4	5	3	4	16
	Duplicated profile of corpses	5	3	12	1	21
		_			_	
	Positive cadaveric identification	4	5	2	3	14



The INTCF has a Histopathology Service at each Department (Barcelona, Madrid, and Seville), with a Histopathology Section at the INTCF Delegation in the Canary Islands (La Laguna, Santa Cruz de Tenerife).

The Histopathology Services and Section, in compliance with the functions entrusted to them, mainly undertake expert activities, but also carry out teaching and research functions, acting as a reference centre, fostering continuous training and promoting research work in their field, as well as in other areas of public interest through collaboration agreements and conventions. As a result of this activity, scientific publications have been published, as well as participation in forensic congresses and conferences and other medical specialities, both nationally and internationally.

The work philosophy is based on the application of permanent updates in terms of knowledge and technical means, under the criteria of quality, promoting teamwork and professional improvement, through training and continuous training processes.

Expert activities are carried out within the scope of the medical speciality of pathological anatomy applied to court cases (Forensic Pathology). Samples from judicial autopsies performed at the Institutes of Legal Medicine and Forensic Sciences (IMLCF) are analysed, providing the necessary diagnosis elements to answer, as far as possible, the different medical-forensic questions that may arise during the judicial investigation. This activity starts with the registration of samples and accompanying documentation and maintaining the chain of custody.

Samples submitted by IMLCFs, whether visceral blocks, viscera, visceral fragments, biological fluids for cytological study and others, are examined, macroscopically studied, photographed and cut. The samples/subsamples obtained are subject to automated processing by paraffin embedding and/or frozen sections. with cryostat when required. Routine staining, histochemical and immunohistochemical techniques, cytocentrifugation in biological fluids, transmission light microscopy, optionally with polarised light, and photomicroscopy are used. The electrolysis technique is used to dissolve metallic elements in hearts with stents implanted in coronary arteries (Madrid Dept.). Once the preceding steps have been performed, the corresponding report is drawn up and submitted in writing. It contains the main facts of the case, including the type of report (investigation objectives), a summary of the information received (according to sample referral form), the study techniques performed and their results, consisting of macroscopic and microscopic aspects of the samples. Subsequently, the pathological diagnoses and conclusions are listed, taking into account the information received. The reports are forwarded to the court and to the relevant IMLCF, indicating the estimated custody time of the samples submitted prior to their destruction.

The investigations most commonly requested are as follows:

- · Sudden and unexpected death
- · Sudden death related to sport
- · Infant sudden death
- · Perinatal death
- · Violent newborn death
- · Pregnancy-abortion diagnosis
- · Death associated with anaphylaxis
- · Death investigation due to alleged medical malpractice-iatrogenesis
- Traumatisms
- Vitality and data study
- · Asphyxia (hanging, strangulation, confinement, suffocation)
- Intoxication death
- Death associated with alcohol or drugs
- Deaths due to physical agents: freezing, hypothermia, heat burns, heatstroke, electricity, radiation
- Death in fires
- · Death in institutions/death in custody
- · Other histopathological studies
- Cytological study of liquids

A significant number of the cases studied involve deaths suspected of involving criminal action, especially in adults and senescence, which at the end of the studies are generally classified as natural deaths. These correspond to the studies classed as sudden unexpected death. There are also numerous suspected cases of sudden death caused by inherited heart disease, the study and diagnosis of which requires a standardised anatomopathological protocol and multidisciplinary collaboration with subsequent genetic counselling. Given its social transcendence, we are actively collaborating in various studies in several autonomous communities and other institutions at a national level.

Vitality and injury dating studies are particularly important, as, from a medico-legal perspective, it is very important to establish whether a wound, or any other type of injury, was suffered while the person was alive or after their death, and to be able to date the time that has elapsed or the chronology of the injuries, from the time an injury occurs until the person dies, are very important factors in the investigation of deaths, especially in homicides.

The most frequent violent deaths involve general intoxication or are related to the consumption of abuse drugs, trauma caused by traffic, work or domestic accidents, drowning and mechanical asphyxiation. Worth particular mention are homicide cases (death by knife or firearms, gender violence, trauma and strangulations), in which the expert activity usually ends with attending court, either in person or by videoconference.

Cases of violent death in infancy and childhood constitute an extraordinarily important group of cases. Differentiating whether a child was born alive or died before birth is something in which the macro- and microscopic study of the lungs is of great help. Likewise, it can be diagnosed whether there was a loss of foetal wellbeing, which is of great value in determining whether the newborn may have died of natural causes or whether there are signs of violence. In children of different ages, violent deaths have an enormous social repercussion, involve significant complexity in relation to the diagnosis and, in many occasions, significant suspicion of involvement of the parents or guardians.

Cases of complaints involving possible medical negligence resulting in the death of the patient are another of the fields covered by this Service, providing the anatomopathological diagnoses essential for the forensic doctor to be able to draw up the final autopsy report with conclusions as to whether or not malpractice was involved.

In 2021, the Histopathology Services/Section of the INTCF registered 5,242 requests corresponding to 5,199 expert cases and 21,683 samples for analysis, issuing 4,194 expert reports (Figure 5.1 and Table 5.1). These data represent an increase of 6.83% in the number of requests received compared to 2020 (3,850 requests received).

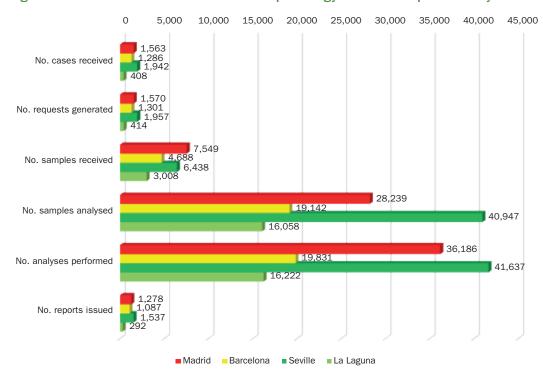


Figure 5.1. Overall data of the INTCF Histopathology Services' Expert Activity in 2021

Table 5.1: Overall data of the INTCF Histopathology Services' Expert Activity in 2021

2021	No. cases received	No. requests generated	No. samples received	No. samples analysed	No. analyses performed	No. reports issued
Madrid	1,563	1,570	7,549	28,239	36,186	1,278
Barcelona	1,286	1,301	4,688	19,142	19,831	1,087
Seville	1,942	1,957	6,438	40,947	41,637	1,537
La Laguna	408	414	3,008	16,058	16,222	292
TOTAL	5,199	5,242	21,683	105,271	114,049	4,194

The composition of the Histopathology Services staff in 2021 is reflected in the following table.

Table 5.2

	INTCF-MADRID	INTCF-BARCELONA	INTCF-SEVILLA	INTCF-LA LAGUNA			
Head of the Service	1	1	-	1*			
Facultatives	7	5	7	1			
Specialist technicians	5	3	6	1			
Laboratory assistants	2	4	3	1			
Clerical staff	1	-	1	-			
*Professional who performs the duties of Service Coordinator.							

5.1. Madrid Department Histopathology Service

At the Department of Madrid, in 2021, histopathological studies were requested for 1,570 deaths, of which 86 were suicides, 37 were homicides, 185 were accidental deaths and the majority were sudden deaths (69%) (Figure 5.1.1 and Table 5.1.1).

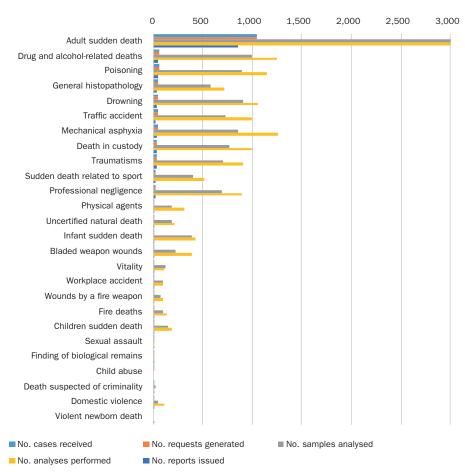


Figure 5.1.1. Casework of the Histopathology Service of the Madrid Department during 2021 by type of report

Table 5.1.1. Casework of the Histopathology Service of the Madrid Department during 2021 by type of report

Type of report	No. cases received	No. requests generated	No. samples analysed	No. analyses performed	No. reports issued
Adult sudden death	1,045	1,047	19,069	24,181	857
Drug and alcohol-related deaths	64	63	998	1,245	50
Poisoning	64	64	898	1,150	50
General histopathology	54	53	581	714	39
Drowning	52	52	911	1,063	43
Traffic accident	47	46	733	990	31
Mechanical asphyxia	47	48	861	1,260	32
Death in custody	37	37	766	998	33
Traumatisms	36	36	706	910	33
Sudden death related to sport	25	25	406	520	21
Professional negligence	23	23	690	891	21
Physical agents	11	12	195	321	7
Uncertified natural death	10	10	188	217	8
Infant sudden death	10	10	388	434	10

Type of report	No. cases received	No. requests generated	No. samples analysed	No. analyses performed	No. reports issued
Bladed weapon wounds	9	10	222	387	7
Vitality	8	8	131	114	11
Workplace accident	6	6	96	106	6
Wounds by a fire weapon	6	6	70	99	6
Fire deaths	5	5	101	134	4
Children sudden death	4	4	148	186	2
Sexual assault	1	1	3	3	1
Finding of biological remains	1	1	2	4	1
Child abuse	1	1	0	0	0
Death suspected of criminality	1	1	21	5	1
Domestic violence	1	1	55	114	3
Violent newborn death	0	0	0	0	1
TOTAL	1,563	1,570	28,239	36,046	1,278

In relation to the 1,278 reports issued in 2021, as was the case in previous years, the most common cases involved sudden deaths (67%); the majority involving victims aged over 50 and 21 associated with sport (Figure 5.1.2). As regards the adults who passed away, 626 were male and 244 female, with a mean age of 55.3 years. The causes of death were cardiovascular in 521 cases (60%), including 392 deaths due to coronary atheromatous disease, with cases identified in individuals as young as 25 years of age. Many of those who died suddenly in 2021 had been vaccinated against COVID-19, given the high vaccination rates in our country, although as part of a study carried out at our department with 44 deaths up to one month after vaccination, between January and July 2021, no pathology associated with the vaccine was found. The main cause of death was ischaemic heart disease as in not vaccinated.

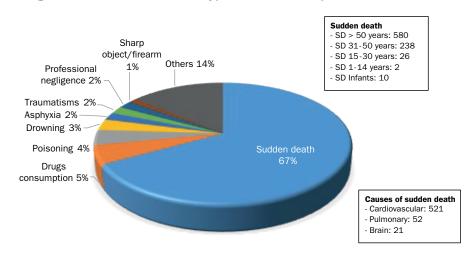


Figure 5.1.2. Distribution of type of death in reports issued in 2021

In 155 cases, no pathological findings were found to explain the cause of death, which in some cases could correspond to deaths due to channelopathies. The cause of death could also not be detected in any of the 10 sudden infant deaths studied. In 87 cases, blood samples were kept for possible genetic analysis for possible familial heart disease and 23 samples were sent to specialised laboratories for this type of study.

The second most frequent group was deaths caused by toxic substances: 64 caused by drug abuse and 56 caused by alcohol and other substances. In 24 cases, the death was associated with drug use, with 14 being suicidal in nature. Forty-three drowning deaths were studied, in eight of which a cardiac pathology was found that may have contributed to the episode of drowning. In other asphyxia deaths, the most common were hanging (10 cases, 9 by suicide). Thirty-two trauma deaths were studied, most of them involving accidental head injuries (one by firearm). The investigation of malpractice allegations was performed in relation to 22 deaths, 7 involving sudden death and 2 involving traumatic brain injury. In 7 cases, skin flaps or other organs with stab wounds were sent and in another 6 cases, with firearm wounds (generally to supplement the forensic study performed to determine the type of weapon), in order to demonstrate the vitality of the injuries, i.e. whether the subject was alive at the time of the aggression.

5.1.1. Forensic case of interest: Sudden death related to sport

Circumstances of the death

A 42-year-old man, with no previous history worth note, was returning from a bike ride and, shortly after greeting a neighbour, was found by another man lying on the ground with his head on the kerb (still wearing his helmet) and convulsing. Basic cardiopulmonary resuscitation (CPR) was performed by neighbours and advanced CPR was performed on arrival of the medical staff, who detected ventricular fibrillation and asystole. CPR was stopped after 45 minutes.

Medicoforensic autopsy findings

Approximate weight: 80 kg. Height: 180 cm. Negative for coronavirus and toxic substances. Incisocontuse wound in the occipital region. CPR rib fractures. Congestive and oedematous lungs.

Possible death cause:

Sudden cardiac death

Samples sent for histopathological study

Brain, heart and lungs.

Histopathological study

External examination of the heart showed increased epicardial fat with some whitish patches in the pericardium (Figure 5.1.1.1 A); heart weight for his body weight and height was normal. In biventricular cross-sections there was extensive scarring with thinning of

the lateral wall of the left ventricle (Figure 5.1.1.1 B). The coronary arteries originated normally and were calcified with a significant reduction in lumen upon macroscopic examination.

Microscopic examination revealed severe coronary atheromatous disease with chronic stenosis >80% of the lumen of the three main coronary arteries (right, anterior descending and circumflex) and recent secondary acute thrombosis on the anterior descending coronary; a posterior branch of the circumflex coronary was rechannelled (Figure 5.1.1.2). In the myocardium, scar fibrosis of the lateral wall of the left ventricle was confirmed as well as other smaller scars on the posterior wall of the left ventricle near the cardiac tip. No signs of acute myocardial infarction were identified in any area of the myocardium.

There were no significant findings in the brain and no CPR-associated lesions in the lung.

Conclusion

Significant acute and chronic ischaemic heart disease was the cause of death.

Comments

Coronary atheromatous disease is the most frequent cause of sudden death in all circumstances (as previously mentioned) and particularly associated with sport, as in this case. The increased myocardial oxygen demand associated with physical exercise could not be met on account of the significant reduction in the lumen of the main coronary arteries (one with added acute thrombosis), which induced ventricular fibrillation due to ischaemia (lack of blood supply). Myocardial scarring from previous infarctions, which may have gone unnoticed during life, may also lead to a higher likelihood of ventricular arrhythmia and sudden death.

In forensic practice, the majority of sudden deaths associated with sport occur in recreational, non-professional athletes (in Spain, mainly associated with cycling and football), meaning having regular heart checks is recommended to detect diseases that make it advisable to reduce the intensity or stop participating in the sport.

Figure 5.1.1.1. A) External view of the anterior face of the heart.

B) Biventricular cross-section

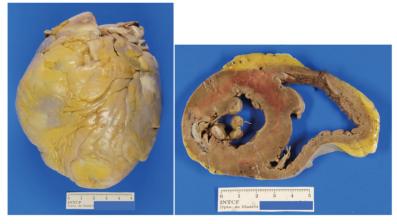
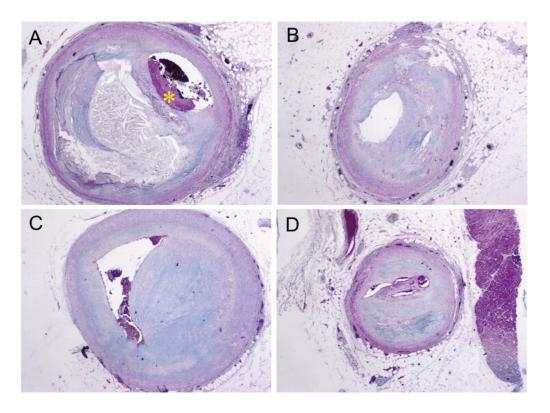


Figure 5.1.1.2. A) Anterior descending coronary artery with thrombosis*.

B) Right coronary. C) Circumflex coronary artery. D) Posterior branch of the circumflex coronary artery



5.1.2 Teaching and scientific activity

5.1.2.1. Contribution in scientific congresses

Suárez Mier MP, Molina Aguilar P, Lucena Romero J, Moyano Corvillo S, Morentin Campillo B, Zorio Grima E, Rueda Soriano J. Risk profile of coronary anomalies originating in the opposite side. 6th National Congress of the Spanish Society of Forensic Pathology (SEPAF), 30th National Congress of the SEAP and 25th National Congress of the SEC. Online, 26-28 May 2021.

Molina Aguilar P, Suárez Mier MP, Lucena Romero J, Moyano Corvillo S, Morentin Campillo B, Rueda Soriano J, Zorio Grima E. Multicentre analysis of coronary anomalies as a cause of sudden death. 6th National Congress of the Spanish Society of Forensic Pathology (SEPAF), 30th National Congress of the SEAP and 25th National Congress of the SEC. Online, 26-28 May 2021.

Chaves Portela S, López García P, Monedero Lázaro P, Costas Costas E, Suárez Mier MP. Paediatric death resulting from accidental ingestion of a button cell battery. Presentation of two cases. 6th National Congress of the Spanish Society of Forensic Pathology (SEPAF),

30th National Congress of the SEAP and 25th National Congress of the SEC. Online, 26-28 May 2021.

García Pérez JL, Suárez Mier MP, Argente del Castillo T, Prieto JL. Accidental embolism in the paediatric age group due to an error in the route of administration. 6th National Congress of the Spanish Society of Forensic Pathology (SEPAF), 30th National Congress of the SEAP and 25th National Congress of the SEC. Online, 26-28 May 2021.

5.1.2.2. Contribution at scientific congresses and meetings

García Pérez, JL. 7th Virtual Meeting of Inherited Cardiopathies and Cardiovascular Genetics Section. Spanish Society of Cardiology. Online course, 4-6 February 2021.

Chaves Portela S, García Pérez JL, López García PL, Sánchez de León Robles, Suárez Mier MP. 6th National Congress of SEPAF (Spanish Society of Forensic Pathology), 30th Congress of SEAP (Spanish Society of Pathological Anatomy)-IAP and 25th Congress of the SEC. Online, 26-28 May 2021.

Martín Gómez M. 10th Cardiogenetic Workshop. Online, 26 November 2021. 12 teaching hours.

García Pérez JL. "Joint ESGFOR-SEPAF seminar. Integrated diagnosis in COVID-19 deaths: microbiology and forensic pathology". Online, 14 December 2021.

5.1.2.3. Scientific publications

Morentin B, Suárez-Mier MP, Monzó A, Ballesteros J, Molina P, Lucena J. Sudden death related to sporting activity in Spain. Multicentre population-based forensic study of 288 cases. *Rev Esp Cardiol*. 2021;74(3):225-32. https://doi.org/10.1016/j.recesp.2020.05.035

Piqueras-Flores J, Delgado-Arenas MJ, Pulgar-Bautista ME, Suárez-Mier MP, Pérez-Díaz P, Antequera-Recio R. Diagnosis of sudden death: collaboration between the Inherited Cardiopathies Unit and the Institute of Legal Medicine. *REC CardioClinics*. 2021. https://doi.org/10.1016/j.rccl.2021.08.001

5.1.2.4. Education activities

Suarez Mier MP. Associate Professor. University of Alcalá. Faculty of Sciences. Degree in Forensic Sciences: Forensic Technologies and Sciences, 2nd semester: Histopathology. 2020/2021 Academic year.

López García PL. Honorary of work placement lecturer. University of Alcalá. Faculty of Sciences. Degree in Forensic Sciences: Forensic Technologies and Sciences, 2nd semester: Histopathology. 2020/2021 Academic year.

García Pérez JL. Collaborating lecturer. University of Alcalá. Faculty of Sciences. Degree in Forensic Sciences: Forensic Technologies and Sciences, 2nd semester: Histopathology. 2020/2021 Academic year.

Suárez Mier MP. "Utility of Forensic Histopathology in violent and suspected criminal deaths". University Master's Degree in Police Sciences. 24 November 2021. University of Alcalá.

Suárez Mier MP. "Update on sudden cardiac death. *Post-mortem* diagnosis of structural heart disease". Organised by the Asturian Public Administration Institute Adolfo Posada. 4 hours. 17-18 March 2021.

García Pérez JL. Work placement tutor on the selective course for the 30th Cycle of Forensic Doctors (CEJ). Lecture: Fundamental aspects of sudden cardiac death. September 2021.

López García PL. Work placement tutor on the selective course for the 30th Cycle of Forensic Doctors. Lecture: Forensic Neuropathology. September 2021.

Chaves Portela S. Work placement tutor on the selective course for the 30th Cycle of Forensic Doctor (CEJ). Lecture: Forensic Paediatric Pathology. September 2021.

Sánchez de León Robles MS. Work placement tutor on the selective course for the 30th Cycle of Forensic Doctor (CEJ). Lectures: Vitality and Asphyxiation. September 2021.

Sánchez de León Robles MS. "Contributions of hystopathological studies in homicides", in the course "Methodology in homicide autopsies. Multidisciplinary approach". Organised by the Valencian Institute of Public Administrations. Course director: Dr. Paloma Hevia. Valencia, 22-24 September 2021.

Suárez Mier MP. Director of the "Practical course on forensic neuropathology". Continuous Training Plan for forensic doctors and INTCF practitioners at the CEJ. Online course, 2-5 November 2021. 10 teaching hours.

Suárez Mier MP. "Reassessment of the diagnosis of myocardial infarction during autopsy in the light of the current clinical classification", on the course "Clinicopathological approach to sudden cardiac death. From the autopsy room to the gene". Led by Joaquín Lucena Romero. Continuous training plan for INTCF forensic doctors and practitioners at the Centre for Legal Studies, organised online on 29 November 2021.

5.1.2.5. Attendance to training activities

Chaves Portela S, García Pérez JL. López García PL, Sánchez de León Robles MS, Suárez Mier MP. Continuous Training Plan 2021. CEJ. "Interpretation of expert opinions issued by the National Institute of Toxicology and Forensic Sciences". Online course, 19-27 April 2021. 10 teaching hours.

Chaves Portela S, Suárez Mier MP. Continuous Training Plan 2021. CEJ. "Basic LIMS: structure, data organisation and queries". Online course, 17-24 May 2021.

Chaves Portela S, García Pérez JL, López García PL, Martín Gómez M, Muñoz Jiménez MT, Sánchez de León Robles MS, Suárez Mier MP. Continuous Training Plan for forensic doctors and INTCF practitioners at the CEJ. "Clinicopathological approach to sudden cardiac death. From the autopsy room to the gene". Online course, 29-30 November 2021.

García Pérez JL. Continuous Training Plan for forensic doctors and INTCF practitioners at the CEJ. "Forensic toxicology. Toxic pathology". Online course, 7-14 June 2021. 10 teaching hours.

Chaves Portela S, García Pérez JL, López García PL. Martín Gomez M, Muñoz Jiménez MT, Sánchez de León Robles MS. Continuous Training Plan for forensic doctors and INTCF practitioners at the CEJ. "Practical course on forensic neuropathology". Online course, 2-5 November 2021. 10 teaching hours.

Chaves Portela S, Martín Gómez M, Muñoz Jiménez MT, Suárez Mier MP. "Practical workshop on the dissemination of the INTCF quality system", from 21 to 28 September 2021. 10 teaching hours. Online.

5.2. Barcelona Department Histopathology Service

In 2021, the Barcelona Histopathology Service experienced two specific developments: the first was the improvement in terms of the COVID pandemic, as over the course of the year, the vaccination programme was rolled out to the population and, as a result, the progressive incorporation of the Histopathology Service in person, maintaining remote working days that were very fruitful and that kept annual productivity at the required values. Secondly, the recruitment of two new pathologists to the team was a major success in overcoming and tackling a historic pendency time. The general casework of the service, in terms of the type of cases for which histopathological studies have been requested, is reflected in Figure 5.2.1 and Table 5.2.1.

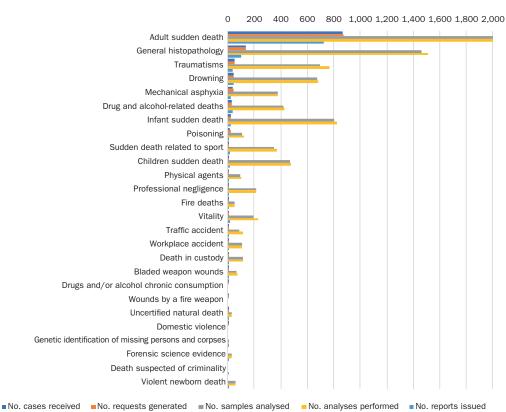


Figure 5.2.1. Casework of the Barcelona Department Histopathology Service during 2021 by type of report

Table 5.2.1. Casework of the Barcelona Department Histopathology Service during 2021 by type of report

Type of report	No. cases received	No. requests generated	No. samples analysed	No. analyses performed	No. reports issued
Adult sudden death	870	875	12,710	13,144	725
General histopathology	135	136	1,465	1,515	99
Traumatisms	50	50	698	764	38
Drowning	44	44	673	679	44
Mechanical asphyxia	40	41	376	378	21
Drug and alcohol-related deaths	32	32	423	428	37
Infant sudden death	22	23	806	826	24
Poisoning	19	20	111	119	9
Sudden death related to sport	11	11	350	367	14
Children sudden death	11	11	469	479	13
Physical agents	9	9	95	102	5
Professional negligence	8	8	216	216	11
Fire deaths	7	7	49	49	4
Vitality	7	9	195	230	13
Traffic accident	6	6	88	112	11
Workplace accident	5	5	111	110	7
Death in custody	5	5	112	112	2
Bladed weapon wounds	4	4	64	70	4
Drugs and/or alcohol chronic consumption	2	2	0	0	0
Wounds by a fire weapon	1	1	0	0	0
Uncertified natural death	1	1	31	31	1
Domestic violence	1	1	0	0	0
Genetic identification of missing persons and corpses	0	0	8	8	1
Forensic science evidence	0	0	33	33	1
Death suspected of criminality	0	0	0	0	1
Violent newborn death	0	0	59	59	2
TOTAL	1,286	1,301	19,142	19,831	1,087

In 2021, the Barcelona Histopathology Service issued 1,087 reports, compared to 1,072 in 2020. In terms of expert activity, the number of cases registered this year totalled 1,286, with 1,301 requests generated, 19,831 analyses carried out and 19,142 samples analysed. This can be attributed to the increase in the number of full autopsies performed in 2021. Suspected and correctly documented cases of COVID referred to the service have been studied. In turn, there has been an increase in the number of cases related to post-vaccination states (anti SARS-CoV-2) for assessment and determination of the

coadjuvant effect that the vaccine may have generated and its contribution to the cause of death.

As always, the most significant number of cases has been made up of those involving sudden death (68%) and/or those being "suspected of criminality" and therefore subject to a court ordered autopsy, which ultimately show a pathology or an injury that justifies a natural death. These include cardiovascular deaths with structural pathology and to a lesser extent those attributed to a genetic alteration (structurally normal heart). The 11 cases of sudden death associated with sport represent 0.8% of the cases received in 2021.

However, there is a group of cases of an uncertain nature, which, for various reasons, including a shortage of samples received, lack of information or presence of complex diseases, had to be generically categorised as "general histopathological study" (10.5% of cases).

Among the causes of death ultimately classified as violent, there is small variation concerning the last years highlighting death in the context of drug dependence (2.5%), various intoxications, especially by alcohol and medicines. These are frequently connected to suicide cases and psychiatric pathology. Drowning (3.42%), different types of mechanical asphyxia (3.11%) and trauma (3.81%), especially head injuries, account for a relevant proportion of violent death. Sudden infant death (22 cases) and sudden child death (11 cases) together account for 2.56% of the cases received. The study of the vitality of injuries (0.54%) and the dating and affiliation of these (chronohistotanatology) have remained stable when compared to 2020.

Furthermore, routine training activities have continued, working on continuous training with two annual CAP (College of American Pathology, USA) intercomparison exercises in forensic pathology and multidisciplinary collaborations, especially in the field of inherited heart disease. In 2021, the full extension and implementation of the LIMS system and the development of digital expert reports in the Barcelona Histopathology Service was completed. In recent years, a higher degree of difficulty has been noted in the diagnostic complexity of expert reports, due to advances in clinical medicine, accompanied by a simultaneous advance in forensic pathology, which have reached a level of importance in the social sphere.

5.2.1. Forensic case of interest: Violent death by suicide involving a mixed mechanism

Circumstances of the death

63-year-old man found dead in his home in a pool of blood, with a bottle of hydrochloric acid and a glass with traces of liquid next to the bedside table, with two knives in the living room and with multiple cuts on his left forearm and abdomen.

Records

Major depression since the death of his seventeen-year-old son, attempted overdose ten years prior, informing neighbours of the intent to self-harm, pending eviction, with debts, separation from partner and alcoholism problems.

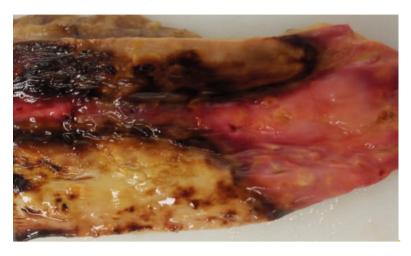
Autopsy findings

Two superficial skin lesions were seen in the abdomen, 8 deep incised wounds on the left forearm with peripheral hyperemia and necrosis in the subcutaneous and muscular cellular tissue, superficial wounds on the palm of the left hand and on the second finger of the left hand (Figure 5.2.1.1), and caustic lesions on the tongue, oesophagus, trachea, bronchi, mediastinum, aortic intima (Figure 5.2.1.2), stomach, diaphragm and peritoneum, with multiple black clots similar to "petroleum" appearance.

Figure 5.2.1.1. Deep wounds and hydrochloric acid lesions on left forearm (courtesy of Dr. Subirana, IMLCFC).



Figure 5.2.1.2. Hydrochloric acid lesions in the aortic wall (courtesy of Dr. Subirana, IMLCFC).



The possible cause of death was determined to be secondary to generalised injuries, in the context of hydrochloric acid ingestion and stab wounds to the forearm, hand and fingers. He most probably started with the superficial abdominal lesions, then the cuts to the left forearm and hands before finally ingesting the hydrochloric acid, as the lesions on the forearm show macroscopic signs compatible with caustic necrosis due to circulation of blood to the cuts during the perimortem period.

Histopathological findings: three fragments of skin from the left forearm and two fragments of the aorta were submitted in paraffin blocks.

Microscopically, on the left forearm there was focal subepidermal detachment, areas with thinning of the skin thickness due to transmural tissue coagulation necrosis, associated with focal haemorrhage in the subcutaneous cellular tissue and occasionally on the epidermal surface (Figure 5.2.1.3), in addition to an acute inflammatory infiltrate predominantly in the subcutaneous cellular tissue, also visible in the dermis with perivascular distribution, and more discreetly in the epidermis and papillary dermis (Figure 5.2.1.4).

The aortic wall contained uncomplicated atherosclerotic plaque and areas of thinning wall thickness at the expense of coagulation necrosis (Figure 5.2.1.5).

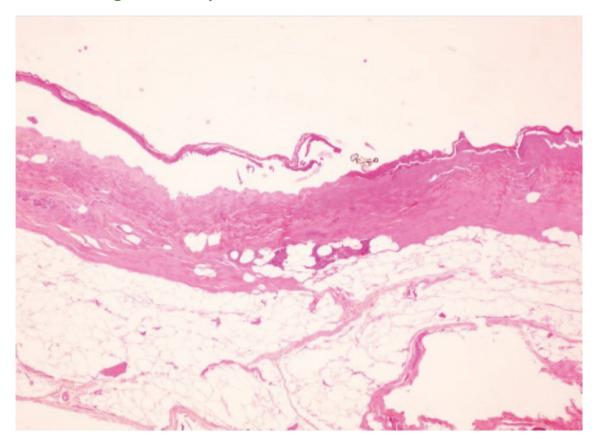


Figure 5.2.1.3. Hydrochloric acid lesion on skin of left forearm

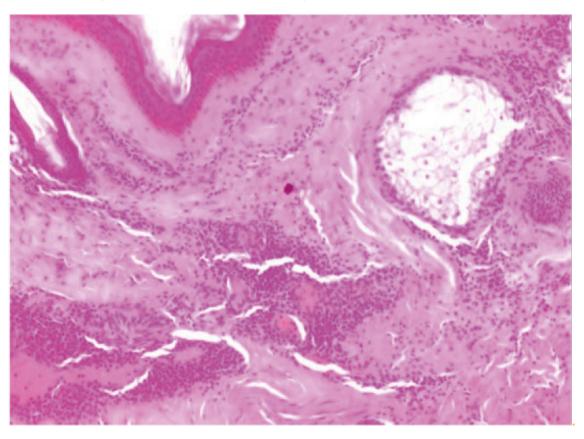


Figure 5.2.1.4. Acute inflammatory infiltrate on left forearm skin

Figure 5.2.1.5. Hydrochloric acid lesion on aortic wall



Conclusion

Skin fragments from left forearm and aortic fragments with morphological changes compatible with caustic lesions secondary to ingestion of hydrochloric acid.

Skin fragments from the left forearm, also with transmural wounds of a few hours' old.

5.2.2 Teaching and scientific activity

5.2.2.1. Contribution in scientific congresses

Multidisciplinary study in the MUSIB study of "sudden death in the Balearic Islands". Three sessions of case closures by videoconference. Collaboration in publications. Collaboration agreement between the Department of Justice, the Health Service of the Balearic Islands and the Cardiology Service of the Son Llàtzer Hospital (Mallorca), signed on 13 March 2018.

Molina Aguilar P, Suárez Mier MP, Lucena Romero J, Moyano Corvillo S, Morentin Campillo B, Rueda Soriano J, Zorio Grima E. "Multicentre analysis of coronary anomalies as a cause of sudden death" (oral communication). 30th National Congress of the SEAP-IAP, 25th Congress of the SEC and 6th Congress of the SEPAF. 26-28 November 2021.

Suárez Mier MP, Molina Aguilar P, Lucena Romero J, Moyano Corvillo S, Morentin Campillo B, Zorio Grima E, Rueda Soriano J. "Risk profile of coronary anomalies originating in the opposite side" (oral communication). 30th National Congress of the SEAP-IAP, 25th Congress of the SEC and 6th Congress of the SEPAF. 26-28 November 2021.

5.2.2.2. Scientific publications

Iglesias M, Ripoll-Vera T, Pérez-Luengo C, García AB, Moyano S, Canos JC, Borondo JC, Alvarez J, Heine-Suñer D, Barcelo B. Diagnostic Yield of Genetic Testing in Sudden Cardiac Death with Autopsy Findings of Uncertain Significance. *J Clin Med.* 2021 Apr 21;10(9):1806. https://pubmed.ncbi.nlm.nih.gov/33919104/

Muñoz-Quirós JM, Mira E, Moyano S, Abad R, García E, Fernández-Rodríguez A. Multidisciplinary medico-legal investigation of death due to SARS-CoV-2 (COVID-19): review of the literature in relation to a case. *Gac. Int. Cienc. Forense*. 2021 oct-dic:41:6-16.

5.2.2.3. Education activities

Borondo Alcázar JC. Lecturer on the "Adaptation Course to the Higher Level for Pathological Anatomy Technicians" held in Valladolid from 27/09/2021 to 1/10/2021 (Directorate General for the Public Justice Service).

Borondo Alcázar JC. Lecturer at the SEPAF congress (20 April 2021, lecture recording): "Vital reactions. From theory to reality".

Canós Villena JC. Lecturer at the CEJ course "Interpretation of expert opinions in the field of forensic medicine. The histopathological expert report: meaning and interpretation of medical terminology". From 19/4/2021 to 27/4/2021.

Moyano Corvillo S. Official tutor to Carmen Alborch Gil during her initial training period (August-November 2021).

Borondo JC. Official tutor to Lorena Díaz Sánchez.

Canós Villena JC. Occasional tutor at newly acquired faculties.

Castro Pons J. Tutor in the collaboration agreement with IES Guineueta (Barcelona), with a student in training (416 h).

Borondo Alcázar JC. Advisory board of the *Revista Española de Medicina Legal* and the AMLC's legal journal.

Canós Villena JC, Moyano Corvillo, S. Coordination/continued training in "Forensic Pathology" with the College of American Pathology (CAP).

Canós Villena JC. Occasional reviewer of the *Revista Española de Medicina Legal* (Forensic Histopathology).

5.2.2.4. Attendance to training activities

Special Corps of Histopathology Professionals: undertaking of two intercomparison exercises (half-yearly) in forensic pathology (continuing education) through the College of American Pathology (CAP).

Moyano Corvillo S, Ladino Orjuela D. "7th Virtual Meeting of Inherited Cardiopathies and Cardiovascular Genetics Section of the Spanish Society of Cardiology". 4-6 February 2021.

Borondo Alcázar JC, Moyano Corvillo S, Ladino Orjuela D. "2nd National Multidisciplinary Congress on COVID-19" (online). 12-16 April 2021.

Moyano Corvillo S, Ladino Orjuela D, Díaz Sánchez L. "30th National Congress of the SEAP-IAP, 25th Congress of the SEC and 6th Congress of the SEPAF" (online). Organised by the Spanish Society of Pathological Anatomy. 26-28 November 2021.

Moyano Corvillo S, Ladino Orjuela D. "Forensic Toxicology. Toxic pathology" (online). Organised by the Centre for Legal Studies. 7-14 June 2021.

Moyano Corvillo S, Díaz Sánchez L. "Practical course on forensic neuropathology" (online). Organised by the Centre for Legal Studies. 2-5 November 2021.

Ladino Orjuela D, Díaz Sánchez L. Update on the expert assessment of sexual violence (2021). From 11/11/2021 to 18/11/2021 (online, 10 hours).

Ladino Orjuela D. New research tools in the field of Forensic Genetics. 21-28/06/2021 (online, 10 hours).

Ladino Orjuela D. Interpretation of expert opinions issued by the National Institute of Toxicology and Forensic Sciences. CEJ course. From 19/04/2021 to 27/04/2021 (online, 10 hours).

Ladino Orjuela, D. Treatment of offences against sexual freedom and integrity in the forensic laboratory. From 14/06/2021 to 21/06/2021 (online, 10 hours).

Ladino Orjuela D. Basic LIMS: structure, data organisation and queries. From 17/05/2021 to 24/05/201 (online, 10 hours).

Ladino Orjuela D. Interpretation of toxicological results and their influence on the expert context in which the analysis is requested. From 10/05/2021 to 17/05/2021 (online, 10 hours).

Díez Espinar R, Muñoz Montoya M, Rodríguez Izquierdo L. "29th Histopathology Technique Workshops. Bonanova Institute" (18 and 19 May 2021; 7.5 hours).

Díez Espinar R, Muñoz Montoya M, Rodríguez Izquierdo L, Chávez Calderón J, Espinosa Gutiérrez Ana. "Multidisciplinary course on drugs: review of toxicity, updating of analytical methods, judicial and social value of analytical expertise". INTCF Edition, Barcelona Dept. (from 4 to 12 November 2021; 15 hours).

Díez Espinar R, Muñoz Montoya M, Rodríguez Izquierdo L, Castro i Pons J, Chávez Calderón J, Espinosa Gutiérrez A. "Quality assurance in the INTCF, quality manuals, standard operating procedures, concepts and definitions". INTCF Edition, Barcelona Dept. (from 23 November to 1 December 2021; 15 hours).

Díez Espinar R, Muñoz Montoya M, Rodríguez Izquierdo L, Castro i Pons J, Chávez Calderón J, Espinosa Gutiérrez A. "Quality. Study of the UNE-EN ISO/IEC 17025:2017 standard. General requirements for the competence of testing and calibration laboratories". INTCF Edition. Barcelona Dept. (15 hours).

Rodríguez Izquierdo L. "Online workshop on basic cardiac dissection" (25 May to 4 June 2021; 6 hours). Galician Association of Forensic Doctors.

Castro i Pons J. Tutoring of trainees in advanced vocational training in pathological anatomy.) A student from IES Guineueta (Barcelona), 416 hrs.

Muñoz Montoya M. "Health staff against gender-based violence". Logoss (13 May-12 July 2021).

Muñoz Montoya M, "Gynecological laboratory tests and body fluids for the senior technician in the clinical laboratory". Logoss (2 May-2 July 2021).

Muñoz Montoya M, "Biosafety and occupational risk prevention for health personnel". Logoss (23 April-22 June 2021).

Muñoz Montoya M. "Urine and microbiological analysis in the emergency laboratory for senior clinical laboratory technicians". Logoss (25 May-27 July 2021).

Rodríguez Izquierdo L. "TAVI in low-risk patients". Webinar (6 May 2021).

Rodríguez Izquierdo L, "Angioplasma of the lower limbs". Webinar (12 May 2021).

Díez Espinar R. "Good weighing practices". GWP Verification (Mettler-Toledo).

5.3. Seville Department Histopathology Service

Concerning the expert activity of the Seville Department Histopathology Service, in 2021, a total of 1,957 requests were received with 40,947 samples analysed through a total of 41,637 analyses, emitting a total of 1,537 expert reports.

As can be seen in Figure 5.3.1, the largest volume of requests for analysis corresponds to the investigation of cases of sudden adult death (684 requests), followed by general histopathological studies (293 requests), the study of deaths related to alcohol and drug abuse (219 requests), the study of general mechanical asphyxia (206 requests), the study of trauma (134 requests), histopathological studies in deaths caused by toxic substances (121 requests), drowning studies (86 requests) and road traffic accidents (32 requests).

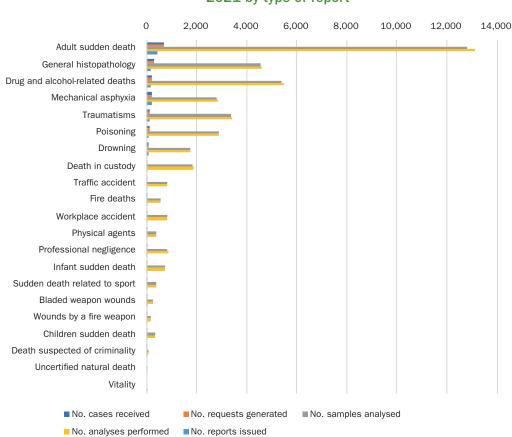


Figure 5.3.1. Casework of the Service of the Seville Department during 2021 by type of report

Table 5.3.1. Casework of the Service of the Seville Department during 2021 by type of report

Type of report	No. cases received	No. requests generated	No. samples analysed	No. analyses performed	No. reports issued
Adult sudden death	679	684	12,799	13,133	453
General histopathology	293	293	4,559	4,626	163
Drug and alcohol-related deaths	220	219	5,401	5,473	179
Mechanical asphyxia	204	206	2,827	2,864	196
Traumatisms	127	134	3,394	3,437	128
Poisoning	121	121	2,881	2,890	101
Drowning	86	86	1,756	1,756	75
Death in custody	53	53	1,823	1,895	53
Traffic accident	32	32	844	844	39
Fire deaths	28	28	574	575	19
Workplace accident	21	21	832	843	28
Physical agents	16	16	389	400	13
Professional negligence	16	17	841	858	24
Infant sudden death	13	13	749	759	19
Sudden death related to sport	10	10	397	397	12
Bladed weapon wounds	9	9	243	249	10
Wounds by a fire weapon	7	8	177	177	9
Children sudden death	5	5	361	361	12
Death suspected of criminality	2	2	90	90	3
Uncertified natural death	0	0	10	10	0
Vitality	0	0	0	0	1
TOTAL	1,942	1,957	40,947	41,637	1,537

5.3.1. Interesting forensic case

Circumstances of the death

A 39-year-old woman with three children and no known personal history died suddenly.

Medicoforensic autopsy findings

No relevant macroscopic data.

Histopathological study

The heart weighed 335 g and the coronary arteries were free from atherosclerotic lesions. Microscopic examination revealed a dissection in the wall of the anterior descending coronary artery resulting in occlusion of the vascular lumen. Furthermore, there was an inflammatory infiltrate in the artery wall consisting predominantly of eosinophils.

Histopathological diagnosis

Spontaneous dissection of the anterior descending coronary artery.

Comments

Spontaneous dissection of the anterior descending coronary artery is a pathology that occurs predominantly in women between 30 and 45 years of age and the bleeding is usually located in the outer third of the arterial wall (Figures 5.3.1.1 and 5.3.1.2). In many cases, the eosinophilic inflammatory infiltrate shown in the picture (Figure 5.3.1.3) is also present, although its origin and role in the disease has not been ascertained, and in other cases is not present at all. The origin of the haemorrhage that dissects the artery wall appears to be in the vessels that supply the artery wall itself, rather than in ruptures of the tunica intima, as occurs in dissecting haematomas of the aorta.

A relationship has been described between this pathology and pregnancy and peripartum, extending up to months after delivery. This disease is less rare than it may appear, and when the coronary arteries are examined by cross-sectioning, it can be mistaken for acute occlusive thrombosis during macroscopic examination.

Figure. 5.3.1.1. This image shows the dissection of the coronary artery wall, with extensive haemorrhaging, in the outer third of the coronary artery, resulting in occlusion of the vascular lumen.

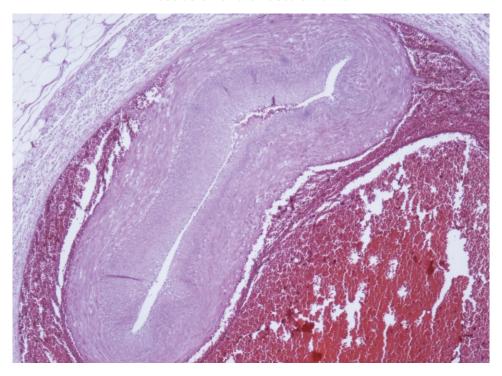


Figure 5.3.1.2. When magnifying further, we can see the dissection of the arterial wall, acute haemorrhaging and inflammatory infiltrate.

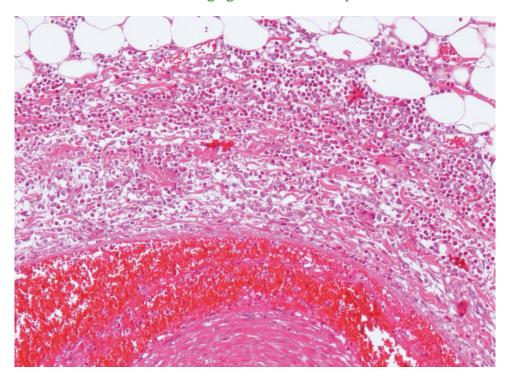
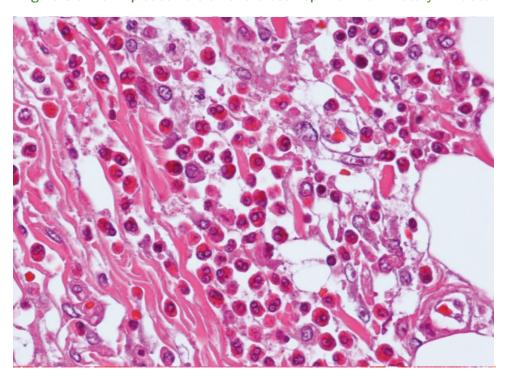


Figure 5.3.1.3. Exploded version of the eosinophilic inflammatory infiltrate



5.3.2 Teaching and scientific activity

5.3.2.1. Education activities

Martínez de Mandojana Pérez AM, Mateo Vico OM, Moro Cárdenas MC, Ronquillo Rubio A. As tutors during the internship period undertaken at the INTCF Seville Department by the forensic doctors in training, between 27 September and 8 October 2021.

Ronquillo Rubio A. As part of the training activity "Forensic Toxicology. Toxic pathology" aimed at INTCF doctors, INTCF doctors on secondment and national police officers undertaking the activity "Pulmonary pathology caused by toxic substances". This training activity was organised by the Centre for Legal Studies as part of the Continuous Training Plan and took place from 07/06/2021 to 14/06/2021 online.

5.3.2.2. Attendance to training activities

Martínez de Mandojana Pérez AM, Mateo Vico OM, Moro Cárdenas MC, Ronquillo Rubio A. "The INTCF forensic sciences service: fields of action, analytical possibilities", lasting 10 hours, from 22 to 26 March 2021, by the Centre for Legal Studies, Ministry of Justice.

Martínez de Mandojana Pérez AM, Mateo Vico OM, Moro Cárdenas MC, Ronquillo Rubio A. "Interpretation of expert opinions issued by the National Institute of Toxicology and Forensic Sciences", lasting 10 hours, from 19 to 27 April 2021, by the Centre for Legal Studies, Ministry of Justice.

Martínez de Mandojana Pérez AM, Mateo Vico OM, Moro Cárdenas MC, Ronquillo Rubio A. "Interpretation of toxicological results and their influence on the expert context in which the analysis is requested", online, lasting 10 hours, from 10 to 17 May 2021, by the Centre for Legal Studies, Ministry of Justice.

Martínez de Mandojana Pérez AM, Mateo Vico OM, Moro Cárdenas MC, Ronquillo Rubio A. "LIMS basic: structure, data organisation and queries", online, lasting 10 hours, from 17 to 24 May 2021, by the Centre for Legal Studies, Ministry of Justice.

Moro Cárdenas MC. Online technical seminar "Safe work during the COVID 19 pandemic: international specification UNE-ISO/PAS 45005", held on 26 May 2021, lasting 2 hours, by the Regional Ministry of Employment, Training, Self-Employment and the Regional Ministry of Economic Transformation, Industry, Knowledge and Universities.

Martínez de Mandojana Pérez AM, Mateo Vico OM, Moro Cárdenas MC, Ronquillo Rubio A. Forensic Toxicology. "Toxic pathology", lasting 10 hours, online, from 7 to 14 June 2021, by the Centre for Legal Studies, Ministry of Justice.

Martínez de Mandojana Pérez AM, Mateo Vico OM, Moro Cárdenas MC, Ronquillo Rubio A. "Treatment of offences against sexual freedom and integrity in the forensic laboratory", lasting 10 hours, online, from 14 to 21 June 2021, by the Centre for Legal Studies, Ministry of Justice.

Martínez de Mandojana Pérez AM, Mateo Vico OM, Moro Cárdenas MC, Ronquillo Rubio A. Practical course in forensic neuropathology, with 10 teaching hours, online, from 2 to 4 November 2021, by the Centre fore Legal Studies, Ministry of Justice.

Martínez de Mandojana Pérez AM, Mateo Vico OM, Moro Cárdenas MC, Ronquillo Rubio A. "Quality assurance in the forensic process. A step forward", lasting 8 hours, online, from 2 to 05 November 2021, by the Centre for Legal Studies, Ministry of Justice.

Moro Cárdenas MC. "Health surveillance in the public administration", lasting 20 hours, online, from 9 to 29 November 2021, by Comisiones Obreras.

Martínez de Mandojana Pérez AM, Mateo Vico OM, Moro Cárdenas MC, Ronquillo Rubio A. Territorial meeting of SEAP Andalucía, Ceuta and Melilla, held on 26 November 2021, online.

Martínez de Mandojana Pérez AM, Mateo Vico OM, Miguel Salas MT, Moro Cárdenas MC, Ronquillo Rubio A. "Clinicopathological approach to sudden cardiac death. From the autopsy room to the gene", lasting 10 hours, remote format, from 29 November to 3 December 2021, by the Centre for Legal Studies, Ministry of Justice.

Larrondo Espinosa FJ, Martínez de Mandojana Pérez AM, Mateo Vico OM, Miguel Salas MT, Moro Cárdenas MC, Ronquillo Rubio A. Joint ESGFOR-SEPAF seminar: "Integrated diagnosis in COVID-19 deaths: microbiology and forensic pathology", held on 14 December 2021, online.

Martínez de Mandojana Pérez AM, Mateo Vico OM, Ronquillo Rubio A. XLIV SEAP-IAP Annual Meeting. Soft tissue tumours. International refresher course in Digestive Pathology. Held on 4 and 5 February 2021. Online edition.

5.4. Histopathology Section at the La Laguna Delegation

Figure 5.4.1 and Table 5.4.1 reflect the Service's expert activity in 2021. The volume of requests received (414) has increased by 45% compared to 2020. There has also been a considerable increase in the number of samples registered, totalling 3,008, and samples analysed, totalling 16,058.

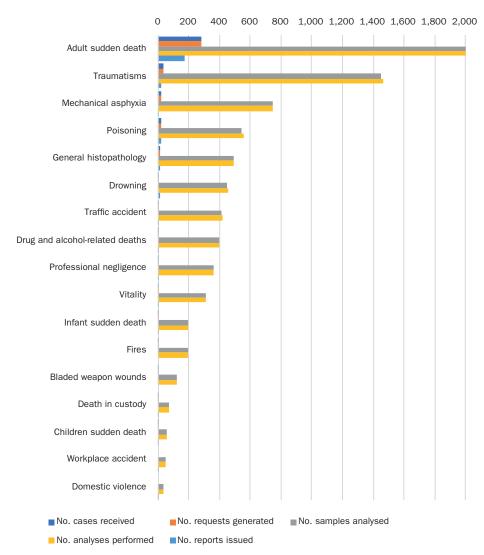


Figure 5.4.1. Casework of the La Laguna Delegation Histopathology Section during 2021 by type of report

Table 5.4.1. Casework of the La Laguna Delegation Histopathology Section during 2021 by type of report

Type of report	No. cases received	No. requests generated	No. samples analysed	No. analyses performed	No. reports issued
Adult sudden death	281	282	10,168	10,286	172
Traumatisms	38	38	1,453	1,464	25
Mechanical asphyxia	22	22	749	749	7
Poisoning	18	18	544	559	24
General histopathology	12	13	496	496	13
Drowning	8	8	446	453	13

Traffic accident	6	6	414	418	6
Drug and alcohol-related deaths	5	5	395	399	5
Professional negligence	5	6	361	361	8
Vitality	5	5	309	311	6
Infant sudden death	4	4	198	198	5
Fires	3	4	192	195	0
Bladed weapon wounds	1	1	121	121	0
Death in custody	1	1	69	69	1
Children sudden death	1	1	60	60	4
Workplace accident	0	0	49	49	2
Domestic violence	0	0	34	34	1
TOTAL	408	414	16,058	16,222	292

A total of 292 reports were issued in 2021, of which 181 (62%) were for sudden death studies, mainly in persons aged over 50 (63%) and in males (67%) (Figure 5.4.2). In 51% of the cases studied, the cause of death was cardiovascular, with ischaemic heart disease being the most frequent cause, followed by cardiac hypertrophy.

Among sudden cardiac deaths, inherited heart disease is worth particular mention, as it usually affects people under the age of 35. In these cases, a specialist cardiological examination of the next of kin is recommended, preferably at the Family Cardiopathy Unit that corresponds to the corresponding health area and a frozen blood sample is kept indefinitely. In 10 cases, the outcome of the histopathological studies suggested a hereditary cardiopathy and, at the request of the families and with the pertinent judicial authorisation, blood samples from 3 cases were sent to external laboratories specialising in genetic studies of inherited heart disease.

Nine sudden deaths were studied in persons aged 0-14 years, 5 sudden infant deaths, corresponding to those under one year of age, and 4 child deaths between the ages of 1 and 14. The gender was male in 55% of cases. In six cases, findings were found that explained the death while none were found in three cases. In these three cases, the possibility of arrhythmias in structurally normal hearts must be ruled out, where changes can be detected at a molecular level and would be included in the 10 cases indicated above, where blood was frozen and a specialised cardiological review was recommended for first-degree relatives.

The most frequent cause of pulmonary sudden death in adults was pulmonary thromboembolism, followed by infections, in many cases related to COVID-19.

In deaths classified as violent, the 29 studies that include deaths due to intoxication and those related to the consumption of drugs and alcohol are worth particular note. Seventy-two per cent of the cases were male and the age range was between 27 and 50 years old

(79%). The most frequent cause of death, as demonstrated in the toxicological analysis, was drug intoxication (48%) in relation to suicides and psychiatric pathologies, followed by adverse drug reaction (41%) and overdose (10%) in the context of drug dependence. In these cases, a histopathological study is decisive to rule out sudden death and to detect pathologies associated with the consumption of certain substances, such as alcohol or cocaine.

Trauma caused by accidental or homicidal falls and those related to occupational and traffic accidents accounted for 11% of the studies undertaken in 2021. The predominant gender was male (63%) and the age was over 50 (58%). In 36% of the studies, death was secondary to traumatic brain injury. In traffic accidents, occupational accidents and, occasionally, in relation to accidental falls, a histopathological study helps to determine the concurrence of any other previous pathologies, such as cardiac pathologies, which could be the cause of accidental traumatic death.

The studied deaths by drowning were 13. The predominant gender was male (85%) and ages ranged from 15 to 89, with a slight predominance of those over 50 (54%). In 46% of cases, a pathology was found, mainly cardiac, which could facilitate or act as a predisposing factor in this type of death (drowning).

42% of mechanical asphyxias were caused by suicide, with hanging being the most frequently used method. The aetiology of the remaining 58% was accidental. In terms of gender distribution, there is a clear predominance of males (71%) and, in 57% of cases, the age range was between 5 and 33 years.

The vitality studies of the injuries acquire relevance in violent homicidal deaths because they help to determine if they were produced alive or dead and to estimate, insofar as possible, the approximate date of the injuries to contribute to establishing a chronology. Seventy-one percent of these studies were conducted on males, with the age range being between 6 to 55. Injuries to the cervical block (skin, muscle and fractures of the laryngeal skeleton) in strangulations were mainly analysed.

Eight cases were studied, in which family members filed malpractice complaints. In 87% of these cases, the age range was between 56 and 89 years old.

The predominant gender was female (62%). The histopathological study was essential to determine the cause of death and to help the forensic doctor establish, together with the other studies carried out, whether or not there was a case of malpractice.

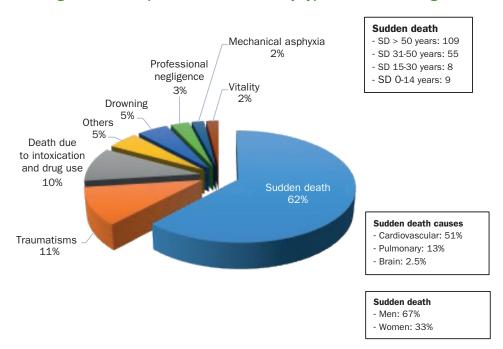


Figure 5.4.2. Reports issued in 2021 by type of death investigated

5.4.1 Forensic case of interest: Death at home during COVID-19 lockdown

Records

A 59-year-old male pensioner, who lived alone, smoked one packet of cigarettes a day for 20 years, obese, with type 2 diabetes mellitus, congestive heart failure secondary to severe chronic ischaemic heart disease, with an automatic defibrillator implant. He was admitted to the Cardiology Department between 17 and 22 December 2020 for heart failure and COVID-19 pneumonia, with his final PCR test being negative. On 30 December 2020, he returned to the hospital for three days, complaining of fever, general malaise, cough, phlegm and difficulty breathing. A physical examination revealed he had a body temperature of 39 °C and a baseline oxygen saturation of 97%. Auscultation revealed decreased vesicular murmur with bibasilar crackles. A chest X-ray showed findings compatible with heart failure, without the characteristic signs of COVID-19. The diagnosis was that of a stable patient who was clinically improving after treatment without the need for admission at the time. Discharge diagnosis: COVID-19 infection and congestive heart failure.

Circumstances of the death:

He was found dead by his family on 2 January 2021 in the bathroom. He was in quarantine, and his family, having noticed that he was not picking up the food left on his doorstep and was not answering the phone, decided to forcibly open the door.

Autopsy findings

White male, 176 cm in height, weighing 95 kg. Positive RT-PCR for SARS-CoV-2 in nasopharyngeal swab of the cadaver. An autopsy was performed in a room with biohazard protection level 3 and appropriate personal equipment. The heart weighed 750 g with signs of chronic ischaemic heart disease and secondary congestive heart failure. The right lung weighed 1,208 grams and the left lung weighed 1,106 grams; they were enlarged and had subpleural bullae. The brain weighed 1,245 g with no significant alterations.

Medicoforensic provisional conclusions

1) This was apparently a natural death (pending laboratory results). There were no signs of defence/fighting. 2) The root and immediate cause is pending laboratory results (COVID-19 positive multi-pathological death). 3) Samples were taken for toxicological, histopathological and biological analysis and sent to the Canary Islands Delegation of the National Institute of Toxicology and Forensic Sciences. Definitive conclusions cannot be made until the results of the additional studies requested are made available.

Histopathological study

Brain, cervical and thoracic blocks with both lungs, both kidneys, spleen, as well as fragments of liver and intestines were received. The most relevant macroscopic findings were severe ischaemic heart disease with dilatation of cavities and atrioventricular valve rings. The lungs were very heavy (2,314 grams overall) and show, when cut, increased consistency and hepatised section planes with bullae and subpleural hollows (Figure 5.4.1.1). Microscopic findings observed in the lung parenchyma were congestion, oedema and intra-alveolar haemorrhage, variable amount of interstitial and perivascular mononuclear inflammatory infiltrate, diffuse alveolar damage with desquamation of abundant type II pneumocytes with cytopathic changes and formation of thick hyaline membranes (Figure 5.4.1.2), strongly immunohistochemically positive for SARS-CoV-2 (Figure 5.4.1.3), as well as thrombi in medium and small vessels (Figure 5.4.1.4).

The **histopathological diagnoses** were: a) bilateral SARS-CoV-2 pneumonia with diffuse alveolar damage in the exudative phase, b) bilateral bullous pulmonary emphysema and c) severe chronic ischaemic heart disease with dilatation of cavities and atrioventricular valvular rings.

Conclusions

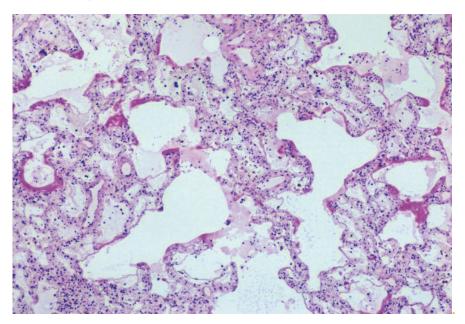
In this case, the histopathological study was essential to establish whether the pulmonary pathology was secondary to congestive heart failure, as established at the hospital consultation two days prior to death, or to bilateral SARS-CoV-2 pneumonia. The presence of the virus in the lung parenchyma could be demonstrated by the use of the anti-SARS-CoV-2 immunohistochemical technique (Nucleocapsid (N) Protein Antibody), and was confirmed by RT-PCR on paraffin sections. The latter technique was performed at the Microbiology Service of the INTCF in Madrid.

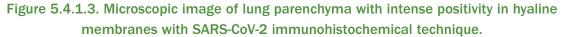
This case highlights the need to perform RT-PCR for SARS-CoV-2 on the cadaver, as well as the importance of a court ordered autopsy on deceased persons during the quarantine period to rule out possible malpractice.

Figure 5.4.1.1. Lungs increased in consistency in section with hepatic-looking section planes and subpleural bullae



Figure 5.4.1.2. Microscopic image of lung parenchyma with diffuse alveolar damage in the exudative phase. Haematoxylin-eosin stain.





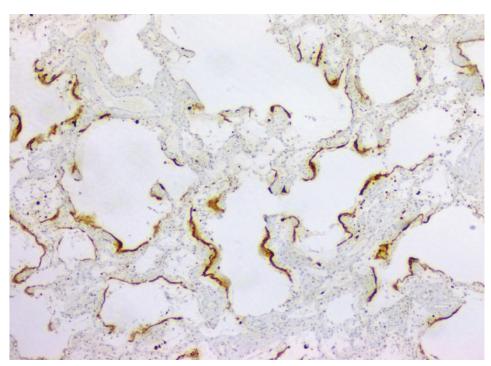
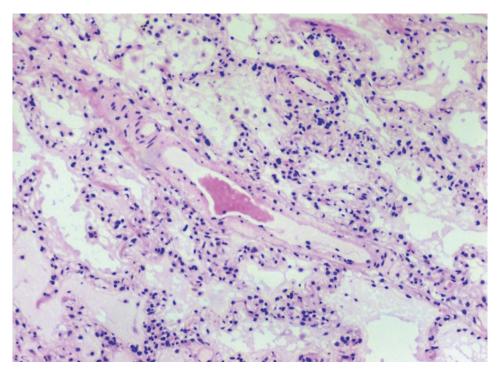


Figure 5.4.1.4. Microscopic image of lung parenchyma with interstitial mononuclear inflammatory infiltrate and fibrin thrombus in small vessel. Haematoxylin-eosin stain.



5.4.2. Teaching and scientific activity

5.4.2.1. Contribution in scientific congresses

Hernández Guerra AI, Quintero Quintero YC, González-Arnay E, Martín Olivera R, Naviera Arratia L. Pulmonary histopathological findings in 8 medical-legal autopsies positive for SARS-CoV-2. Oral communication. 6th National Congress of the Spanish Society of Forensic Pathology. Held in Santiago de Compostela from 26 to 28 May 2021.

Quintero Quintero YC, Hernández Guerra A I, Tapia-Chinchón, J. Disseminated cryptococcosis: medical-legal autopsy findings. Poster format. 6th National Congress of the Spanish Society of Forensic Pathology. Held in Santiago de Compostela from 26 to 28 May 2021.

5.4.2.2. Contribution at scientific congresses and meetings

Quintero Quintero YC. XLIV Annual Meeting of the Spanish Society of Anatomic Pathology, held online on 4 February 2021.

Hernández Guerra Al y Quintero Quintero Y. Virtual Meeting of Inherited Cardiopathies and Cardiovascular Genetics Section of the Spanish Society of Cardiology. Online, from 4 to 6 February 2021.

Hernández Guerra AI, Quintero Quintero YC. 6th National Congress of SEPAF (Spanish Society of Forensic Pathology), 30th Congress of SEAP (Spanish Society of Pathological Anatomy)-IAP and 25th Congress of the SEC. Online, 26-28 May 2021.

Hernández Guerra and Quintero Quintero YC. "10th Cardiogenetic Workshop". Hospital Clínico Universitario Virgen de la Arrixaca. CSUR-ERN Family Cardiopathies Unit. Online, 26 November 2021.

Hernández Guerra AI, Quintero Quintero YC. Joint ESGFOR-SEPAF seminar. Integrated diagnosis in deaths caused by COVID-19: Microbiology and Forensic Pathology. Held online on 14 December 2021.

5.4.2.3. Education activities

Hernández Guerra AI, Quintero Quintero YC. Training of anatomical pathology residents in foetal and autopsy pathology as part of the collaboration agreement between the Histopathology Section of the Canary Islands Delegation of the INTCF and the Anatomical Pathology Service at Complejo Hospitalario Universitario de Canarias.

Quintero Quintero YC. Arrhythmogenic cardiomyopathy. Presentation of medico-legal autopsy cases at the annual interdepartmental accredited course "Advances in Anatomical Pathology". Complejo Hospitalario Universitario de Canarias. Anatomical Pathology Service, 3 June 2021.

Hernández Guerra Al, Quintero Quintero YC. As tutors of the internship period undertaken at the INTCF-La Laguna Delegation by a forensic medical intern, between 13 and 17 September 2021.

Quintero Quintero YC. Non-inflammatory degenerative pathology of the aorta: nomenclature and diagnostic criteria. Presentation of autopsy cases at the annual interdepartmental accredited course "Advances in Anatomical Pathology". Complejo Hospitalario Universitario de Canarias. Anatomical Pathology Service, 21 October 2021.

Hernández Guerra AI. Histopathological lung findings in a series of medical-legal autopsies positive for SARS-COV2, at the annual interdepartmental accredited course "Advances in Anatomical Pathology". Annual interdepartmental accredited course. Complejo Hospitalario Universitario de Canarias. Anatomical Pathology Service, 27 October 2021.

Hernández Guerra AI. Practical approach to inherited heart disease. From the patient's bedside to the gene through the autopsy room (presentation of case studies), on the course "Clinicopathological approach to sudden cardiac death. From the autopsy room to the gene". Director: Joaquín Lucena Romero. Continuing Education Plan for 2021 at the Centre for Legal Studies, held online 29 and 30 November 2021.

Quintero Quintero YC. Macroscopic study of the encephalon, in the First Course on Adult Autopsy, organised by the Canarian Territorial Association of the Spanish Society of Pathological Anatomy, held online on 16 December 2021.

Hernández Guerra AI. Protocol for macroscopic study of the heart, in the First Course on Adult Autopsy, organised by the Canarian Territorial Association of the Spanish Society of Pathological Anatomy, held online on 16 December 2021..

5.4.2.4. Attendance to training activities

Quintero Quintero YC. Update on Digestive Pathology, organised by the Spanish Association of Pathological Anatomy, held online, 4 and 5 February 2021.

Quintero Quintero YC. Update in Forensic Chemistry and Toxicology. Continuing Education Plan for 2021 at the Centre for Legal Studies, held online between 8 and 16 March 2021.

Quintero Quintero YC. Update on Nephrology in its adaptation to the new paradigm of the SARS-COV-2 pandemic, endorsed by the Spanish Society of Nephrology, held online on 17 March 2021.

Hernández Guerra Al y Quintero Quintero Y. The INTCF Forensic Sciences service: fields of activity, analytical possibilities. Continuing Education Plan for 2021 at the Centre for Legal Studies, held online between 22 and 26 March 2021.

Hernández Guerra Al y Quintero Quintero Y. Interpretation of expert opinions issued by the National Institute of Toxicology and Forensic Sciences. Continuing Education Plan for 2021 at the Centre for Legal Studies, held online between 19 and 27 April 2021.

Quintero Quintero Y. How to write a scientific article. Dr. Antoni Esteve Foundation, online between 14 April to 26 May 2021.

Hernández Guerra AI y Quintero Quintero Y. Interpretation of toxicological results and their influence on the expert context in which the analysis is requested. Continuing Education Plan for 2021 at the Centre for Legal Studies, held online between 10 and 17 May 2021.

Hernández Guerra AI. Basic LIMS: structure, data organisation and queries. Continuing Education Plan for 2021 of the Centre for Legal Studies, held online 17-24 May 2021.

Quintero Quintero Y. Forensic Toxicology. Toxic pathology. Continuing Education Plan for 2021 at the Centre for Legal Studies, held online between 7 and 14 June 2021.

Hernández Guerra AI y Quintero Quintero Y. Practical course on Forensic Neuropathology. Continuing Education Plan for 2021 at the Centre for Legal Studies, held online between 2 and 04 November 2021.

Hernández Guerra AI. Forensic medicine and public health. Continuing Education Plan for 2021 of the Centre for Legal Studies, held online between 18 and 24 November.

Quintero Quintero YC. Clinicopathological approach to sudden cardiac death. From autopsy to the gene. Continuing Education Plan for 2021 at the Centre for Legal Studies, held online 29 and 30 November 2021.

5.4.2.5. Participation in investigation projects

Pathology associated with Covid-19 infection: results of autopsy series at the IMLCF of Santa Cruz de Tenerife. Raquel Martín-Olivera¹, Emilio González-Arnay², Yamilet C. Quintero-Quintero³, Ana I. Hernández-Guerra³, Jesús M. Vega-González⁴, Eduardo C. Salido-Ruiz^{4, 5}. Project approved by the Directorate General for Relations with the Justice Administration of the Government of the Canary Islands on 21 May 2020.

¹ Legal Medicine and Forensic Sciences of Santa Cruz de Tenerife.

² Applied Anatomy Laboratory. Anatomy, Histology, and Neuroscience Department. Universidad Autónoma de Madrid.

³ Histopathology Section. National Institute of Toxicology and Forensic Sciences. Canary Islands Delegation.

⁴ Pathological Anatomy Service. Hospital Universitario de Canarias.

⁵ Basic Medical Sciences Department. Universidad de La Laguna.



The Criminalistics Service is located in the Madrid Department, attending requests received from across Spain.

The required studies fall into the following areas:

- Study of injuries
- Study of traces
- Anthropological studies
- Forensic entomology study
- · Document and handwriting analysis

Distribution of staff in the Forensic Sciences Service during 2021.

Table 6.1: Madrid Department Forensic Sciences Service

	Forensic Sciences Service INTCF MADRID
Head of the Service	1
Facultatives	11
Specialist technicians	4
Laboratory assistants	3
Clerical staff	1

In 2021, the INTCF Criminalistics Service registered 595 cases, having generated a total of 965 requests; 984 reports were issued, 4,059 samples were received, a total of 4,874 were analysed, and 23,065 analyses were performed. Compared to the 2020 data, there has been a significant increase in both the number of requests and reports issued, reflecting a return to normality.

In addition to the forensic activity at the Forensic Sciences Service, it also participates in teaching and training activities in collaboration with the Institutes of Forensic Medicine and various university centres.

Figure 6.1. Overall data on the INTCF Forensic Sciences Service expert activities during 2021

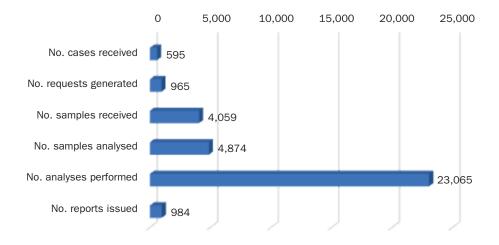


Table 6.2: Overall data on the INTCF Forensic Sciences Service expert activities during 2021

2021	No. cases received	No. requests generated	No. samples received	No. samples analysed	No. analyses performed	No. reports issued
Madrid	595	965	4,059	4,874	23,065	984
TOTAL	595	965	4,059	4,874	23,065	984

Figure 6.2. Casework of the Madrid Department Criminalistics Service during 2021 according to the type of report

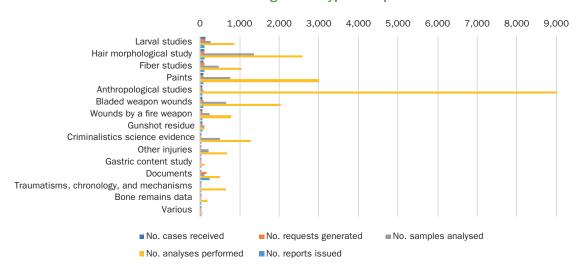


Table 6.3: Casework of the Madrid Department Criminalistics Service during 2021 according to the type of report

Type of report	No. cases received	No. requests generated	No. samples analysed	No. analyses performed	No. reports issued
Larval studies	127	129	276	867	102
Hair morphological study	103	121	1,354	2,576	101
Fiber studies	91	116	483	1,039	103
Paints	75	77	765	3,008	73
Anthropological studies	66	66	82	9,155	72
Bladed weapon wounds	57	74	646	2,041	73
Wounds by a fire weapon	55	58	249	795	55
Gunshot residue	49	48	117	119	47
Criminalistics evidence	30	39	502	1,277	42
Other injuries	28	31	211	681	34
Gastric content study	23	23	21	118	20
Documents	18	162	118	498	244
Traumatisms, chronology, and mechanisms	6	9	18	652	10
Bone remains data	3	7	15	184	7
Various	1	5	17	55	1
TOTAL	595	965	4,874	23,065	984

Cases received by the Forensic Sciences Service often require different types of analysis, leading to the creation of more than one request for the same case involving different areas of the Service. These requests may come from the analysis requested by forensic doctors and/or judges or created by the Service itself following the study of the samples to be analysed.

The facultatives, laboratory technicians and laboratory assistants who form part of the Service's staff require specialist training depending on the area of work to which they are assigned. Particular mention must be made of the fact that the work performed in a forensic laboratory is not always taught in curricula, meaning staff need to receive training by participating in specialist work groups, attending courses, continuously reviewing specialist publications and personal experience gained during years of work.

Study of injuries

In this sense, injuries are taken to mean wounds in which the integrity of the skin is affected: gunshot wounds, stab wounds and blunt injuries mainly, without overlooking other types of injuries, such as grooves caused by hanging, strangulation, umbilical cord sections, etc.

In general, the types of sample to be studied are skin flaps or, in other words, skin cuttings. This service is the only forensic laboratory in Spain that carries out this study on body samples.

The study of gunshot injuries is focused on determining the entry and exit wound and the estimated gunshot distance. To determine the gunshot distance, all garments or surfaces that a projectile passes through, i.e. garments on top of the skin wound, are required. Based on the morphology and the presence of characteristic gunshot residues, it is possible to assess which is the entry and exit wound and the distance at which the shot was fired.

In 2021, a total of 58 requests for gunshot wound studies were received and 55 reports were issued. A total of 249 samples were analysed, resulting in 795 analyses.

From the 55 expert reports elaborated, 26 gunshots were done through the clothes and other interposed materials, not having received the same in 8 cases, what impedes obtaining the distance from gunshot results in 15% of cases.

The analysis of residues on hands is related to gunshot studies, employing SEM-EDX (Scanning Electron Microscopy - Energy Dispersive X-ray spectroscopy), based on the determination of specific particles from the primer of the ammunition.

The INTCF Criminalistics Service supplies to all the IML of Spain a gunshot residue collection kit which specifies the protocol to follow for the right sample collection. The purpose of using a kit is to practice the sample taking at the scene to avoid losses of residues on the hands that will end up returning false negatives.

In 2021, a total of 48 requests were received and 47 reports were emitted with 117 samples analysed and 119 analyses performed.

Stab wounds involve the use of a sharp-edged object or sharp instruments. Laboratory examination of this type of injury includes determining the type of wound sustained and trying to deduce the weapon or generate a match with the suspected weapon in case the weapon has been referred.

In 2021, 74 such requests were received and 73 reports were issued, corresponding to 646 samples studied, with a total of 2,041 analyses carried out.

The study of blunt wounds includes the ones produced by diverse mechanisms: lacerations of contusive origin produced by the action of a hard object on the body surface, hangings, and strangulations, as well as those produced by other more complex mechanisms such as bites. With these types of wounds, it is particularly important to examine the possible presence of foreign material inside the wounds to determine the type of object causing it or the compatibility with the noose in the case of hangings and strangulations.

The exam of these samples during 2021 generated 31 report requests, emitting 34 reports, with 211 samples studied and 681 analyses performed.

Often, the study of stab wounds and blunt injuries in skin flaps, in addition to being studied by the forensic sciences service, includes a vitality study, with samples being shared with the Histopathology Service. To this end, skin flap samples are first studied fresh and then fixed in formaldehyde for a histological study.

As part of this area of study, an exam is performed for signs of violence on clothing with a view to determining if the continuity solutions on clothing were caused by a cut or a tear.

Both clothes involved in the examination of signs of violence and suspicious objects in the case of stab wounds or blunt force injuries are commonly shared with the Biology Services and sent to the relevant department depending on the geographical origin of the samples.

Study of traces

The area of traces includes the fibers, paintings, plastics, ropes, inorganic stains, adhesives, and all those samples of unknown origin, not organic, that may be of interest.

As regards the study of hairs, the Criminalistics Service carries out a morphological study beforehand, with a view to selecting the hairs that may be useful to a genetic study. In 2021, a total of 121 requests were received, 101 reports were issued, 1,354 samples were analysed and 2,576 analyses were carried out.

Regarding fibres, 116 study requests were received and 103 reports were issued, corresponding to 483 samples analysed, with 1,039 analyses carried out. Most of these studies are based on the analysis of fibres collected from the fingernails of victims of violence.

Concerning paints, the corresponding study involves the active participation of the INTCF Criminalistics Service in the creation of the database for the European Collection of Automotive Paints (EUCAP) in relation to the Paint and Glass Working Group of ENFSI, the European Network of Forensic Science Institutes, providing samples of vehicles manufactured in Spain. After the pause in 2020 caused by the pandemic, samples were once again received from the Spanish automotive manufacturers assigned to the Institute. In 2021, 77 requests were received for paint studies, with 73 reports issued, leading to the examination of 765 samples and a total of 3,008 analyses performed. Of the reports generated, 65 related to the study of car samples for the creation of the European database and the remainder to paint samples from multiple sources.

The remainder in this group also includes those considered "miscellaneous", which includes the study of different samples, which could be samples of an unknown origin that are sent for identification or small remains found in different supports (inside wounds, embedded in bones, on the surface of clothes, etc.) studied by the Service and that are individualised to identify them and try to determine their origin. A total of 40 requests were made, with 43 reports issued, 519 samples analysed and 1,332 analyses carried out.

Study of forensic anthropology

Includes the study of total or partially skeletonised and bone remains in which it is requested: bone identification, species determination, the minimum number of individuals, biological profile, injuries study, and estimated death date.

The genetic study is included in a high proportion of these samples, either for the identity confirmation or inclusion in the missing persons databases.

In relation to the biological profile, a total of 66 requests were received in 2021, with 72 reports issued and 82 samples studied, with 9,155 analyses carried out.

The study of injuries or trauma in bone remains includes both the examination of skeletonised remains, soft parts samples in which the study of injuries in skin tissue and bone is requested. A total of 9 requests were made, with 10 reports issued, 18 samples analysed and 652 analyses carried out.

Data from bone remains accounted for 7 requests, with 7 reports issued, 15 samples analysed and 184 analyses carried out.

Forensic entomology

These studies try to determine the death date based on the fauna colonising a corpse, taking into account the circumstances in which the body was found such as in open or closed areas, death cause, environmental conditions (temperature, humidity, season of the year), etc.

It is one of the most requested studies that the Service receives. The majority of them are related to corpses located in closed areas and predominantly by a non-violent death.

In 2021 they received 129 requests, with 102 reports emitted, analyzing 276 samples, which supposes 867 analyses.

Gastric content

The examination of gastric content includes the study of food present in the stomach at the time of autopsy and depending on the type of food ingested, the volume present, the possible pathologies of the individual and the simultaneous consumption of medication and/or alcohol, aim to establish the estimated time from the last intake and time of death. The study is based on the macroscopic identification of food as well as microscopic studies that aim to identify the animal or plant origin and the macroscopic structures that make it possible to establish what type of food is.

Samples of gastric contents are often shared with the Chemistry Department for the analysis of possible toxic substances found in the contents and with the Biology Department for the determination of possible traces of blood.

During 2021, a total of 23 requests were received, issuing a total of 20 reports, with 21 samples analysed and 118 analyses carried out.

Documents

This area includes requests for the examination of manuscripts, whether to identify the author of a text, the authenticity of a signature, and, in case that it is false, the audit thereof.

During 2021, a total of 162 requests were received with 244 reports issued, with 118 samples studied and a total of 498 analyses.

Interlaboratory controls were also performed by the Forensic Service in practically all areas of study, facilitating the analysis of the quality and efficiency of the analyses.

6.1. Interesting forensic cases

6.1.1. Gunshot residue study

A gunshot residue kit developed and supplied by the INTCF was received in order to investigate gunshot residue from a person belonging to the State Security Forces who had allegedly committed suicide.

Specific gunshot residue particles for conventional ammunition as well as particles from a nontox ammunition were found as part of the study. The case fired was requested and was found to be nontox and non-conventional ammunition.

If we had not had the percussion case, we would have given a false positive for a different cartridge than the one used.

This is on account of a memory effect, i.e. residues from previous shots remain in the barrel of the gun and mix with those produced by subsequent shots, resulting in the mixture of particles. This case clearly reflects the need to compare the results obtained in the sample holders taken from a person against those found on the case fired.

6.1.2. Natural death or murder?

The body of a 41-year-old woman was found in an emphysematous state in bed in the guesthouse she was living in. Because the body was found several days after the crime, the advanced state of decomposition made it difficult to determine the cause of death of the victim. Although it was initially considered a natural death given the victim's medical history, the case was later investigated as a possible homicide caused by gender violence, with the hypothesis that the victim's partner may have suffocated the victim with a pillow.

Several samples were sent to the INTCF forensic sciences service to try to clarify the cause of death and the date of death:

- Fresh lower lip skin flap.
- Clipping from the pillowcase found on the victim's head, as well as a number of cosmetics found on the bedroom dresser that presumably belonged to the victim.
- Gastric content.
- Entomological samples.

The lower lip sample was referred for lesion study. It was in an advanced state of decomposition, which limited the criminalistic study. After studying both with the naked eye and under a stereoscopic microscope, a slight erosion and three small, slightly depressed and aligned areas were detected, meaning it could not be ruled out that they had been caused by the action of teeth from the upper jaw, although the condition of the sample made it impossible to generate a match with the victim's teeth.

In relation to the pillowcase clipping, a match between the cosmetics submitted and the stains on the pillowcase was requested.

After studying the undoubted samples, or reference samples, the stains on the pillowcase cutting were studied using the same study methodology as for cosmetics: physical and chemical characteristics of the cosmetics, using microscopic techniques and infrared spectroscopy (FTIR).

The pillow cut-out consisted of a rectangular fragment of white fabric with a floral pattern in pink, green and blue (Figure 6.1.2.1), which had dirt and several stains of what might be cosmetics.



Figure 6.1.2.1. Cutting of the pillowcase subject to study

When magnifying the possible stains compatible with cosmetics, two main types of stains were visible: shiny light brown (Figure 6.1.2.2) and black (Figure 6.1.2.3).

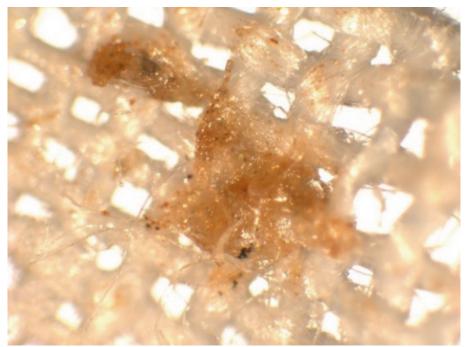


Figure 6.1.2.2. Brown stain found on pillowcase cutting

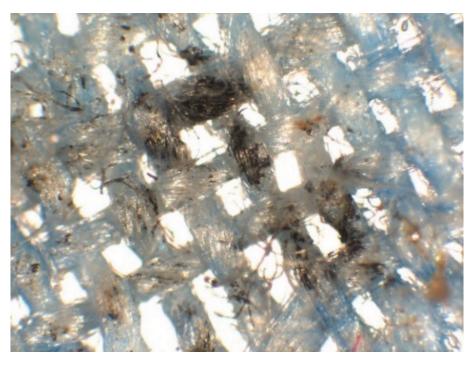


Figure 6.1.2.3. Black stain found on pillowcase cutting

The colouring and composition of the light brown shiny stains were presumably a match with some of the cosmetics received, in particular a palette of eye shadows, meaning it could not be ruled out that they could have come from the same origin.

However, the black stains did not match any of the conclusive cosmetics in colour; however, on account of their colour and composition, it could not be ruled out that they came from a black mascara or eyeliner that could have been used by the victim and were not found during the visual inspection.

Although multiple difficulties were encountered during the analysis, it could be concluded that the stains present on the pillow cutting could be compatible with cosmetic residues.

The sample of gastric contents facilitated the identification of the food ingested by the victim, and it was possible to determine that the digestion time of the last intake was less than 4 hours and probably less than 2 hours.

From the entomological samples submitted, it was determined that the date of death was between 15 and 19 days prior to the autopsy.

The reports provided by the Forensic Service determined the time that had elapsed since the last intake, the date of death compatible with the account of events and the possibility of death by asphyxiation. All this resulted in the accused confessing to and acknowledging the facts.

6.1.3. Male and female?

In 2020, bone remains consisting of the right femur, tibia and fibula of human origin were found together with several animal remains (Figure 6.1.3.1) in a temperate mountain climate. The remains were in an advanced state of decomposition, with little soft tissue remains.

The anthropological study of the remains established that they corresponded to an adult male with an estimated height of between 167.66 cm and 183.02 cm (possibly between 174.56 cm and 179.55 cm).

All the changes seen in the bones were compatible with having occurred in fresh bone, possibly as a result of the taphonomic action of carnivorous animals/carrion eaters.

Given the macroscopic characteristics of the remains, they seemed to correspond to remains dating back over a period of less than five years.

The genetic study helped to identify them as belonging to a man who disappeared in the summer of 2018 with his partner.

In 2021, new cadaver remains were found in the same area, consisting of the skull and jaw, elements of the shoulder girdle and pelvic girdle, elements of both upper limbs and of the left lower limb, ribs and elements of the laryngeal skeleton. These remains were partially covered with stones. Together with them, severely deteriorated and stained fabric remains (skirt and sweatshirt) were found, as well as fragments of skin and a bracelet with the name of the woman who disappeared in 2018, which is why it was initially thought that they could correspond to her.

The anthropological study of the injuries detected:

- Blunt object trauma: Figures 6.1.3.2 to 6.1.3.5.
- · Possible contusive trauma, without being able to rule out the effect of taphonomy.

All these lesions are compatible with having occurred in fresh bone, with no capacity for bone regeneration.

The injuries observed on the skin fragments were consistent with the effect of taphonomy and a dual cutting and tearing component was found in the clothing.

Ultimately, as was the case in 2020, the genetic study confirmed that the remains under study corresponded to the man who disappeared in 2018. The remains of his partner have not been found to date.

Figure 6.1.3.1



Figure 6.1.3.2

Figure 6.1.3.3





Figure 6.1.3.4

Figure 6.1.3.5





6.2. Teaching and scientific activity

6.2.1. Participation in investigation projects and collaborations with other institutions

In collaboration with the ENFSI Working Group Paint and Glass, the maintenance of the EUCAP automotive paint database has been carried out.

In collaboration with the ENFSI Working Group Paint and Glass, participation in the creation of the adhesive tapes database.

Creation of an internal photographic database of microscopic structures from different foods, for application in the study of gastric contents.

Agreement between the Ministry of Justice and the state agency CSIC, M.P. to carry out RAMAN analyses in the Research Group on Optical Spectroscopies in Plasmonic Nanostructures of the Institute for the Structure of Matter.

CSIC Interdisciplinary Platform. Open Heritage Research and Society. Study of pigments.

Collaboration with the Ministry of the Interior for the integration of data at national level on the discovery of unidentified human remains and the identification of missing persons.

Collaboration with the University of Alcalá de Henares in the Forensic Sciences Degree (Electron microscopy and X-ray energy dispersive microanalysis).

6.2.2. Contribution in scientific congresses

Trinidad Argente del Castillo Sánchez, Resurrección Gomez Garví, Ana María Pérez Cao, Mar Nogal Ruiz and M.ª Soledad Sánchez de León Robles. Should the medical examiner determine the aetiology of suicide? About a case of firearm injury. SPAF Congress, Barcelona, 26-28 May 2021.

Teresa Cabellos and Amparo Jiménez. Presentation as co-authors of the oral communication "The complex recovery and identification of Eloy Campillo: lessons learned". Authors: Serrulla, F.; Herrasti, L.; Etxeberria, F.; Zubiria, R.; Izaguirre, A.; Abascal, M.; Carnicero, S.; Jiménez, A.; Cabellos, T.; González-Albo, M.C.; Martin, P.; Merino, P. Presented at the 13th Scientific Meeting of the Spanish Association of Anthropology and Forensic Odontology (AEAOF): Online edition Organised by the AEAOF and the University of Granada, 17 November 2021.

(Electron microscopy and energy dispersive X-ray microanalysis). Attendance at the "Virtual FASE Advanced Course and One-day Symposium". Organised by the Forensic Anthropology Society of Europe. 11-13 November 2021.

Ana María Beltrán. Attendance at the 28th Annual ETHG Meeting of the ENFSI. June 2021. Online.

Soledad García. Attendance at the annual meeting of the RLFOE of Forensic Documentology. June 2021.

6.2.3. Scientific publications

Serrulla Rech F, Teijeira Álvarez R, Serrulla Blanco M, López López M, Santamaría Lozano M, Cherrez Bermejo C. The importance of context in forensic anthropology. *Revista Internacional de Antropología y Odontología Forense*. 2021 Oct;4(3):29-37. https://aeaof.com/media/revista/9/LA%20TRASCENDENCIA%20DEL%20CONTEXTO%20EN%20 ANTROPOLOG%C3%8DA%20FORENSE.pdf

Jiménez Sánchez MA, Cabellos Panadés T. The intervention of the anthropology laboratory of the National Institute of Toxicology and Forensic Sciences. In: Serrulla Rech F. coordinator. *Democratic memory. Communal graves and exhumations. The recovery and identification of the remains of Eloy Campillo.* Madrid Ministry of the Presidency, Relations with Parliament and Democratic Memory; 2021. p. 39-55. https://www.mpr.gob.es/servicios/publicaciones/Documents/ELOY%20CAMPILLO_ACC.pdf

6.2.4. Teaching and training activities

(Electron microscopy and energy dispersive X-ray microanalysis). Co-supervisor of the Master's thesis "Study of bone trauma with single-sharp instruments involved in cases of forensic interest". Interuniversity Master's Degree in Criminology and Police Investigation. Catholic University of Avila.

(Electron microscopy and energy dispersive X-ray microanalysis). Co-supervisor of the Master's thesis "Application of stable isotopes in Forensic Anthropology". University Master's Degree in Police Sciences. University Institute for Research in Police Sciences. University of Alcalá.

Amparo Jiménez, Teresa Cabellos. "Forensic Anthropology". Lecturers. Public Security Management degree course. University Centre of the Guardia Civil, Carlos III University, Aranjuez (Madrid), November 2021.

Margarita Santamaría. Coordinator and tutor at the Forensic Sciences Service in the practical phase of the selective course for the 30th cycle of access to the National Corps of Forensic Doctors.

Margarita Santamaría. Speaker on the course "The Forensic Sciences Service of the INTCF: Fields of action and analytical possibilities". From 22 to 26 March 2021. Centre for Legal Studies. Online.

Margarita Santamaría. Speaker on the course "Interpretation of expert opinions issued by the National Institute of Toxicology and Forensic Sciences". From 19 to 24 April 2021. Centre for Legal Studies. Online.

Margarita Santamaría. Speaker on the course "Methodology in homicide autopsies. Multidisciplinary approach". From 21 to 24 September 2021. Valencian Institute of Public Administration. Online.

The role of the crime laboratory in the study of firearm injuries. Director and speaker on the course "The INTCF forensic sciences service: fields of activity, analytical possibilities". 22 to 26 March 2021. Centre for Legal Studies. Online.

Ana María Beltrán. Tutor at the Forensic Sciences Service in the practical phase of the selective course for the 30th cycle of access to the National Corps of Forensic Doctors.

Soledad García. Speaker on the course "The INTCF forensic sciences service: fields of activity, analytical possibilities". From 22 to 26 March 2021. Centre for Legal Studies. Online.

Professionals. Attendance on the course "Basic LIMS: structure, data organisation and queries". From 17 to 24 May 2021. Centre for Legal Studies. Online.

Professionals. Attendance on the course "Practical workshop on the dissemination of the INTCF quality system". From 21 to 28 September 2021. Centre for Legal Studies. Online.

Professionals. "Interpretation of expert opinions issued by the National Institute of Toxicology and Forensic Sciences". From 19 to 24 April 2021. Centre for Legal Studies, Online.

Professionals. Attendance on the course "Comprehensive forensic perspective on suicide". From 4 to 12 May. Centre for Legal Studies. Online.

Professionals. Attendance on the course "Detection and identification of chemical weapons in forensics. Toxicity and impact on the environment: On the case study". From 4 to 11 May. Centre for Legal Studies. Online.

Professionals. Attendance at the Operational Ballistics Conference: Firing Trajectories and Ballistic Effects". Organised by the Guardia Civil. From 14 to 18 June.

Professionals. Attendance on the course "Advanced biological SEM: making the most of your microscope". Organised by Oxford Instruments. Online.

Professionals. "New research tools in the field of Forensic Genetics". June 2021. Centre for Legal Studies. Online.

Professionals. "The INTCF forensic sciences service: fields of activity, analytical possibilities". From 22 to 26 March 2021. Centre for Legal Studies. Online.

Professionals. "Criminal databases: operational and legal aspects". From 13 to 20 April 2021. Centre for Legal Studies. Online.

Professionals. 1st Afore WP3:2 Webinar on the Validation of Analytical Methods in Forensic Science. May 2021.

Professionals. 2nd Afore WP3:2 Webinar on the Validation of Analytical Methods in Forensic Science. December 2021.

Professionals. "Multidisciplinary forensic intervention in multi-victim incidents". From 15 to 22 November. Centre for Legal Studies. Online.

Professionals. "Forensic medicine and public health". From 18 to 24 November 2021. Centre for Legal Studies. Online.

Laboratory technicians and laboratory assistants. Attendance of the "Multidisciplinary course on drugs: review of toxicity, updating of analytical methods, judicial and social value". From 18 to 26 October 2021. Subdirectorate General for Access and Promotion of Justice Administration Staff. Online.

Laboratory technicians and laboratory assistants. Attendance of the course "Quality assurance in the INTCF, quality manuals, standard operating procedures, concepts and definitions". From 11 to 30 November 2021. Subdirectorate General for Access and Promotion of Justice Administration Staff. Online.



The Toxicological Assessment and Environmental Service (hereinafter SVTMA) was created in 1998 to meet the growing demand from the courts for expert reports on environmental offences and to respond to the request for analysis and assessment of results in alleged environmental offences.

The Toxicological Assessment and Environmental Service have as objective the emission of reports and dossiers that the Judicial Authorities and the Public Prosecutor's Office request. It also analyses and investigates practices ordered by Judicial Authorities, Governmental Authorities, and the Public Prosecutor Office related to the judicial procedures or in the preliminary proceedings carried out by the Public Prosecutor's Office in fields of the investigation of alleged offences against the environment and natural resources.

The analyses and tests carried out at laboratories, as well as the assessment reports issued by the Toxicological Assessment and Environmental Service at the Barcelona, Madrid and Seville Departments, are aimed at determining the possibility that an alleged criminal offence included in Articles 325 to 331 of the Criminal Code, in Chapter III of Title XVI, regulating the so-called crimes against natural resources and the environment, may cause substantial damage to the quality of the receiving environment, water, soil or atmosphere, or may pose a risk of serious damage to the balance of natural systems. The cases included in Chapter IV, relating to the protection of plantlife, wildlife and domestic animals (Organic Law 10/1995, of 23 November) are also analysed and assessed.

To meet this objective, requests for analyses and tests by the judicial authority are dealt with, in addition to collaboration with or consultancy offered to the judiciary police in the sample taking process. In cases considered necessary, and always at the request of the Public Prosecution Service or the judicial authority, studies and fieldwork are performed to round off the expert report.

The opinions issued by this service include the results of analyses and tests carried out on numerous samples. A detailed study of the area and activity as well as an in-depth bibliographical review and specific regulatory review is needed; Community law, Spanish law and regional law as well as municipal regulations (spillages, water quality, residues, soil, emissions into the atmosphere, air quality, etc.).

The majority of the cases are related to the following investigations:

- Environmental impact study of urban or industrial wastewater spills of hydraulic public domain
- · Environmental impact study of discharges of slurry and sewage sludge
- Residues and leachates analyses and environmental impact study
- · Environmental investigations about polluted soils
- · Environmental investigation of atmospheric pollution
- Environmental investigations about poisoned flora and fauna samples
- · Environmental investigation in fires
- · Scientific-technical and regulatory assessment of applicable environmental regulations

The analysis techniques, studies, and tests carried out for this purpose are:

- · Physico-chemical techniques.
- Ecotoxicity bioassays.
- · Microbiological Analysis.
- · Fieldwork.
- · Assessment of environmental reports and documents.
- Bibliographic reviews.
- · Study of specific legislation.

Concerning the management of quality about the activity of the laboratories, all the Toxicology Assessment and Environmental Service participate actively in the continuous implementation of the INTCF quality system. It is done through the continuous revision and actualisation of internal work procedures and its correspondent validation tests; participation in interlaboratory exercises of the parameters and tests established in each laboratory, and their participation in the correspondent internal and external audits.

The Toxicological and Environmental Assessment Service of the Barcelona, Madrid and Seville Departments has accredited several of its laboratory tests under ISO 17025. ENAC accreditation dossiers: 297/LE639, 297/LE1366, 297/LE2239.

The Toxicological Assessment and Environmental Services is staffed by laboratory assistants, laboratory technicians, facultatives and heads of service. The distribution of staff in each department is shown in Table 7.1.

Table 7.1: Staff of the VTMA Services of the different Departments (2021)

	INTCF MADRID	INTCF BARCELONA	INTCF SEVILLA		
Head of the Service	1	1	1		
Facultatives	5 (4*)	1 (0*)	2		
Specialist technicians	2	2	1		
Laboratory assistants	2	-	1		
Clerical staff	-	-	-		
* Occupation for several months.					

The VTMA Services at the INTCF registered during a total of 242 requests for expert reports in 2021, issuing 233 reports after the analysis of 1,474 samples.

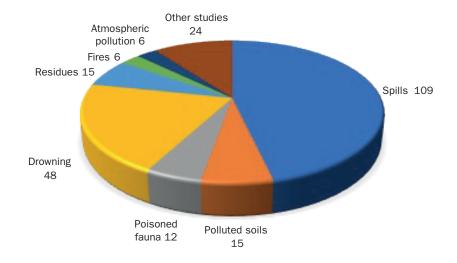
500 1,000 1,500 2,000 No. cases received No. requests generated 236 No. samples received 787 161 No. samples analysed 249 1.242 No. analyses performed 1,906 855 No. reports issued Seville Madrid Barcelona

Figure 7.1. Overall data on the INTCF VTMA Services expert activity during 2021

Table 7.2: Overall data on the INTCF VTMA Services expert activity during 2021

2021	No. cases received	No. requests generated	No. samples received	No. samples analysed	No. analyses performed	No. reports issued
Madrid	26	38	236	697	1,242	38
Barcelona	132	159	787	528	1,906	134
Seville	26	45	161	249	855	61
TOTAL	184	242	1,184	1,474	4,003	233

Figure 7.2. Expert activity of the VTMA Services at the INTCF during 2021 distributed by type of report



Hereunder we collect the scientific and expert activities like the teaching and formative activities developed during 2021 for each VTMA Services from the different Departments. Each Service has included a forensic interesting case description to publish the expert labour.

7.1. Toxicological Assessment and Environmental Service of the Barcelona Department

With regard to the expert activity of the VTMA Service at the Barcelona Department, during 2021, 159 requests were generated and 528 samples were analysed as part of a total of 1,906 analyses, issuing a total of 134 expert reports, according to Figure 7.1.1 and Table 7.1.1, which shows the casework of the VTMA Service in Barcelona during 2021 broken down by type of report.

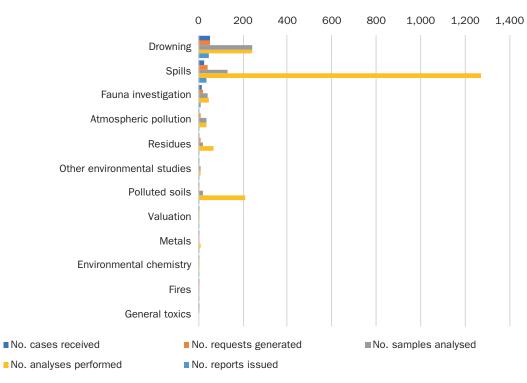


Figure 7.1.1. Casework of the VTMA Service of the Barcelona Department during 2021 by type of report

Table 7.1.1. Casework of the VTMA Service of the Barcelona Department during 2021 by type of report

Type of report	No. cases received	No. requests generated	No. samples analysed	No. analyses performed	No. reports issued
Drowning	53	53	243	243	48
Spills	25	40	133	1,271	36
Fauna investigation	17	20	44	46	12
Atmospheric pollution	7	8	37	37	6
Residues	7	9	22	68	6
Other environmental studies	5	5	11	11	4
Polluted soils	5	6	21	209	7
Valuation	5	5	2	2	6
Metals	4	5	6	8	3
Environmental chemistry	4	4	5	7	4
Fires	3	3	4	4	2
General toxics	1	1	0	0	0
TOTAL	132	159	528	1,906	134

7.1.1. Casework of the SVTMA Barcelona Department during 2021 by type of report

Concerning environmental crime investigation, during 2021, our casework increased 19 % compared to 2020. The increase is due to a continuous and direct collaboration with judiciary police. They have strengthened confidence in our expert service as a laboratory and as an issuer of valuation reports to the Justice Administration service.

In relation to the type of report, during 2021, most cases related to environmental analysis and assessment reports continue to be related to liquid discharges into the public water domain, with investigations related to industrial discharges being more frequent; fundamentally in Catalonia.

It should be noted that during 2021, several reports were generated, including both analytical and toxicological assessment reports on matters related to discharges into the marine environment, all of which were carried out as a result of judicial investigations carried out by teams from the Nature Protection Service (SEPRONA) of the Guardia Civil, mainly in the Balearic Islands. These requests required the adaptation of analytical methods to suit the analysis of pollutants in the seawater matrix.

We have incorporated, in our analytical routines, the investigation of pollutant gases that deplete the ozone layer, such as refrigerant gases or chlorofluorocarbon compounds (HCFC); likewise, the Service has carried out analyses and reports related to episodes of

atmospheric pollution, as well as several reports in which, on behalf of the Court of Instruction/Environmental Prosecutor's Office, we have been asked to provide a documentary assessment for the preparation of toxicological reports in cases related to serious episodes of atmospheric pollution that have occurred in Catalonia.

During 2021, a total of 10 field trips were performed, generating highly useful data for the preparation of environmental assessment reports related to the receiving media, in situ analysis and data obtained as part of the visual inspections of companies under investigation.

With a view to guaranteeing the quality of our analyses, we regularly participate in interlaboratory exercises involving several organisations that cover a huge spectrum of determinations performed by this Service: heavy metals, general pollution parameters, toxicity tests, microbiological tests, and the determination of combustion accelerants in cases of fires.

7.1.2. Types of analysis performed by the VTMA Service at the Barcelona Department during 2021

Concerning the analyses and studies performed at this Department in relation to the received requests, the following table and graphic shows the distribution of the different works performed by the Service:

Table 7.1.2.1. Types of analysis performed by the VTMA Service at the Barcelona Department during 2021

	No. of analyses	No. of analyses (%)
Inorganic compound determinations	282	14.8
Determination organic compounds	573	30.1
Globalising parameters of contamination	659	34.6
Microbiological analysis	158	8.3
Ecotoxicity tests	178	9.3
Study/valuation of documents	48	2.5
Field outings/determinations in situ	8	0.4
TOTAL	1,906	100.0

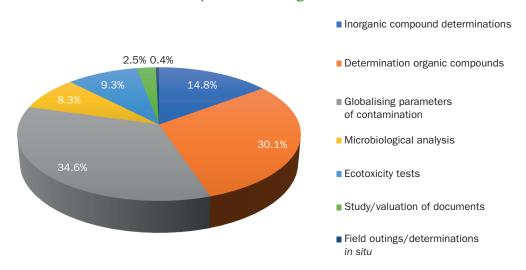


Figure 7.1.2.1. Types of analysis performed by the VTMA Service at the Barcelona Department during 2021

7.1.3. Forensic case of interest: Expert investigation in a case related to the re-use of quarries as waste landfills

The town of Seva (Barcelona) and the surrounding area has been a site of special interest for the extractive industry, with several quarries located in the town. The two main examples of this are the Fitó quarry and the Pinós quarry (Figure 7.1.3.1).

The extraction phase at the Pinós quarry finished approximately two decades ago and since then it has been used as a dumping ground for supposedly inert substances. The geological characteristics of this quarry were not suitable for its use as a landfill, and its steep slope resulted in the movement of the waste deposited, posing a serious risk of the slope falling towards the C-17 motorway and towards an industrial area located in the town of Centelles (Barcelona). Around 2018, the Pinós landfill reached its maximum filling point; since then, and due to the high risk of landslides, the landfill has been emptied to a level that guarantees its safety.

Previous analyses carried out in 2019 by the Toxicological Assessment and Environmental Service at the INTCF in Barcelona demonstrated that the waste stored at the Pinós landfill contained biological activity indicating that the waste stored was not exclusively inert, but that waste of all types, including hazardous waste, had been deposited there.



Figure 7.1.3.1. Location of the Fitó quarry (red) and the Pinós quarry (orange) next to the town of Centelles.

The neighbouring Fitó quarry has maintained its extraction activity until the present day, where a small amount of aggregate extraction is still performed. However, part of this quarry was fully depleted years ago and the main extraction area at the old quarry (excavation basin) has been used as a dumping ground for inert substances.

Given the proximity between the two landfill areas and the fact that the owner of the two activities is the same person/company, waste is being transferred from the Pinós landfill to the Fitó quarry.

In anticipation of the removal of non-inert waste from the Pinós landfill and its future deposition at the Fitó quarry, a series of preventive samples were taken from the surroundings of the Fitó quarry to check the quality of the environment, especially the aquatic environment surrounding the quarry. The results obtained in 2019 demonstrated that the water quality in the surrounding area was good.

The Fitó quarry was adapted for the deposition of solid inert waste by placing a plastic waterproofing mesh at the bottom of the extractive vessel, as well as a network of drainage pipes for channelling and collecting the leachate. Subsequently, work started to fill it with waste from the Pinós landfill, as well as external waste.

Between 15 and 25 January 2020, storm Gloria hit the Iberian mainland, with Catalonia particularly hard hit. The high volume of rainfall caused a rise in the water table in the area where the Fitó quarry is located, resulting in the old quarry basin filling with water, forming an artificial lake. This artificial lake made up of rainfall had already formed prior to storm Gloria, but the significant increase in the volume of water during this storm and the sudden rise in the water table in the quarry basin seriously affected the waterproofing system at the Fitó landfill, lifting the plastic layer that served to waterproof the basin and protect the groundwater from any type of seepage (Figure 7.1.3.3).

Figure 7.1.3.2: Water in the quarry basin and the Guardia Civil taking a sample.



Figure 7.1.3.3. Satellite images of the Fitó quarry in different years. The red circle in the image corresponding to 2020 shows where the plastic insulation came free.



Following the removal of the waterproofing layer, a large number of analyses were performed in the area around the quarry, both on the surface water and groundwater. To check the quality of the groundwater, both wells and piezometers in the surrounding area of the activity were analysed.

Analyses carried out by the Toxicological Assessment and Environmental Service at the INTCF in Barcelona returned high levels of contamination in the lake that had formed in the excavation basin; these waters contained a high concentration of cations (e.g. sodium, potassium, calcium) and anions (e.g. chlorides, sulphates) which suggest that the water had been salinised by dissolution of the natural substrate and the waste deposited in the quarry.

Likewise, the samples show a high concentration of organic matter in solution subject to both biotic and abiotic decomposition processes (e.g.: COD, BOD5) which resulted in the proliferation of microorganisms (faecal and total coliforms and streptococci).

The high concentrations of salts (especially sulphates, possibly from the dissolution of the gypsum contained in the waste), together with the consumption of the oxygen dissolved in the water column by the oxidation of the organic matter in solution and bacterial activity, encourage anoxic conditions that favour the production of sulphides, from the dissolved sulphates, together with other fermentation products (e.g. methane, ammonium or mercaptans).

Both the salinity and the presence of high concentrations of sulphides and methane turned the rainwater that had accumulated in the quarry into waste water as a result of the leaching of the waste deposited in the quarry, with the consequent risk to the balance of natural systems or the health of those exposed.

These leachates are highly toxic (EC50), close to the level established in the regulations for their consideration as toxic and hazardous (EC50 \leq 0.3%) pursuant to the sectoral waste regulations, and should therefore have been treated appropriately to minimise their environmental risk.

It was also possible to observe the contamination, due to the accumulation of waste in the quarry basin, in the river into which the water extracted from the excavation basin is discharged, as well as in the surrounding wells and piezometers analysed. Both in the river and in the wells, high values for pollution parameters such as COD, conductivity, nitrates, nitrites, ammonium, chlorides, sulphates, magnesium, toxicity and total coliforms have been detected.

It has been found that the levels of pollutants in the excavation basin have decreased since storm Gloria. This is because, after an abrupt rise in the water table and the upwelling of the waterproof layer, the pollutants deposited at the bottom of the lake rose; now, however, as time has passed, they are settling again, reducing the previous levels of contamination. This "decontamination" phenomenon is misleading, as the pollutants are still present in the sludge settled at the bottom of the basin.

The company managing the Fitó landfill has set up a small treatment plant to treat the water taken from the quarry before discharging it into the public water domain. However, the analyses performed on the treated water from the bottom of the quarry's basin have

shown a very limited capacity for purification, discharging a variety of pollutants into the receiving environment.

Figure 7.1.3.4. Images showing the appearance of the water at the outlet of the treatment plant





7.1.4. Teaching and scientific activity

7.1.4.1. Contribution in scientific congresses

Herminia Bueno Cavanillas: Conference on "Noise pollution in the field of criminal law". ACUSTI.CAT Congress. 14 February 2021.

Herminia Bueno Cavanillas: Conference "The role of Forensic Sciences in the Investigation of Environmental Crime". Organised by Aula Emilio Herrera Linares de Ciencia y Tecnología, Life Quality and Environment Seminar and La Cátedra de Salud. La Madraza Palace. University of Granada. 26 November 2021.

7.1.4.2. Teaching

Herminia Bueno Cavanillas: Lecture on "Interpretation of the expert reports issued by the toxicological and environmental assessment service". Course: Interpretation of expert opinions issued by the National Institute of Toxicology and Forensic Sciences. Organised by CEJ as part of the continuous training programme and aimed at INTCF professionals. 19-27/04/2021.

Herminia Bueno Cavanillas: Lecture on "INTCF equipment. Equipment management. Part II. Chemical and Drug, VTMA and general equipment". 26 November 2021.

7.1.4.3. Training activities

Herminia Bueno Cavanillas: Practical course on sampling, taxonomic classification of benthic macroinvertebrates and calculation of biotic indices for the study of environmental crimes. Valdemoro (Madrid). From 15 to 18 February 2021.

Miguel Ángel Serrano and María Ángeles Lázaro Cardenal. Online course on "Quality assurance in the INTCF, quality manuals, standard operating procedures, concepts and definitions". Organised by the Subdirectorate General for Access and Promotion of Justice Administration Staff. 23/11-01/12/2021.

Miguel Ángel Serrano and María Ángeles Lázaro Cardenal. Online course "Quality. Study of the UNE-EN-ISO/IEC 17025:2017 standard. General requirements for the competence of testing and calibration laboratories". Organised by the Subdirectorate General for Access and Promotion of Justice Administration Staff. 15-22/11/2021.

Miguel Ángel Serrano and María Ángeles Lázaro Cardenal. "Multidisciplinary course on drugs: review of toxicity, updating of analytical methods, judicial and social value of analytical expertise". Organised online by the Subdirectorate General for Access and Promotion of Justice Administration Staff. 04-12/11/2021.

7.2. Toxicological Assessment and Environmental Service of the Madrid Department

The expert activity carried out during 2021 by the Toxicological and Environmental Assessment Service at the Madrid Department (hereinafter SVTMAM) involved 26 cases received, 38 requests generated and 38 reports issued (Figure 7.2.1 and Table 7.2.1).

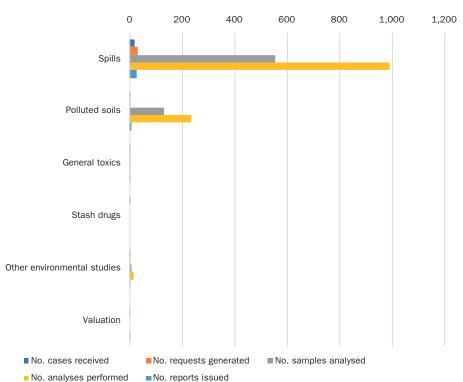


Figure 7.2.1. Casework of the VTMA Service of the Madrid Department during 2021 according to the type of report

Table 7.2.1. Casework of the VTMA Service of the Madrid Department during 2021 according to the type of report

Type of report	No. cases received	No. requests generated	No. samples analysed	No. analyses performed	No. reports issued
Spills	18	30	557	992	29
Polluted soils	4	5	133	234	6
General toxics	2	1	1	2	1
Stash drugs	1	0	0	0	0
Other environmental studies	1	1	6	14	1
Valuation	0	1	0	0	1
TOTAL	26	38	697	1,242	38

Furthermore, adequate quality management, a fundamental part of the development of the laboratory's activity that ensures its technical quality, competence and capacity to generate valid results, involved 23 requests being generated (21 related to participation in intercomparison exercises, 1 with method validation, 1 referring to ENAC external audit) and 24 reports issued (21 related to participation in intercomparison exercises, 1 with method validation, 1 referring to ENAC external audit and 1 relating to internal audit).

Analytical activity in relation to requests for analysis and reporting was performed on 697 samples, with a total of 1,242 analyses.

The number of analyses carried out can be distributed by types of reports into which the reports issued are classified (Figure 7.2.2).

Polluted soils

Polluted soils

234

Other environmental studies

General toxics

2

0 200 400 600 800 1000 1200

Figure 7.2.2. Number of analyses carried out by type of report issued

Thus, the largest volume of analytical work was performed in the investigation of spill-related requests (992 analyses), followed by analyses related to contaminated soil investigation (234 analyses), other environment-related studies (14 analyses) and general toxic investigation analyses in collaboration with other INTCF services (2 analyses).

The expert opinions/reports issued consist (depending on the request and relevance) of a physico-chemical analysis report, a microbiological analysis report, an ecotoxicity test report and an expert report on the scientific-technical assessment of both the analyses carried out and the documentation submitted by the applicant, a study of related environmental regulations, an environmental study of the surroundings and other documentation of interest relevant to the investigation.

The types of analyses and the determination of different parameters are related to the types of reports that make up the opinions, with physico-chemical analyses being the most frequently performed (Figure 7.2.3).

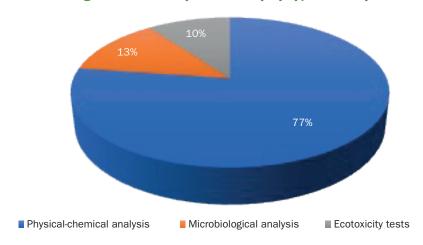


Figure 7.2.3. Analytical activity by type of analysis

The nature of the samples received and analysed (Figure 7.2.4) are as follows:

- Inland waters (coastal waters, transitional waters, groundwater, rivers, lakes, artificial or heavily modified water bodies).
- Seawater
- Waste water (urban waste water and industrial waste water).
- Soil.
- Sediments (river beds).
- Sludge.
- Samples related to forensic investigations (of inorganic origin).

Soil 14.29
Sediments
8.33
Sediments
8.33

Waste water
36.51
Seawater 0.13

Figure 7.2.4. Nature of samples for analysis (%)

The cases received were requested by the Community of Madrid, the Basque Country, the Canary Islands, Castilla-La Mancha, Castilla y León and Galicia (Table 7.2.2).

Table 7.2.2. Distribution of registered cases by autonomous communities

Autonomous Region	No. cases registered	% cases registered
Community of Madrid	38	74.50%
Basque Country	5	9.80%
Canary Islands	3	5.90%
Castilla y León	3	5.90%
Castilla-La Mancha	1	2.00%
Galicia	1	2.00%
TOTAL	51	100.00%

7.2.1. Forensic case of interest. Environmental impact due to the inadequate management of urban waste water. Septic tank overflows

7.2.1.1. Records

As a result of overflowing septic tanks, urban wastewater is being discharged into the ground.

Property development resulting in discharges is located on protected (extended) undeveloped land (Figure 7.2.1.1.1). Undeveloped or rustic land is land on which constructions cannot be erected and, therefore, cannot be transformed into urban land. Urban subdivisions are prohibited, as well as other subdivisions, divisions or segregations.

Clase de suelo literal del documento: Suelo No Urbanizable Protegido Clase I
Espacios ZEPA-Area de Tolerancia
Clase de suelo literal del documento: Suelo No Urbanizable Protegido
Clase de suelo según Ley de aprobación: Suelo No Urbanizable de Protección
(APLAZADO)

Clasificación
Suelo urbano / urbano consolidado
Suelo urbano no consolidado
Suelo urbano no consolidado
Suelo urbano no consolidado
Suelo urbano / urbano consolidado
Suelo urbano no consolidado
Suelo urbano consolidado
Suelo urbano no consolidado
Suelo urbano de arbanta de a

Figure 7.2.1.1.1 Urban qualification of the land affected by the discharges. Source: Urban Planning (www.madrid.org/cartografia/planea/index.htm)

As part of this classification of undeveloped land, the exclusive use of *camp* sites was permitted. Any permanent construction or building was incompatible with that classification and use, and was therefore not permissible. Over time, however, the area has been subject to unlawful subdivision and to a large extent has been developed, with temporary mobile dwellings also set up.

If there are no administrative regulations addressing the resolution of an urban planning problem, it may not be possible to classify these actions as a criminal offence. Without going into all the urban planning issues and possible land use planning offences, the point is that an urban settlement requires proper management of the waste and waste water generated. Environmental impact studies leading to an environmental impact statement or discharge authorisation would be important.

The wastewater generated as part of the development under investigation is managed by means of septic tanks. In theory, they fulfil the function of wastewater management in urban areas without a sewerage system. Septic tanks are constructions (reservoirs) dug into the ground which, through decantation, help to separate solid organic matter and liquid wastewater, resulting in a natural purification process by anaerobic microorganisms that partially digest the organic matter.

To avoid affecting the soil, they must be properly managed, which requires constant maintenance and removal of waste by an authorised manager (collection in tanks for subsequent treatment at waste water treatment plants (WWTPs) and other facilities). This requires regular and regulated emptying and cleaning, with the collection and appropriate treatment of the accumulated matter.

Risks to human health and the environment must be avoided. The discharge of the contents of a septic tank may pose a risk to human health on account of the microbiological characteristics of the wastewater. Their composition can lead to contamination of the public water domain, affecting catchment areas of water for human consumption, bird protection areas, areas vulnerable to nitrate pollution (groundwater), sensitive areas

(phosphorus, nitrogen and organic matter compounds) and other areas declared as special protection areas for habitats and species.

Soil contamination must be reduced to ensure that pollutants do not reach harmful levels. Furthermore, it may affect the balance of nutrients in the soil. When left unchecked, soil contamination can prevent the proper ecological and chemical condition of surface waters and the good chemical and quantitative status of groundwater.

The Natura 2000 protected areas (Sites of Community Importance [SCIs], Special Areas of Conservation [SACs] and Special Protection Areas for Birds [SPAs]) are part of a European ecological network aimed at maintaining or restoring biological diversity and protecting natural habitat types and species of wild flora and fauna classed as being of Community interest.

The area under investigation is SPA territory, designated for the conservation of wild bird species, included in Directive 2009/147/EC of the European Parliament and of the Council of 30 November 2009 on the conservation of wild birds. It is a key area for the conservation of several unique bird species, such as the Iberian imperial eagle, the golden eagle, the peregrine falcon, the black vulture and the black stork.

The catchment areas of the rivers in which the discharges in question are located include formations and species representative of the Mediterranean area, such as pastures, grasslands, herbaceous vegetation, forests, scrubland and arable land.

The affected land, according to the hydrological planning of the Tagus Hydrographic Confederation, is declared a vulnerable area, affecting the northern sector of the groundwater body 030.015 (Talavera) and is located close to water abstraction points for the supply of water (Figure 7.2.1.1.2).

Figure 7.2.1.1.2. Location of the discharge area within the area designated as vulnerable and location of ground and surface water supply catchment areas.

Source: Tagus Hydrographic Confederation Viewer

(visor.chtajo.es/VisorCHT)



7.2.1.2. Complaint and investigation

The SEPRONA department at the Guardia Civil carried out a visual inspection and then sampled wastewater from these septic tanks, at two different points, M1 and M2 (Figure 7.2.1.2.1). They were at the limit of their capacity, clogged, overflowing into the ground and appeared as if they had been abandoned.

ORTOFO DE LA ZONA Y PUNTO APROXIMADO DEL VERTIDO

Visur SEPRONA

Urbanización

Urbanización

Zona marcada con un circulo rojo donde se localizan las fosas objeto de las presentes actuaciones.

Figure 7.2.1.2.1. Photographic report by SEPRONA. Location of sampling points

It was noted that the cesspools had not been maintained, which led to the discharges. The decision was made to subject them to temporarily monitoring to check that the discharges were corrected, taking successive samplings between the months of March and July (Table 7.2.1.2.1).

All examination is a result of the investigation ordered by the Area Prosecutor's Office under criminal investigation proceedings.

The samples were sent in due time and form to the Madrid Department of the INTCF for analysis and reporting, together with the chain of custody documents, received on the same day as the samples and proceeding with the analysis by SVTMAM, as requested: "pH, conductivity, BOD, COD, SS, TN, PT, faecal coliforms, E. *coli*, faecal streptococci/ enterococci, as well as any other parameter of interest deemed necessary [...] relating to the discharge of the waste water generated on the land [...]".

Table 7.2.1.2.1. Dates of field monitoring and investigation sampling and investigation of discharges from septic tanks and advice given

DATE OF SAMPLE COLLECTION		OPINION. ISSUE DATE		
First submission	Mar-21	First OPINION	Mar-21	
Second submission	Apr-21	1st EXTENSION	May-21	
Third submission	May-21	2 nd EXTENSION	June-21	
Fourth submission	July-21	3 rd EXTENSION	Sept-21	

Two samples of waste water were received for each sample, taken at the two points corresponding to the cesspools, collected in sterile packaging (suitable for microbiological determinations), duly labelled, inside a SEPRONA bag labelled and sealed with plastic ties with unique and unambiguous numbering (Figure 7.2.1.2.2).

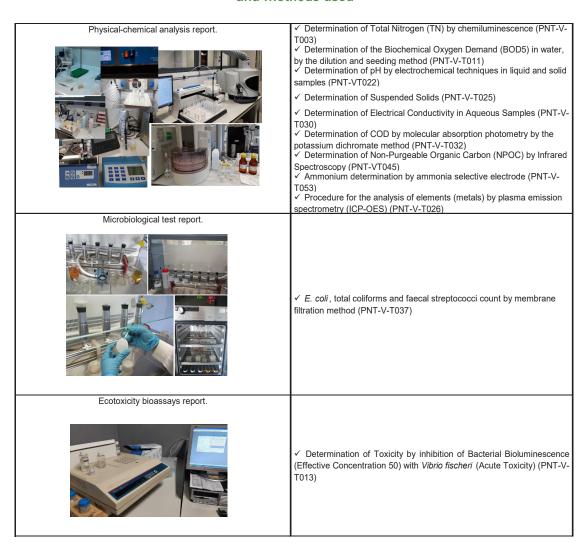


Figure 7.2.1.2.2. Samples received at the SVTMAM

Aliquots for the determination of the requested and relevant parameters were adapted, following the quality and sample management/chain of custody criteria at all times, as well as the determination of the analyses and tests (Table 7.2.1.2.2).

The opinions issued consist of a physico-chemical analysis report, a microbiological analysis report, an ecotoxicity test report and a report on the assessment of the results obtained, together with all the information received, applicable regulations and any other reference that allows for a comprehensive scientific-technical assessment.

Table 7.2.1.2.2. Determinations made in the investigation. Studies conducted and methods used



7.2.1.3. Results obtained

Based on the results of the physico-chemical analyses, the samples correspond to untreated urban waste water. They show high values for organic pollution indicator parameters, with maximum values for ammonium (\sim 60 mg NH4+/I), non-purgeable organic carbon (NPOC) (\sim 700 mg C/I), COD (\sim 2000 mg O2/I), BOD5 (\sim 800 mg O2/I), phosphorus (\sim 37 mg P/I) and total nitrogen (\sim 140 mg N/I), as well as high conductivity and suspended solids, which remain elevated at both sampling points over time.

Furthermore, according to the results obtained in the microbiological tests, the samples contain a high load of faecal contamination, and this was maintained throughout the samples and monitoring performed, with values >100,000 CFU/100 ml (Figure 7.2.1.3.1).

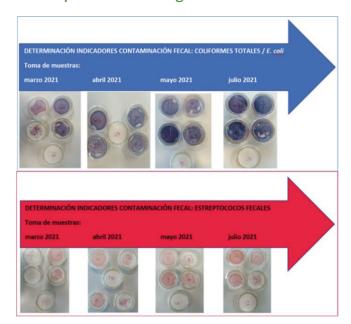


Figure 7.2.1.3.1. Culture plates of micro-organisms indicative of faecal contamination

The ecotoxicity bioassay performed indicates that the samples tested show toxicity that is maintained throughout the samples for the test organism used, *Vibrio fischeri*.

Samples collected at sampling point M1 showed EC50 values (15 min, 15 $^{\circ}$ C) between 7.7 and 44.3 toxic units/between 13% and 2.3% throughout the samples.

Samples collected at sampling point M2 showed EC50 values (15 min, 15 °C) between 17.2 and 44.4 toxic units/between 5.8% and 2.5% throughout the samples.

7.2.1.4. Conclusions

The use of septic tanks as a primary waste water treatment system should aim to protect water resources by preventing the pollution and degradation of aquifers. The main problem with septic tanks is the leaching or seepage of wastewater into aquifers, with the risk of contamination.

The accumulation of inadequately treated waste water discharge on land may lead to the alteration of the physical, chemical and microbiological characteristics of the groundwater that may be affected. Point-source pollution of the soil by the introduction of unusual concentrations of substances may result in the loss of the soil's properties.

Waste water from septic tanks must be authorised for discharge to avoid endangering the quality of groundwater. This would require studies describing the hydrogeological and geomorphological characteristics of the area.

In terms of serious and potential harm to human health, this depends on:

- The hazard identified, i.e. the biological and chemical agents identified by analysis, in this case primarily faecal bacteriological contamination.
- The exposure of people to the contamination identified, i.e. the possibility of people coming into contact with the waste water, directly or indirectly, by different routes: oral, dermal or inhalation.
- In relation to the results of the analyses, waste water with the microbiological load should not come into contact with food and water intended for consumption.
- The presence of micro-organisms indicative of faecal contamination can affect human health. *Escherichia coli* is a bacterium that lives in the intestine of mammals. Most *E. coli* are harmless. However, some types can cause illness and diarrhoea, sometimes leading to kidney failure and even death, especially in children and adults with weakened immune systems. Infection can be caused by consuming food or water that has been in contact with sewage.

In terms of damage to the balance of natural systems, soil is an element of the ecosystem that is difficult to recover; the degradation of its initial properties (physical, chemical and biological), as well as the loss of nutrients and of its finer particles, makes the regeneration process very slow.

Depending on the characteristics of the soil and mainly on its permeability, the infiltration of waste water with a high organic matter may affect its composition, enriching the soil and groundwater with organic matter and salts.

For the determination of serious or potential damage to the balance of natural ecosystems, a hydrogeological report will be required to determine whether there is a risk of contamination of aquifers.

Having verified the significant contamination load of the samples submitted after successive samplings:

- 1. It is considered that such waste water should not be discharged without appropriate treatment so as not to contribute to the deterioration of the environment in which it is found. In other words, they need the corresponding administrative authorisation and correct management of septic tanks. Discharges made without the corresponding authorisation or those that do not comply with the conditions under which they have been authorised are likely to pollute any element of the public water domain and may cause damage. The damage depends, among other things, on the hazard posed by the spill, the volume of the spill and the duration of the spill.
- 2. The discharge of nutrients and organic matter, the pollutant load of the discharge samples submitted, can contribute to the eutrophication and deterioration of surface water bodies, and thus to the vulnerability of groundwater.

3. If people come into contact with such a spill, it can be hazardous to their health.

Given that the septic tanks, the purification system used by the population centre/ urbanisation, have been neglected and are a constant source of pollution for the environment and a risk to people's health, who is responsible for these situations? Is responsibility shared between the owners and the administration?

7.2.2. Teaching and training activities

7.2.2.1. Education activities

Collaboration with the University of Alcalá de Henares (UAH) in the teaching of the subject "Forensic Instrumental Analysis (652010)" 2021/2022 academic year. Honorary professor on the degree in Forensic Sciences. Forensic Sciences and Technologies. Pilar García de Yébenes Torres.

Collaboration with external internships carried out at the Madrid Department of the INTCF involving students from Higher Vocational Training courses in Health from the Technical School of Specialised Teaching. 2020/2021 academic year. Margarita de Pablo López.

7.2.2.2. Training activities

"Practical workshops on the implementation of fieldwork for the determination of critical biotic and chemical indices in the investigation of ecological crimes". Central Operational Environmental Unit (UCOMA), Headquarters of SEPRONA at the Guardia Civil. From 15 to 18 February 2021. Jorge Muñoz Conejero. Francisco Javier Piga de la Riba.

Update in Forensic Chemistry and Toxicology. LSA. Continuous Training Plan 2021, online, from 8 to 16 March 2021. Juan José Rivero Herrera.

INTCF Forensic Sciences Service: Fields of action, analytical possibilities. LSA. Continuous Training Plan 2021, online, from 22 to 26 March 2021. Juan José Rivero Herrera.

Forensic detection and identification of chemical weapons. Toxicity and impact on the environment. LSA. Continuous Training Plan 2021, online, from 4 to 11 May 2021. Pilar García de Yébenes Torres. Jorge Muñoz Conejero. Sergio Sánchez Pérez. Juan José Rivero Herrera.

Interpretation of toxicological results and their influence on the expert context in which the analysis is requested. LSA. Continuous Training Plan 2021, online, from 10 to 17 May 2021. Pilar García de Yébenes Torres. Juan José Rivero Herrera.

Basic LIMS: structure, data organisation and queries. LSA. Continuous Training Plan 2021, online, from 17 to 24 May 2021. Pilar García de Yébenes Torres.

Pesticides in forensic toxicology. LSA. Continuous Training Plan 2021, online, from 31 May 2021 to 7 June 2021. Jorge Muñoz Conejero. Juan José Rivero Herrera.

Practical workshop on the dissemination of the INTCF quality system. LSA. Continuous Training Plan 2021, online, from 21 to 28 September 2021. Pilar García de Yébenes Torres.

WEBINAR Safety, use, maintenance and design of fume cupboards. Waldner. 09 and 11/02/2021. Lara García Mínguez. Margarita de Pablo López. Pilar Vázquez Codias.

Course on the preparation of master formulas. Carpe Diem University and Vocational Training Centre. Online. 21/06/2021. Lara García Mínguez.

Introductory course to Photoshop. Carpe Diem University and Vocational Training Centre. Online. 15/06/2021. Lara García Mínguez.

Basic Internet course. Carpe Diem University and Vocational Training Centre. Online. 15/06/2021. Lara García Mínguez.

Outlook course. EGS Training Group (accredited by the Antonio de Nebrija University). Online. 25/05/2021 to 24/06/2021. Lara García Mínguez.

Introductory course to Excel 2019. EGS Training Group (accredited by the Antonio de Nebrija University). Online. 25/04/2021 to 24/06/2002. Lara García Mínguez.

Office365-CS Judicial BodiesEXPRES/138. Online classroom, Ministry of Justice. October 2021. Juan José Rivero Herrera.

Course on Good Electrochemistry Practice Sensors (pH, ISE, Conductivity). Metller Toledo. Online. 17/03/2021. Lara García Mínguez. Margarita de Pablo López.

Course on Good UV/Vis Spectrophotometry Practices. Multiple applications. Metller Toledo. Online. 04/03/2021. Lara García Mínguez.

Course entitled "European Pharmacopeia: Balances for analytical purposes". Metller Toledo. Online. 17/06/2021. Pilar Vázquez Codias.

Course on how to minimise the product effect. Metller Toledo. Online. 24/02/2021. Pilar Vázquez Codias.

Quality assurance in the INTCF, quality manuals, standard operating procedures, concepts and definitions. Secretariat of State for Justice, Ministry of Justice. Online. 22/11/2021 to 30/11/2021. Lourdes García Lojo. Lara García Mínguez. Margarita de Pablo López. Pilar Vazquez Codias.

Quality. Study of the UNE-EN ISO/IEC 17025:2017 standard. General Requirements for the Competence of Testing and Calibration Laboratories. Secretariat of State for Justice, Ministry of Justice. Online. 02/11/2021 to 12/11/2021. Lourdes García Lojo. Lara García Mínguez. Margarita de Pablo López. Pilar Vázquez Codias.

Multidisciplinary course on drugs: review of toxicity, updating of analytical methods, judicial and social value of analytical expert opinions. Secretariat of State for Justice, Ministry of Justice. Online. 18/10/2021 to 26/10/2021. Lourdes García Lojo. Lara García Mínguez. Margarita de Pablo López. Pilar Vázquez Codias.

7.2.2.3. Other activities

Participation in the master's degree in Chemical Science and Technology (UNED), February-June 2021; subjects: Infrared and Raman Microscopy (6 ECTS credits), Catalysis in Organic Chemistry (6 ECTS credits), High Resolution Nuclear Magnetic Resonance (6 ECTS credits), X-Ray Diffraction, Thermal Analysis and Gas Adsorption for the Characterisation of Compounds (6 ECTS credits), Application of Inorganic Solids in Green Chemistry (6 ECTS credits), Chemistry on Surfaces and Principles of Heterogeneous Catalysis (6 ECTS credits). Juan José Rivero Herrera.

7.3. Toxicological Assessment and Environmental Service of the Seville Department

As was the case in 2020, during 2021, the cases and requests received at the Service have continued to be affected by the influence of the pandemic, since both for the courts and prosecutors' offices and for the SEPRONA teams of the Civil Guard, the backlog accumulated over several months required the prioritisation of resolutions and actions.

7.3.1. Casework of the VTMA Service of the Seville Department during 2021 by type of report

Among the cases/petitions received this year, the largest number concerned wastewater spills mainly urban, and discharges that have not undergone any treatment. The other spills mainly correspond to the olive industry.

The casework of the VTMA Service at the Seville Department during 2021 is reflected in Figure 7.3.1.1 and Table 7.3.1.1 by type of report.

0 100 200 300 400 500 700 800 600 Spills General toxics Residues Others Polluted soils Valuation ■ No. cases registered ■ No. requests registered ■ No. samples analysed ■ No. analyses performed ■ No. reports issued

Figure 7.3.1.1: Casework of the VTMA Service of the Seville Department during 2021 by type of report

Table 7.3.1.1. Casework of the VTMA Service of the Seville Department during 2021 by type of report

Type of report	No. cases registered	No. requests registered	No. samples analysed	No. analyses performed	No. reports issued
Spills	18	34	199	725	41
General toxics	3	2	4	4	5
Residues	2	5	5	26	7
Others	2	2	2	2	2
Polluted soils	1	1	38	97	2
Valuation	1	1	1	1	4
TOTAL	27	45	249	855	61

7.3.2. Casework of the SVTMA Service at the Seville Department during 2021 by type of report

Most of the samples analysed are waste water and affected surface water. In 2021, the number of soil and leachate samples has increased.

Technical documents 8

Surface water 215

Lands 126

Liquid waste 17
Seawater 54
Sludge 17

Waste water 666

Figure 7.3.2.1. Casework of the VTMA Service at the Seville Department during 2021 by type of report

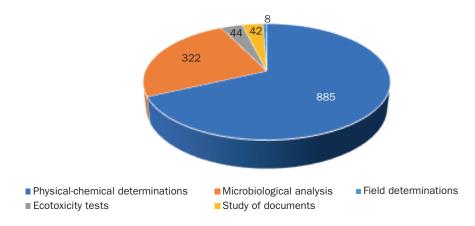
7.3.3. Types of analysis performed by the VTMA Service at the Seville Department during 2021

Concerning the analyses and studies performed at this Department in relation to the received requests, the following table (and corresponding graph) shows the distribution of the different works performed by the Service:

Table 7.3.3.1. Types of analyses carried out by the SVTMA at the Madrid Department during 2021

	No. of analyses	No. of analyses (%)
Physico-chemical determinations	885	67.55
Microbiological analysis	322	24.58
Ecotoxicity tests	44	3.35
Study/valuation of documents	42	3.20
Field outings/determinations in situ	8	0.61
TOTAL	1,310	100

Figure 7.3.3.1. Distribution of analyses by type of determinations



7.3.4. Forensic case of interest: Investigation of discharges from the olive industry by a cooperative association in a town in the province of Seville.

In 2019, the Environmental Prosecutor's Office of Seville opened criminal investigation proceedings to investigate a possible offence against natural resources and the environment caused by industrial olive activity in relation to untreated discharges into the sewage system of a town in the province of Seville. These discharges, without any kind of treatment, end up in Arroyo de la Ribera.

In January 2019, SEPRONA team in Ecija took samples from the industry's outlet chamber and from the point of discharge into the stream and the analyses performed revealed high levels of organic matter, nitrogen and phosphorus nutrients and phenolic compounds derived from the decomposition of the olive industry's waste.

In the municipal sewer, it combines with urban wastewater and flows untreated into the stream, allegedly causing substantial damage to water quality.

In June 2019, during a visual inspection of the discharges and the stream carried out by VTMA's staff, it was found that the discharges were still occurring and that the effect on the stream, given its *in situ* parameters and organoleptic properties, spanned more than 2 km in length, causing a depletion of dissolved oxygen, indicative of substantial damage to water quality.

The following images depict the appearance of the spill at the point of impact on the stream and the impact of the spill 2 km downstream.

Figure 7.3.4.1. Appearance of the discharge at the point of impact on the stream Figure 7.3.4.2. Impact on the stream 2 km downstream





Figure 7.3.4.3. Details of the site where the spill occurred

The area of influence of the discharge is located in the Sierra y Mioceno de Estepa groundwater body, corresponding to identification code 051.043(1) within the Demarcación Hidrográfica del Guadalquivir. It consists of permeable geological formations, specifically the permeable limestone and dolomite geological formations of the Sierra de Estepa, which, in the area in question, are highly permeable.

The receiving medium for the discharge is Arroyo de la Ribera, which rises near the town and flows into the River Blanco.

The report concludes that a discharge with the characteristics described causes substantial damage to the quality of the receiving waters and, depending on the flow and continuity of the discharge, may pose a risk to the Sierra y Mioceno de Estepa groundwater body and may seriously damage the balance of the natural systems.

In January 2021, at the request of Magistrate's Court No. 2 in Estepa, a new inspection was performed and samples taken with the Civil Guard's SEPRONA team in Ecija, to check whether the discharges were still occurring and whether the stream was affected.

During the inspection, it was found that, despite the fact that the manhole that diverts the discharges to the sewer had been closed, they were still occurring, with very high parameters in relation to pH, organic matter and nutrients, although at a very low flow rate.

It was also found that the industry was piping waste water to ponds several kilometres away, where it was to be treated by a waste manager.

At the time of the inspection, these ponds were overflowing into one another and eventually trickled out of the last pond into the adjoining farmland, where the surface runoff joined the same stream.

The following pictures show the appearance of the ponds and the seepage from the slope of the ponds.

Figure 7.3.4.4: Series of three basins where the waste accumulates; the communication by overflow due to overfilling can be seen.



Figure 7.3.4.5: This picture shows the filling level of the third basin and the overflow of the third basin.



Figure 7.3.4.6. Appearance of the lateral slope of the third pond; the run-off of the liquid waste produced by the overflow and seepage of the third pond can be seen.



The report issued by the Service concluded that both the discharge of this waste water and its disposal in ponds without an adequate management system, as described above, may seriously damage the balance of the system of the Sierra y Mioceno de Estepa groundwater body.

7.3.5. Teaching and scientific activity

7.3.5.1. Contribution in scientific congresses

Lhoëst Mathijsen F. Sampling techniques in marine environments. "1st Environmental Training Course on Marine Pollution and Sampling in the Marine Environment". Online training from 4 to 7 October 2021. Bay of Cadiz. Practical workshops, two shifts, 26 October and 16 November 2021.

7.3.5.2. Teaching and training activities

Teaching

Lhoëst Mathijsen F. "Practical workshops on the implementation of fieldwork for the determination of critical biotic and chemical indices in the investigation of ecological crimes". From 15 to 18 February 2021. Valdemoro, Madrid.

Training

Cano Rodríguez, M. E. Interpretation of expert opinions issued by the National Institute of Toxicology and Forensic Sciences. From 19 to 27 April 2021. Centre for Legal Studies. Online Continuous Training.

Cano Rodríguez, M.E.; Lhoest Mathijsen, F. Detection and identification of chemical weapons in forensics. Toxicity and impact on the environment. From 4 to 11 May 2021. Centre for Legal Studies. Online Continuous Training.

Cano Rodríguez M.E. Interpretation of toxicological results and their influence on the expert context in which the analysis is requested. From 10 to 17 May 2021. Centre for Legal Studies. Online Continuous Training.

Cano Rodríguez, M.E.; Gómez Bujedo, S.; Lhoest Mathijsen, F. Basic LIMS: structure, data organisation and queries. From 17 to 24 May 2021. Centre for Legal Studies. Online Continuous Training.

Cano Rodríguez, M.E.; Gómez Bujedo, S.; Lhoest Mathijsen, F. Pesticides in forensic toxicology. From 31 May to 7 June 2021. Centre for Legal Studies. Online Continuous Training.

Cano Rodríguez M.E. Forensic toxicology. Toxic pathology. From 7 to 14 June 2021. Centre for Legal Studies. Online Continuous Training.

Cano Rodríguez M.E. Practical workshop on the dissemination of the INTCF quality system. From 21 to 28 September 2021. Centre for Legal Studies. Online Continuous Training.

Cano Rodríguez M.E. Multidisciplinary forensic intervention in multi-victim incidents. From 15 to 22 November 2021. Centre for Legal Studies. Online Continuous Training.

Cano Rodríguez M.E. Practical workshop on validation of forensic toxicology methods. From 4 to 08 October 2021. Centre for Legal Studies. Online Continuous Training.

Cano Rodríguez M.E. Databases of criminal interest: Operational and legal aspects. From 13 to 20 October 2021. Centre for Legal Studies. Online Continuous Training.

Cano Rodríguez M.E. Quality assurance in the forensic process. A Step Forward. From 2 to 05 November 2021. Centre for Legal Studies. Online Continuous Training.

Training of laboratory technicians and assistants

"Multidisciplinary course on drugs: review of toxicity, updating of analytical methods, judicial and social value of analytical expertise". 10-26 October 2021. Centre for Legal Studies. Online Continuous Training.

"Quality assurance in the INTCF, quality manuals, standard operating procedures, concepts and definitions". From 8 to 11 November 2021. Centre for Legal Studies. Online Continuous Training.

"Quality Study of the UNE-EN ISO/IEC 17025:2017 standard. Testing and calibration laboratories". From 16 to 23 November 2021. Centre for Legal Studies. Online Continuous Training.

7.3.6 Other merits

Credential awarded to Françoise Lhoest Mathijsen, Head of the VTMA Service of the Seville Department: Cross of Merit of the Guardia Civil with white distinction, in recognition of his professionalism, dedication and love of service, Order of 29 June 2021, awarding the Cross of the Order of Merit of the Guardia Civil.



Each INTCF department has a Quality Assurance Service, which is essential for managing the reliability of results. At the La Laguna Delegation, where there is no Quality Assurance Service, these functions are assumed by an expert appointed by the Director of the Delegation as head of Quality Assurance together with the doctors also appointed by the Director of the Delegation as coordinators of the Services.

One of the most important functions of the Quality Assurance Service is to collaborate with the different services of the Department to which it belongs in the implementation, maintenance and improvement of a quality system based on ISO 17025. General requirements for the competence of testing and calibration laboratories, through the management and control of all quality assurance aspects at the different Analytical Services of the INTCF.

Concerning the implementation and maintenance of accredited test scopes based on ISO 17025, and in all matters relating to the process and scope of accreditation as test providers based on the UNE-EN ISO/IEC 17043 Standard, the Quality Assurance Services of each Department act as the main interlocutor with the National Accreditation Body (ENAC).

Thus, the Quality Assurance Service ensures two of the functions of the INTCF as defined in Article 480 of the Organic Law 6/1985 of 1 July 1985 on the Judiciary, which are "to contribute to the unity of scientific criteria" and "to the quality of analytical expertise", and its actions reflect the recognition of the INTCF as a centre of reference in toxicology and forensic sciences.

Although each Quality Assurance Service carries out its functions within its Department/ Delegation, during 2021 the following tasks were carried out jointly:

- Fulfilling one of the objectives established for the Quality Assurance Services, in the 2020-2022 Action and Research Plan, the general functions have been reviewed among the Departments and the Delegation, to harmonise their classification and include them in the web portal for their knowledge, with the following being agreed:
- · Management and control of the quality system documentation
- Elaboration of standard operating procedures related to quality management
- Follow-up and closure of non-conformities, corrective actions, complaints and risk, opportunity management and improvement actions
- Internal audits management
- Development/coordination of the interlaboratory comparisons organised by the INTCF, as a reference centre
- Control and evaluation of the interlaboratory comparison results in which the centre participates

- Advice and monitoring of validations
- · Collaboration with the different INTCF Services in:
 - The implementation, maintenance, and improvement of a quality system based on ISO 17025. General Requirements for the Competence of Testing and Calibration Laboratories
 - · Management of staff training
 - The elaboration and revision of the calibration, verification, and maintenance program equipment
 - The elaboration and revision of the Standard Operating Procedures for Technical Work
 - Maintenance of accreditations and in the process of new scopes

As a result of these meetings, a consensus was reached on the proposal to the National Directorate to update the list of posts with a view to adjusting the tasks to those that have been consolidated, adding a future perspective.

- The design and monitoring of the project for the management of equipment from the LIMS application has been carried out with a view to harmonising, streamlining and controlling the different operations required under ISO 17025:2017.
- Within the programmes as part of the General Framework for Quality Improvement of the General State Administration (AGE), established by the Directorate General of Public Governance, Subdirectorate General of the General Inspection of Services, the Quality Assurance Services have carried out a survey to reflect the activity of the INTCF in terms of quality (Report on the Activity of the Spanish Ministries, ISAM 2020).
- The Heads of the Quality Assurance Services of the different Departments have actively participated and collaborated within the Quality group of the Network of Official Spanish Forensic Laboratories (RLFOE), virtual attending the annual meeting. At these meetings, new developments in international standards that apply to the forensic field are shared. It serves as a forum to exchange opinions related to the management and implementation of quality with the other quality managers of Spanish forensic laboratories.
- In May 2021, following negotiations between the Ministry of Justice and the trade unions, the 2021 Reinforcement Plan for the National Institute of Toxicology and Forensic Sciences and the Institutes of Legal Medicine and Forensic Sciences was approved. This plan resulted in an increase in the management of training by the Quality Assurance Services.

 In 2021, Quality Assurance Services staff participated as lecturers at national and international training activities held virtually. These include three courses on quality assurance organised by the Centre for Legal Studies and the two courses organised by the Ministry of Justice through the National Institute of Public Administration.

In relation to the international activities, worth particular mention were the collaborations with the Twinning Project with Turkey, TR16 IPA JH 03 18, with the Spanish Agency of International Cooperation for Development (AECID) and with the ICrime Project for the reinforcement of investigation units, forensic institutes, networks and criminal investigation procedures at the Central American Integration System.

The personnel resources available to the Quality Assurance Services for the development of all the activities carried out during 2021 are as follows in Table 8.1.

Table 8.1: Staff of the Quality Assurance Services at the different Departments

	INTCF-MADRID	INTCF-BARCELONA	INTCF-SEVILLA
Head of the Service	1	1	1
Facultatives	2	1 + 1*	2
Specialist technicians	1	1**	-
Laboratory assistants	-	-	-
Clerical staff	1	-	-

 $[\]ensuremath{^{\star}}$ Professional with partial dedication in the Service.

Overall data on the number of requests registered and reports issued by the INTCF Quality Assurance Services during 2021 are collected in Figure 8.1.

Figure 8.1 Overall data of the number of requests registered and reports issued by the INTCF Quality Assurance Services during 2021



^{**} Specialist technician that does activities related to the management focused on equipment and MRs of the Department

Table 8.2: Overall data of the number of requests registered and reports issued by the INTCF Quality Assurance Services during 2021

2021	No. requests generated	No. reports issued
Madrid	447	456
Barcelona	112	107
Seville	217	102
TOTAL	776	665

Not all the activities carried out by the different Quality Assurance Services are recorded in the LIMS system; to this end, the detailed distribution of the activities carried out by each of the Quality Assurance Services during 2021 is shown in the different tables shown throughout this chapter (Tables 8.1.1.1, 8.2.1.1 and 8.3.1.1).

These activities would be defined as follows.

1 and 2. Management of system documents. Standard Operating Procedures (SOPs) are written documents that describe how an activity included within the Quality System has to be carried out. The annexes are part of the SOPs and include specific information. Due to its importance, constitute an independent document. Data collection sheets (DCSs) are used to record the activity As the SOPs, the DCSs, and the annexes related to them are the documentary basis of the quality system implemented in the Department. The review of their validity and adaptation to new quality requirements and to the operational changes that need to be implemented is important to ensure the correct functioning of the laboratories. The Quality Assurance Service reviews the technical preparation and the design of all of them before they are put into effect. Once approved, the SGC is in charge of its management and distribution.

- **3 and 4. Staff training.** The training and qualification of the personnel working in the different Services are one of the pillars of the quality of the work carried out in the laboratory. To this end, the ISO 17025 quality standard establishes as a requirement that the laboratory is sure that all the staff has the necessary competence to carry out the activities for which they are responsible. The training programs and all the registers derived until the obtainment of the qualification certificate are supervised by the SGC.
- **5.** Management of internal/external equipment calibrations. The SGC collaborates with the Services in the preparation and review of the equipment calibration, verification, maintenance program, and the management of external and internal calibrations. It also coordinates and manages the inventory of equipment, reference materials, standards, and reference data.
- **6. Validation studies.** The validation of a test method involves working out studies to check that the methods are suitable for their intended purpose. Although the Services are responsible for carrying out the validation studies, the role of SGC is to advise and guide

the design (parameters to be studied, the matrices to be tested, the evaluation criteria to be applied to evaluate the results), the development and the evaluation of results.

7 and 8. Intercomparison exercises in which the INTCF participates. Participation in Quality controls, called Intercomparison Exercises, is one of the most useful tools that laboratories have for their self-assessment and assurance of the validity of the results they issue. The Services of the different Departments/Delegations of the INTCF receive samples similar to those of the casework, analyse them, and evaluate the results obtained against criteria previously established by the suppliers. The SGC manages the reception of the samples from these exercises. In some cases, it is in charge of sending results after their analysis. Once the provider issues a report, the SGC evaluates the participation result of each Service. Each new supplier is assessed on the basis of a test developed by the Quality Assurance Services to evaluate the suitability of the characteristics and technical aspects of the new testing exercise.

- 9 and 10. Intercomparison exercises that the INTCF organises. The INTCF Regulation recognises its role as a reference centre. Among its functions as a reference centre is to organise quality assurance controls that allow self-evaluation of the different laboratories in the methods. In this regard, the INTCF acts as a provider of Intercomparison Exercises organising three exercises from the Quality System Services. A quality control for forensic and paternity laboratories realised annually by the INTCFM, in collaboration with the Spanish and Portuguese Speaking Group of the ISFG, a four-monthly one organised and carried out by the INTCFS and focused on laboratories performing blood alcohol and volatiles analysis, and an annual one, organised by the INTCFB, which allows laboratories to analyse drugs of abuse commonly found in stashes.
- **11. Internal audits.** By carrying out internal audits, the laboratory executes a continuous monitoring of compliance with the requirements of the implemented Quality System. They are implemented allowing the detection of deviations from the work procedures and established policies. These audits are done in planned intervals as it is established in the Internal Audits Programme elaborated by the Quality Assurance Service (SGCs). They are approved by the Direction when the circumstances of the moment recommend it (when changes are introduced in the work systematics, it is suspected non-compliance with the established Quality requirements, etc.).
- 12. Quality system review. This activity, together with the internal audits, is of great interest to obtain information about compliance with the requirements of the Quality System implemented and with the requirements of ISO 17025, and whether they are correctly implemented. The quality system is reviewed periodically in a meeting with Management at least once a year. This is to assure its efficiency and, if necessary, implement corrective or improvement actions. The information derived from this review is duly documented in a comprehensive and detailed report drawn up by the Quality Assurance Service. It does not include only the review findings, but also any need for change detected and the proposed actions for improvement.

13, 14 and 15. Non-compliant work, corrective actions, actions to address risks and improvement actions. When any aspect of the activities carried out under the Quality System does not comply with the established procedures or requirements, a non-conformity or non-conforming work (NC) is opened to study the causes of the deviation, assess the influence it may have had in other areas and the risk that it may pose for the activity of the laboratory. Corrective actions (AC) must be established to correct the causes that have given rise to this and avoid a similar situation in the future. The SGC documents all the NCs, evaluates the corrective actions proposed by the Service and monitors them.

The new risk-based approach of the new ISO 17025 quality standard requires laboratories, through the SGC, to identify and assess the risks and opportunities associated with the activities performed.

16. Management of claims and complaints. The SGC performs the initial management of the communications (professions, requests, etc.) in which it is suspected that there may be implicitly a claim or that, in the case of not taking the appropriate measures, may generate a claim, as well as the management of user complaints in relation to any activity of the INTCF. It is also responsible for the management when a citizen submits or sends a complaint.

8.1. Madrid Department Quality Assurance Service

8.1.1. Activities performed by the Service

The activities and functions that the Quality Assurance Service has carried out during 2021 are reflected in Table 8.1.1.1.

Table 8.1.1.1. Activity data for 2021

Activities	
Development of new Standard Operating Procedures (SOPs) and Data Collection Sheets (DCSs). Modification of versions of procedures and sheets	43 (PNT) 40 (DCS)
2. New annexes elaboration and modification of annexes versions	33
3. Training programs and qualification certificates of the staff	47
4. Initial and going training of personnel in the Quality System	20
5. Management of external equipment calibrations	1
6. Validation studies of analysis methods	7
7. Assessment of participation in intercomparison exercises	102 (107)
Conformity assessment of organisers of intercomparison exercises in which the Department participates	-
9. Evaluation of external participant (reports)	4
10. Evaluation of external participant (certificates)	346
11. Internal audits	6
12. Management review of the quality system by the Direction	2

Activities	
13. Records of non-conformities or non conformity works	58
14. Records of corrective actions	21
15. Records of actions to address risks and opportunities	5 + 37
16. Actions to address risks and opportunities	5
17. Claims and complaints management	24

The following is detailed to fully describe the data corresponding to the activities collected in this table.

1 and 2. Management of system documents. Out of a total of 43 working procedures in force, 32 existing versions have been modified, mainly in terms of techniques and reagent processing, and to a lesser extent in terms of equipment management and operation, and 11 new technical procedures have been developed. From the 40 DCSs put into force, 22 were new. The remainder were modifications to existing versions. 33 annexes were managed.

3 and **4**. Staff training. In 2021, 47 training programmes were reviewed and managed with the corresponding records. 24 of these were training programmes to access the centre and the rest were created for training staff on new techniques.

Likewise, as part of the initial training, the SGC gave seminars to raise awareness of the Quality System implemented at the INTCFM. During 2021, training in Quality was imparted to 18 people (2 assistants, 13 experts and 3 specialist laboratory technicians). This seminar was also imparted to two trainees who were on a training course at the centre. Over the course of the year, all the queries made by the Services were answered, especially about incidents or concerns related to the management of samples, technical activities, and the issuing of reports.

- **5. Management of external equipment calibrations.** In 2021, the calibration, verification and maintenance programme of each Service was reviewed. Annually, a total of 92 pipettes were externally calibrated by an ISO 17025 accredited company for automatic pipettes with a volume of 10 ml or less once again. The SGC has coordinated with the Services to send them, subsequently supervising the evaluation of the external calibration carried out by them. The incidents detected after calibration and their possible transcendence in the laboratory's analytical work was evaluated.
- **6. Validation studies.** During 2021, 6 validations were launched (Table 8.1.1.2), 4 of them having been completed during the same year and 3 launched in previous years.

Table 8.1.1.2. Validations launched in 2021

SERVICE	Test/technique method	Status				
	Panbio-COVID-19 Ag Rapid Test Device (nasopharyngeal) antigen detection test (Abbott)					
BIOLOGY	Modification of the diatom research protocol by organic digestion with nitric acid (PNT-B-T001). Use of the minidigiprep system for organic digestion with nitric acid	Finalised				
DRUGS	Confirmation and quantification by GC-MS-MS in blood of 11-NOR-D9- TETRAHIDROCANNABINOL-CARBOXYL-CARBOXYL (THC-COOH), TETRAHIDROCANNBINOL (THC), HYDROXY-TETRAHIDROCANNBINOL (THC-OH), CANNABINOL (CBN), CANNABIDIOL (CBD) in the Q-222 instrument	Finalised				
	THC quantification by HPLC with DAD	Finalised				
CHEMICAL	Quantitative determination of ethyl alcohol in biological fluids by GC-HS using 500 µl and 100 µl sample in the Q-239 instrument.	In process				
	Confirmation by GC-MS/MS in urine of THC-COOH and THC	In process				
Launched in previous years and completed in 2021						
DRUGS	Confirmation and quantification by GC-MS-MS in blood of 11-NOR-D9- TETRAHIDROCANNABINOL-CARBOXYL-CARBOXYL (THC-COOH), TETRAHIDROCANNBINOL (THC), HYDROXY-TETRAHIDROCANNBINOL (THC-OH), CANNABINOL (CBN), CANNABIDIOL (CBD) in the D190 instrument	Finalised				
	Verification of the Poroshell 120 EC-C18 column and HEROPOROSHELL method for use in the PNT-D-T006 accredited procedure on UPLC 1290 Infinity II (D-192) equipment.	Finalised				
TOXICOLOGICAL VALIDATIONS AND ENVIRONMENT	Procedure for the analysis of elements by plasma emission spectrometry (ICP-OES)	Finalised				

Furthermore, 20 spreadsheets associated with tests or techniques have been validated, 18 by the Drugs Service and 2 by the Toxicological Assessment and Environment Service. Furthermore, the validation of worksheets generated as a result of the implementation of the interoperability of the equipment with the OpenLab management system was performed.

7. Intercomparison exercises in which the INTCFM participates. During 2021, we participated in 53 exercises, resulting in 169 evaluation reports from SGC, as some exercises consist of two or more rounds per year.

After the first participation in an Intercomparison Exercise, the laboratory must assess whether the programme meets the requirements to be used as quality control. In 2021, two compliance assessments were carried out on the exercise organised by ARVECON GmbH, which is the provider of the Serum and Urine GHB Presence Exercise and the SFD Exercise: Toxicological Analysis in Drivers. Both assessments were satisfactory, with the Drugs Service maintaining its participation in both.

9 and **10**. Intercomparison exercises that the INTCFM organises. One more year INTCFM has organised annually collaborating with the Spanish and Portuguese Speaking Group of the ISFG, a quality control directed to forensic and paternity laboratories known as "Study of DNA polymorphisms in bloodstains and other biological samples". After the evaluation

of the results remitted by the participants in 2021, a summary was drawn up as well as a final participation and results report with the methodologies and results of each laboratory and with the assigned value, preparing a report for each level: basic, advanced and animal item.

In relation to the evaluation certificates, a total of 346 certificates were issued corresponding to 3 types of participation certificates with the assessment of results: basic level kinship module and forensic module and animal identification (see 8.1.4. Case of interest).

- **11.** Internal audits. In 2021, 4 audits were carried out: 1 to review the management system of the intercomparison exercise, 2 to assess the management of equipment in the Chemistry and Histopathology Departments, and 1 pre-drug destruction audit. As a result, 4 deviations were detected and corrected by the Services.
- **12. Quality system review.** In 2021, testing activities and activities as a supplier of the forensic intercomparison exercise were reviewed. The results of both reviews were recorded in the corresponding minutes prepared by the SGC staff.
- 13, 14 and 15. Non-conforming works, improvement, and corrective actions. In 2021 we have managed 58 NCs, 3 of these related to some coordination aspect in the Intercomparison Exercise that the INTCFM organises ("Study of DNA polymorphisms in bloodstains and other biological samples"). Corrective actions were established for only 21 of the NCs, 17 of which were implemented during 2021.

The main sources of detection were the deviations of the proper staff services (37%) and the evaluation activities in the quality of the tests (41%).

The SGC also manages actions, either at the proposal of the Service or by the SGC itself, to improve the management system and the laboratory's activities (standardisation of processes, optimisation of Quality management, optimisation of methods, etc.). In 2021, 37 improvement actions were opened, 13 of which were developed in 2021.

5 risks were identified and evaluated: 1 related to equipment; 3 to test methods and 1 to sample management.

16. Management of claims and complaints. In 2021, a total of 23 complaints were registered, 4 of which originated from requests for counter-analysis, and the rest were requests for different Services.

In terms of complaints, one was received, related to the management of sample referrals.

8.1.2. Intercomparison exercises in which the INTCFM Services participated in 2021

Table 8.1.2.1 lists the inter-comparison exercises in which INTCFM Services participated in 2021.

Table 8.1.2.1. Intercomparison exercises in which the INTCFM Services participated in 2021

Participation in intercomparison exercises of the Biology Service

Program: Analysis of DNA polymorphism in blood stains and other biological samples

Organised by: INTCF-GHEP-ISFG

Frequency: Annual

Parameters: forensic and kinship genetics and preliminary studies in blood, hair and other matrices

Program: GEDNAP Proficiency test

Organised by: GEDNAP-ENFSI (German Speaking Working Group of the International Society for Forensic Genetics)

Frequency: Annual

Parameters: forensic and kinship genetics in blood stains and other biological fluids

Program: Vitreous Fluid Post-mortem

Organised by: College of American Pathologists (CAP)

Frequency: twice a year

Parameters: glycemia in vitreous body

Program: Bacteriology

Organised by: Sociedad Española de Enfermedades Infecciosas y Microbiología Clínica (SEIMC)

Frequency: per month

Parameters: cultivation, identification, and resistance to antibiotics

Program: Streptococcus pneumoniae and Legionella sp antigen detection (BAS)

Organised by: College of American Pathologists (CAP)

Frequency: twice a year

Parameters: determination of bacterial antigens

Program: Amplification of nucleic acids of respiratory virus (ID-2)

Organised by: College of American Pathologists (CAP)

Frequency: twice a year

Parameters: molecular analysis of the following viruses: adenovirus, coronavirus/rhinovirus, influenza, parainfluenza

and respiratory syncytial virus in liquid samples

Program: SARS-CoV-2, Molecular

Organised by: College of American Pathologists (CAP)

Frequency: twice a year

Parameters: molecular detection of SARS-CoV-2 virus

Participation in intercomparison exercises of the Forensic Sciences Service

Program: Adhesive Tape Analysis

Organised by: Collaborative Testing Services (CTS)

Frequency: Annual

Parameters: adhesive tape analysis

Program: Questioned Documents Examination - Forensic Testing Programme

Organised by: Collaborative Testing Services (CTS)

Frequency: Annual

Parameters: document analysis

Program: Fibers analysis

Organised by: Collaborative Testing Services (CTS)

Frequency: Annual Parameters: fibre analysis

Program: Human vs Non Human Bone Origin Determination

Organised by: Collaborative Testing Services (CTS)

Frequency: Annual

Parameters: bone origin determination

Program: Handwriting Examination-Forensic Testing Program

Organised by: Collaborative Testing Services (CTS)

Frequency: Annual

Parameters: written documents and signatures study

Participation in intercomparison exercises of the Forensic Sciences Service

Program: Paint analysis

Organised by: Collaborative Testing Services (CTS)

Frequency: Annual

Program: GSR (Gun Shoot Residues). Distance Determination

Organised by: Collaborative Testing Services (CTS)

Frequency: Annual

Parameters: shooting distance on clothing samples

Program: ENFSI Proficiency test on identification of GSR (Gun Shoot Residues) by SEM/EDX

Organised by: ENFSI Firearms/GSR by SEM Working Group

Frequency: Annual

Parameters: analysis of gunshot residues in firing kits

Program: ENFSI Proficiency test on shot range estimation by SEM/EDX

Organised by: ENFSI Firearms/GSR by SEM Working Group

Frequency: Annual

Parameters: firing distance analysis

Program: Collaborative Exercise Fiber analysis

Organised by: ENFSI European Textile & Hair Working Group

Frequency: Annual Parameters: fibre analysis Program: EDEWG QA Trial

Organised by: ENFSI European Document Expert Working Group (EDEWG)

Frequency: Annual

Parameters: document analysis.

Program: Collaborative Exercise ENFSI (ENFHEX)
Organised by: ENFSI European Handwriting Expert

Frequency: Annual

Parameters: analysis of handwritten documents
Program: Hair-Examination Collaborative Exercise

Organised by: ENFSI European Textile & Hair Working Group Frequency: Annual

Parameters: hair study
Program: EPG Paint Test
Organised by: ENFSI
Frequency: twice a year
Parameters: study of paintings

Program: Interlaboratory documentography Test (TIGE)

Organised by: Spanish network of official forensic laboratories (RFLOE)

Frequency: Annual

Parameters: analysis of handwritten documents

Participation in intercomparison exercises from the Drugs Service

Program: Proficiency study AQA

Organised by: National Measurement Institute of Australian Government (NMI)

Frequency: four-monthly

Parameters/samples: heroin, cocaine, amphetamine compounds in powder-solid samples

Program: International Quality Assurance Programme (IQAP) Seized materials Group

Organised by: United Nations Office on Drugs and Crime (UNODC)

Frequency: twice a year

Parameters/samples: Drugs in powder-solid samples

Program: Interlaboratory Exercise on Drugs of Abuse Commonly Abused in Addictions

Organised by: INTCF-BARCELONA

Frequency: Annual

Parameters/samples: Drugs in powder-solid samples

Program: International Quality Assurance Programme (IQAP-UNODC) Biological Specimens Group.

Organised by: United Nations Office on Drugs and Crime (UNODC)

Frequency: twice a year

Parameters/samples: identification and quantification of the most common drugs of abuse in urine

Program: Forensic Blood Toxicology Proficiency Testing (Quartz)

Organised by: LGC Frequency: quarterly

Parameters/samples: drugs of abuse and psychotropics in the blood

Participation in intercomparison exercises from the Drugs Service

Program: Drugs in Hair Proficiency Test (DHF)

Organised by: Arvecom Gesellschat für Toxikologishe und Forensisde Chemie (GTFCh)

Frequency: four-monthly

Parameters/samples: drugs of abuse and psychotropics in the hair

Program: Drugs in Serum

Organised by: Arvecom Gesellschat für Toxikologishe und Forensisde Chemie (GTFCh)

Frequency: four-monthly

Parameters/samples: drugs of abuse and psychotropics in serum

Program: Drugs in serum and urine Proficiency Test

Organised by: Arvecom Gesellschat für Toxikologishe und Forensisde Chemie (GTFCh)

Frequency: four-monthly

Parameters: GHB in serum and urine

Program: Toxicological analysis for drivers fitness determination

Organised by: Arvecom Gesellschat für Toxikologishe und Forensisde Chemie (GTFCh)

Frequency: four-monthly

Parameters/samples: drugs and pharmaceuticals in urine

Participation in intercomparison exercises of the Chemistry Service

Program: Whole blood Alcohol/Volatiles survey Organised by: College of American Pathologists

Frequency: four-monthly

Parameters/samples: alcohol, volatiles, and ethylene glycol in blood

Program: Forensic Toxicology (criminalistics)
Organised by: College of American Pathologists

Frequency: twice a year

Parameters/samples: medicines and drugs in blood and urine

Program: Blood Oximetry Survey

Organised by: College of American Pathologists

Frequency: four-monthly

Parameters/samples: carboxyhaemoglobin in blood

Program: Flammable analysis

Organised by: Collaborative Testing Service

Frequency: Annual

Parameters/samples: combustion-accelerating substances in various media

Program: Forensic Blood Toxicology Proficiency Testing (Quartz)

Organised by: LGC Frequency: quarterly

Parameters/samples: drugs of abuse and psychotropics in the blood

Program: Drugs in Hair Proficiency Test (DHF)

Organised by: Arvecom Gesellschat für Toxikologishe und Forensisde Chemie (GTFCh)

Frequency: four-monthly

Parameters/samples: drugs of abuse and psychotropics in the hair

Program: Blood Drug Analysis

Organised by: Collaborative Testing Service

Frequency: Annual

Parameters/samples: drugs of abuse and psychotropics in the blood

Program: Quality control of ethyl alcohol in the blood

Organised by: INTCF-SEVILLA Frequency: four-monthly

Parameters/samples: ethyl and methyl alcohol in blood

Program: Drugs in Oral Fluid PT Scheme Organised by: LGC Proficiency Testing

Frequency: quarterly

Parameters/samples: drugs of abuse in oral fluid

Program: Vitreous Fluid, Post-mortem

Organised by: College of American Pathologists

Frequency: twice a year

Parameters/samples: sodium and potassium, and ethyl alcohol and acetone in vitreous body

Participation in intercomparison exercises of the Toxicology and Environmental Assessment Service

Program: Non Specific Determinands. Aquacheck - Group 11.

Organised by: LGC Standards Frequency: twice a year

Parameters: DBO, DQO, MBAS, COD/COT, suspended solids in aqueous matrix

Program: Aquacheck. Grupo 17 D Organised by: LGC Standard Frequency: Annual

Parameters/samples: total phenol, ammonia, total phosphorus and total nitrogen in wastewater.

Program: Aquacheck. Grupo 17 C Organised by: LGC Standard Frequency: Annual

Parameters/samples: metals in waste water

Program: Aquacheck. Grupo 12 C Organised by: LGC Standard Frequency: twice a year

Parameters/samples: chromium VI in effluent matrix

Program: Aquacheck. Group 12 Organised by: LGC Standard

Frequency: Annual

Parameters/samples: metals in effluent matrix

Program: Quality in Water Analysis Scheme (QWAS) WT 419

Organised by: LGC Standards Frequency: twice a year

Parameters/samples: total coliforms, fecal coliforms, and fecal streptococci in waters

Program: Quality in Water Analysis Scheme (QWAS) WT 422

Organised by: LGC Standards

Frequency: Annual

Parameters/samples: total coliforms, fecal coliforms and fecal streptococci in marine waters

Program: Effluent, waste water, Contaminated Land and Hazardous waste Organised by: Laboratory Environmental Analysis Proficiency (LEAP)

Frequency: twice a year

Parameters/samples: pH and conductivity in aqueous matrix

Program: Effluent, waste water, Contaminated Land and Hazardous waste Organised by: Laboratory Environmental Analysis Proficiency (LEAP)

Frequency: twice a year

Parameters/samples: Settled solids in aqueous matrix

Program: Effluent, waste water, Contaminated Land and Hazardous waste Organised by: Laboratory Environmental Analysis Proficiency (LEAP)

Frequency: twice a year

Parameters/samples: nitrate, nitrite, ammonium, chloride, orthophosphate, total phosphorus, total nitrogen in

aqueous matrix

Program: Effluent, waste water, Contaminated Land and Hazardous waste Organised by: Laboratory Environmental Analysis Proficiency (LEAP)

Frequency: twice a year

Parameters/samples: bromide and fluoride in aqueous matrix

Program: Effluent, waste water, Contaminated Land and Hazardous waste Organised by: Laboratory Environmental Analysis Proficiency (LEAP)

Frequency: twice a year

Parameters/samples: calcium, magnesium, potassium, sodium, hardness, alkalinity in aqueous matrix

Program: Wastewater: Toxicity (GSCAR4) Organised by: Quality Services Office (GSC)

Frequency: Annual

Parameters/samples: toxicity (inhibitory substances) in waste water

8.1.3. Accreditation scopes

The Madrid Department has two test accreditation files, Accreditation No. 297/LE1367 and Accreditation No. 297/LE1366, that bring together testing methods in the forensic and environmental areas respectively. During 2021, ENAC conducted a reassessment audit of both dossiers, which was satisfactory, with both scopes remaining unchanged.

It is also responsible for the accreditation of the INTCF under ISO 17043, as Intercomparison Programme provider in Accreditation No. 8/PPI016. During 2021, it successfully passed the follow-up audit conducted by ENAC.

8.1.4. Case of interest. Intercomparison exercise of "Study of DNA polymorphisms in blood stains and other biological samples"

Since 1992, the Quality Assurance Service at the Madrid Department has coordinated an annual quality control, the intercomparison exercise of "Study of DNA polymorphisms in bloods tains and other biological samples". This exercise provides laboratories performing paternity testing and forensic investigation with various parentage items, which must be genetically identified, and forensic items to perform fluid nature analysis and genetic analysis. The exercise also provides theoretical kinship and forensic cases for biostatistical calculations.

This is divided into two levels: Basic and Advanced: The Basic is divided into two modules: kinship and forensic, and the Advanced has just one forensic module. Furthermore, the option of genetically identifying a non-human item is available.

It should be noted that the kinship and forensic modules of the basic level of this exercise have been accredited under the criteria set out under ISO 17043 since 2014.

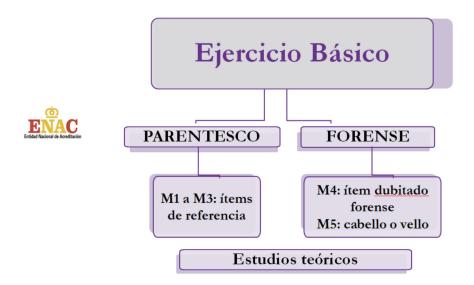


Figure 8.1.4.1 Modules of the EIADN 29 intercomparison exercise (2021)

A non-human item was also available this year.

Figure 8.1.4.2. Schedule of the Intercomparison Exercise "Study of DNA polymorphisms in blood stains and other biological samples"

Calendario 2020



Calendario 2020-2021

	DIC	CIEME	RE		
Ма	Mi	Ju	Vi	Sá	Do
1	2	3	4	5	6
8	9	10	11	12	13
					20
					27
			25	26	27
29	30	31			
	Ma 1 8 15 22 29	1 2 8 9 15 16 22 23	1 2 3 8 9 10 15 16 17 22 23 24	1 2 3 4 8 9 10 11 15 16 17 18 22 23 24 25	1 2 3 4 5 8 9 10 11 12 15 16 17 18 19 22 23 24 25 26



In 2021, the exercise schedule for item preparation, homogeneity studies and contamination control (fluid nature studies and genetic analysis) prior to shipment and distribution of items to participants followed the planned schedule. However, the development and subsequent validation of a new, more intuitive and user-friendly form led to a two-month delay in the opening of the online form for the submission of results by participants.

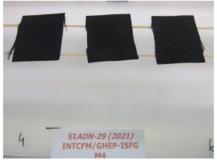
On account of the pandemic, no samples were taken from donors, rather frozen samples left over from other years were used. In the absence of sufficient samples, only the Basic Level Exercise and the non-human item exercise could be organised and no samples could be prepared for the Advanced Level Exercise.

Figure 8.1.4.3. Items sent from the Basic Level, kinship and forensic module and non-human item













Kinship module: items M1 to M3, two cards each with blood stains and a saliva swab. Their genetic analysis was requested.

Forensic module: items M4 and M5, mixture of two males —semen— (fluid analysis and genetic identification) and hair sample (genetic analysis), respectively.

The basic level also included a theoretical kinship exercise and a theoretical forensic exercise.

Non-human item 6: badger blood on the edge of a knife.

The following is the general data on the participation of the laboratories during the Exercise of 2021.

Regarding the **non-human item**, 27 laboratories sent results.

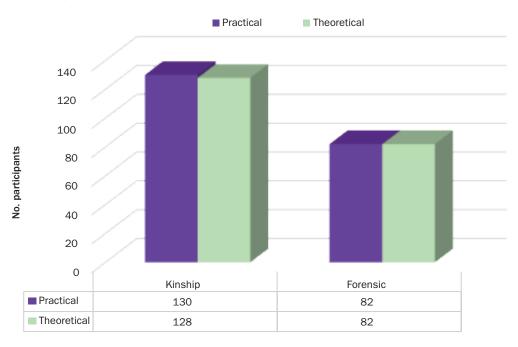


Figure 8.1.4.4. Distribution of participation in Basic Level modules

Figure 8.1.4.5. Geographical distribution of participants. The arrow indicates a new laboratory



Figures 8.1.4.6. and 8.1.4.7. Type of laboratories (%) distributed by continent. Public laboratories are mainly linked to Justice/Judiciary and Security Forces; to a lesser extent they belong to hospitals and research centres

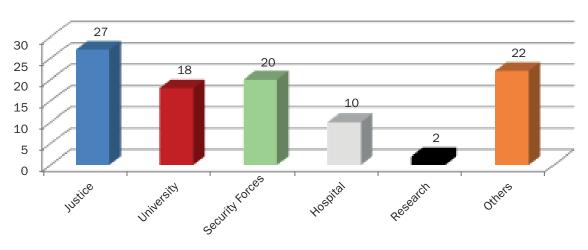
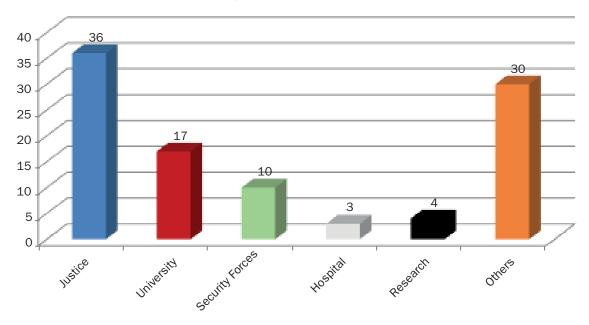


Figure 8.1.4.6. Europe





After evaluating the results, a non-reporting summary was issued for each level and a final report of participation and results, with the methodologies and results of each laboratory and the assigned values.

Subsequently, each participant received an individual certificate for each Basic Level module they had participated in, as well as all those who had participated in the non-human item.

8.1.5. Teaching and scientific activity

8.1.5.1. Participation in investigation projects and collaborations with other institutions

The Quality Assurance Service at the Madrid Department on behalf of the INTCF, has participated in the DNA working group of the European Network of Forensic Science Institutes (ENFSI) in charge of updating the *Manual of Good Practices* related to the Training of Personnel.

We have participated as experts in two international projects. Staff from this Service have collaborated as teachers and advisers in a range of activities that form part of the Twinning Project with Turkey TR16 IPA JH 03 18, managed by the International and Ibero-American Foundation for Public Administration and Policies (FIIAPP). As teaching staff on activities 2.13 Accreditation assistance for PCR and typing experiment method with Y-Filer Plus kit and PCR Powerplex Y23 System kit and 1.32 Workshop on Cytochrome B Analysis for Species Diagnosis and as advisers in activity 2.7 Additional follow up activity for accreditation programme.

They have also collaborated with the Spanish Agency for International Development Cooperation (AECID) to provide online training on ISO 17025: General Requirements for the Conformity of Testing and Calibration Laboratories.

8.1.5.2. Contribution in scientific congresses

Fernández Oliva K. Participation in the Quality Working Group. 45th ENFSI DNA expert working group meeting-online. ENFSI DNA group. 20 April 2021.

Fernández Oliva K. Results of the Intercomparison Exercise "Study of DNA polymorphisms in blood spots and other biological samples". Presentation. 26th Meeting of Forensic Genetics organised by the Spanish and Portuguese Speaking Group of the ISFG (GHEP-ISFG). Online. 18-20 October 2021.

8.1.5.3. Teaching and training activities

Education activities

Fernández Oliva K. Standardisation in the forensic laboratory. Reliability of results and interoperability between laboratories Organised by AECID. From 31 May to 11 June 2021.

Practical workshop on the dissemination of the INTCF quality system. Organised by the Centre for Legal Studies (CEJ). From 21 to 28 September 2021.

Data exchange. Course. Quality assurance in the forensic process. A step forward. Organised by the Centre for Legal Studies (CEJ). 05 November 2021.

What is quality? How is it achieved at a forensic laboratory? What is it worth? Organised by the Centre for Legal Studies (CEJ). 02 November 2021. Madrid, Seville (16 November 2021) and Barcelona (15 November 2021) editions.

Documentation of the INTCF quality system. Quality manual, procedures, annexes, data collection sheets. Types of procedures. Control of documentation. Organised by the Training Service of the Subdirectorate General for Access and Promotion of Justice Administration Staff. 22 November 2021. Madrid edition.

INTCF facilities and environmental conditions: factors and impact on the different INTCF activities. Organised by the Training Service of the Subdirectorate General for Access and Promotion of Justice Administration Staff. 23 November 2021. Madrid edition.

Methods of analysis. What is needed according to the INTCF quality system? Organised by the Training Service of the Subdirectorate General for Access and Promotion of Justice Administration Staff. 26 November 2021. Madrid and Barcelona editions (25 November 2021).

Intercomparison exercises. How does the INTCF work? Organised by the Training Service of the Subdirectorate General for Access and Promotion of Justice Administration Staff. 26 November 2021. Madrid and Barcelona editions (25 November 2021).

Dealing with deviations and complaints. How to overcome a non-conformity or non-conforming work? System improvements and risk analysis. Why is this necessary? Organised by the Training Service of the Subdirectorate General for Access and Promotion of Justice Administration Staff. 29 November 2021. Madrid edition.

Internal audits: What are they for? How are they performed? Who performs them? Organised by the Training Service of the Subdirectorate General for Access and Promotion of Justice Administration Staff. 29 November 2021. Madrid edition.

INTCF reports from a quality perspective. Organised by the Training Service of the Subdirectorate General for Access and Promotion of Justice Administration Staff. 29 November 2021. Madrid edition.

Accreditation of a laboratory. How is this achieved? Organised by the Training Service of the Subdirectorate General for Access and Promotion of Justice Administration Staff. 29 November 2021. Madrid edition.

Quality management. Forensic laboratories. UNE-EN ISO/IEC 17025 Standard. Organised by the University of Alcalá de Henares (UAH). Subject: Forensic Instrumental Analysis. 15 October 2021.

Chain of custody. Traceability. Organised by the University of Alcalá de Henares (UAH). Subject: Forensic Instrumental Analysis. 29 October 2021.

Evaluation of the quality of tests: Intercomparison exercises. Organised by the University of Alcalá de Henares (UAH). Subject: Forensic Instrumental Analysis. 29 October 2021.

Generation of reports and opinions. Content. Organised by the University of Alcalá de Henares (UAH). Subject: Forensic Instrumental Analysis. 16 November 2021.

Pérez Vergas R. Nonhuman DNA in the Forensic Genetics Laboratory: an Overview. Activity: 1.32 Workshop on Cytochrome B Análisis for Species Diagnosis. Twining Project TR16 IPA JH 03 18. 21 June 2021.

Species Identification: Standard Procedures. Activity: 1.32 Workshop on Cytochrome B Analysis for Species Diagnosis. Twining Project TR16 IPA JH 03 18. 22 June 2021.

Accreditation. Activity: 2.13 Accreditation assistance for PCR and typing experiment method with Y-Filer Plus kit and PCR Powerplex Y23 System kit. Twining Project TR16 IPA JH 03 18. 31 May 2021.

Internal Validation Example. Activity: 2.13 Accreditation assistance for PCR and typing experiment method with Y-Filer Plus kit and PCR Powerplex Y23 System kit. Twining Project TR16 IPA JH 03 18. 04 June 2021.

INTCF staff: Personnel management to ensure competence. Goals. Activity: 1-TOX-2021: Quality assurance in the INTCF, quality manuals, standard operating procedures, concepts and definitions. Madrid edition. Organised by the National Institute of Public Administration. 23 November 2021.

INTCF equipment. Equipment management. Part 1. Biology, forensic sciences and histopathology teams. Activity: 1-TOX-2021: Quality assurance in the INTCF, quality manuals, standard operating procedures, concepts and definitions. Madrid edition. Organised by the National Institute of Public Administration. 24 November 2021.

INTCF equipment. Equipment management. Part 2. Chemistry and Drugs, Assessment and General equipment. Activity: 1-TOX-2021: Quality assurance in the INTCF, quality manuals, standard operating procedures, concepts and definitions. Madrid edition. Organised by the National Institute of Public Administration. 24 November 2021.

INTCF equipment. Equipment management. Part 1. Biology, forensic sciences and histopathology teams. Activity: 1-TOX-2021: Quality assurance in the INTCF, quality manuals, standard operating procedures, concepts and definitions. Barcelona edition. Organised by the National Institute of Public Administration. 26 November 2021.

Training activities

Fernández Oliva K

- Interpretation of Expert Opinions issued by the INTCF. Organised by the Centre for Legal Studies (CEJ). Online. 19-27 April 2021.
- Basic LIMS: structure, data organisation and queries. Organised by the Centre for Legal Studies (CEJ). Online. 1-24 November 2021.
- New research tools in the field of Forensic Genetics. Organised by the Centre for Legal Studies (CEJ). Online. 21-28 June 2021.

Pérez Vergas R.

- Interpretation of Expert Opinions issued by the INTCF. Organised by the Centre for Legal Studies (CEJ). Online. 19-27 April 2021.
- Forensic detection and identification of chemical weapons. Toxicity and impact on the environment. Organised by the Centre for Legal Studies (CEJ). Online.
 4-11 November 2021.
- Interpretation of toxicological results and their influence on the expert context in which the analysis is requested. Organised by the Centre for Legal Studies (CEJ).
 Online. 10-17 November 2021.
- New research tools in the field of Forensic Genetics. Organised by the Centre for Legal Studies (CEJ). Online. 21-28 June 2021.
- Proficiency Testing Masterclass 2021. LGC AXIO Proficiency Testing. Online.
 26-27 October 2021.

8.2. Quality Assurance Service of Barcelona Department

8.2.1. Activities performed by the Service

The activities and functions that the Quality Assurance Service at the Barcelona Department has carried out during 2021 are reflected in Table 8.2.1.1.

Table 8.2.1.1. Activity data for 2021

Activities	
Development of new Standard Operating Procedures and modification of procedure versions	60
2. New annexes elaboration and modification of annexes versions	83
3. Training programmes and qualification certificates for staff	43
4. Initial and going training of personnel in the Quality System	11
5. Management and evaluation of external and internal equipment calibrations and physical patterns	189
6. Validation studies of analysis methods	2
7. Assessment of participation in intercomparison exercises	47
8. Conformity assessment of organisers of intercomparison exercises in which the Department participates	3
9. Evaluation of external participants (reports)	28
10. Evaluation of external participants (certificates)	-
11. Internal audits	5
12. Management review of the quality system by the Direction	2
13. Records and monitoring of non-conformities or non conformity works and incidents	13 + 92
14. Records of corrective actions	5
15. Records of actions to address risks and opportunities	17
16. Claims and complaints management	46
17. SGC valuation reports	10

In more detail, the related activities have consisted of:

1 and **2**. Management of system documents. In 2021, a total of 60 work procedures were drawn up and/or revised, as well as 83 annexes, documents that provide additional data (tables, specific calculations, etc.), and/or the necessary records for data collection for the activities described in the procedures.

This year, the efforts made in the development of new working procedures are worth particular mention: more than 75% of the documents implemented this year are newly drafted documents. The staff at the Quality Assurance Service has participated very actively in the drafting of the new procedures published, as many of them are procedures in non-technical areas with staff with little experience in the preparation of this type of documentation.

3 and **4**. Staff training. During 2021, the specific training and coaching programs carried out (9 programmes) and the internal training and coaching records for trainees submitted (34 records) were reviewed. A large part of the time is dedicated to advising the staff who have to prepare them and on bringing the documents already drawn up into line with the pre-established requirements.

Quality training was imparted to new staff (11 new employees) and trainees to familiarise them with the Quality System implemented at the Department and all documentation of training records, records of authorised signatures and all other documentation related to the training and qualification of laboratory staff was archived.

Furthermore, they participated as teaching staff in several training activities on quality assurance in forensic laboratories for INTCF staff:

- A course organised by the Centre for Legal Studies (CEJ) for professionals.
- Two courses aimed at specialist technicians and laboratory assistants organised by the Ministry of Justice through the Training Service of the Subdirectorate General for Access and Promotion of Justice Administration Staff.
- **5.** Management of internal and external equipment calibrations. The Quality Assurance Service has collaborated with the different services in the preparation and review of calibration, verification and maintenance programmes for 28 types of equipment. These programmes include the maintenance, control and evaluation of their measuring capacity to ensure their correct operation. From the programmed activities that can be carried out internally, the Quality Assurance Service has reviewed and assessed the conformity of the results of 74 calibrations (automatic pipettes and scales).

In relation to the calibrations that, due to technical requirements, have to be performed externally, the Quality Assurance Service handled the following:

the contracting of external calibration services for platform scales (3 scales);

- coordination of the sending of automatic pipettes with a volume of 10 μ l or less for calibration (51 automatic pipettes);
- and the assessment of the conformity of the external calibrations performed (112 calibration assessments).
- **6. Validation methods studies.** As in previous years, the Quality Assurance Service has actively participated in the design, monitoring and study of the results obtained in the validations carried out by the different Services. In 2021, work was carried out in collaboration with the Biology, Chemistry and Drugs, and Toxicological Assessment and Environment Services on the validation of the following analytical methods:

Test/technique method Amplification and sequencing of mitochondrial Cytochrome B mitochondrial DNA with ABI3500 Genetic Analyser with Finalised ABI3500 Genetic Analyser **BIOLOGY** Microscopic visualisation of spermatozoa by Christmas Finalised Tree staining Quantification of ketamine in samples of seizures and other In process seizures by GC-FID CHEMISTRY AND DRUGS Determination of carboxyhaemoglobin (COHB) in blood using new In process UV-VIS spectrometry equipment. TOXICOLOGICAL VALIDATIONS Determination of anions by ion chromatography In process AND ENVIRONMENT

Table 8.2.1.2. Validations launched in 2021

7 and 8. Intercomparison exercises in which the INTCFB participates. In relation to this type of activity, the Quality Assurance Service has worked on:

- updating and monitoring the Quality Assessment Activity Plan which includes internal and external control activities (inter-laboratory exercises);
- requesting budgets for the interlaboratory exercises in which the Department participates;
- filing the requests for participation in each of the exercises and monitoring the approval of the purchase orders generated;
- resolving the incidents that occur while receiving the samples for the different controls that form part of the exercises;
- and assessing the outcome of the INTCFB's participation in these external quality control activities.

During 2021, the technical services of the INTCF Barcelona Department participated in a total of 24 intercomparison exercises, some of them with several annual rounds that are processed and evaluated independently. This participation has generated 47 evaluation reports from the Quality Assurance Service.

Furthermore, the SGC participated in the assessment of the characteristics and technical aspects of 3 new intercomparison exercise programmes for inclusion as an external control activity in the annual programme of the Chemistry and Drugs Service's participation in interlaboratory exercises.

9 and **10**. Intercomparison exercises that the INTCFB organises. Once again this year, the INTCFB has organised a new control of the "Interlaboratory Exercise in relation to Drugs of Abuse in seizures (DAHA)", an intercomparison exercise for the external quality control of national public laboratories that analyse drugs of abuse (see the case of interest).

After evaluation of the results submitted by the participants, the SGC issues a full report with information on the organisation of the exercise, the preparation of the samples, the methodologies used for data processing and evaluation of the data, the results obtained, additional information provided by the participants, results obtained with the data processing and re-evaluation of results and any other issues that may be of interest.

One new development in 2021, in addition to the report on the results of the exercise, was the preparation of a personalised individual report, which was sent to each participant, summarising the results obtained by the participant and including trend graphs by substance.

- **11. Internal audits.** In 2021, the Quality Assurance Service staff performed 3 horizontal internal audits on purely administrative activities.
- **12. Quality system review.** In 2021, two Quality System review meetings were held in which information was gathered on the conformity of activities with the Quality System implemented and the requirements of ISO 17025, compliance with objectives, identified needs for change and proposals for action. All this information was documented in two comprehensive and detailed reports written by the Quality Assurance Service.
- 13, 14 and 15. Non-conforming works, corrective actions, risks and opportunities for improvement. When any aspect of the activities carried out under the Quality system does not comply with the established procedures or requirements, an incident is recorded. It is usually resolved with a remedial action without the need to establish corrective action. A Non-Compliant Work is opened when the deviation or incident is repetitive, or the importance or seriousness of the non-compliance or incident that has occurred requires it. When a non-conforming work (or non-conformity) is opened, INTCFB staff study the causes of the deviation, assess the influence it may have had on other areas and the risk it poses to the laboratory's activity. Once the true causes of the deviation are known, the necessary corrective actions (CA) are established to rectify the causes of the deviation and prevent it from recurring. The SGC documents the incidents occurring in some Services and those detected in the exercise of its functions of supervision and control of technical and administrative activities, it also documents all the TNCs identified, evaluates the corrective actions proposed and monitors their implementation and effectiveness.

During 2021, 92 incidents and 13 Non-Conforming Works were recorded and followed up on, with a total of 5 corrective actions have been documented, the rest being remedial actions.

In 2021, 11 risks were identified. For some of these, action has already been taken to eliminate or minimise them. Regardless of the risks identified, 11 actions to improve activities and processes have been documented and are in the process of being implemented

16. Management of claims and complaints. In 2021, the evaluation of the 46 communications registered as complaints or claims (including this year's report returns communications) has revealed errors in 22 requests, the main cause of complaints being transcription errors in reports and complaints about the late issuance of cases.

In addition to the above, the staff of the Quality Assurance Service carries out the following activities:

- Completion of surveys related to the Department's Quality Management (e.g. ISAM 2021, audit assessment questionnaire, etc.).
- Design and management of the 2021 INTCFB staff feedback questionnaire.
- Drawing up of internal reports requested by the Department Management or National Management.
- Drawing up reports for the management, information, or assessment of matters related to issues dealt with by the Quality Assurance Service.

The staff of the Quality Assurance Service also participates in additional activities related to the centralised management for the acquisition of external controls and standards necessary for the performance of the tests:

- Requesting quotations for the interlaboratory exercises in which the Department participates.
- Requesting interlaboratory exercises using the purchasing application, monitoring the approval of orders, and resolving any incidents that occur.
- Preparation of the necessary documentation (customs authorisation, etc.) for the delivery of external controls and standards to the Department when required.
- Request, management, and filing of Import Authorisations for narcotic and psychotropic substances required as external quality controls (interlaboratory exercises) or reference materials for the Chemistry and Drugs Service.

8.2.2. Intercomparison exercises in which the INTCFB Services participated in 2021

Table 8.2.2.1. Intercomparison exercises in which the INTCFB Services participated in 2021

Participation in intercomparison exercises of the Biology Service

Program: Analysis of DNA polymorphism in blood stains and other biological samples

Organiser: INTCF-GHEP-ISFG

Frequency: Annual

Parameters: forensic and kinship genetics and preliminary studies in blood, hair and other matrices

Program: GEDNAP Proficiency test

Organiser: GEDNAP-ENFSI (German Speaking Working Group of the International Society for Forensic Genetics)

Frequency: Annual

Parameters: forensic and kinship genetics in blood stains and other biological fluids

Participation in intercomparison exercises of the Chemistry and Drugs Service

Program: Proficiency study AQA

Organiser: National Measurement Institute of Australian Government (NMI)

Frequency: four-monthly (participated in 2 out of 3 rounds)

Parameters/samples: heroin, cocaine, amphetamine compounds in powder-solid samples

Program: ENFSI Proficiency test Organiser: ENFSI Drugs Working group

Frequency: Annual

Parameters/samples: heroine, cocaine, others in powder-solid samples

Program: International Quality Assurance Programme (IQAP) Seized materials Group

Organiser: United Nations Office on Drugs and Crime (UNODC)

Frequency: twice a year

Parameters/samples: Drugs in powder-solid samples

Program: Interlaboratory Exercise on Drugs of Abuse Commonly Abused in Addictions

Organiser: INTCF-BARCELONA

Frequency: Annual

Parameters/samples: Drugs in powder-solid samples Program: Blood Alcohol Intercomparison Exercise

Organiser: INTCF-SEVILLA Frequency: four-monthly

Parameters/samples: ethyl alcohol and other volatile compounds in blood and plasma

Program: Whole blood Alcohol/Volatiles Survey (AL1)

Organiser: College of American Pathologists

Frequency: four-monthly

Parameters/samples: ethyl alcohol, volatiles and ethylene glycol in blood

Program: Toxicology Blood (quantitative)

Organiser: LGC Standards Frequency: per month

Parameters/samples: carboxyhaemoglobin in blood

Program: Toxicology (T)

Organiser: College of American Pathologists

Frequency: four-monthly

Parameters/samples: drugs and psychotropic drugs in serum and urine

Program: International Quality Assurance Programme (IQAP) Biological Specimens Group

Organiser: United Nations Office on Drugs and Crime (UNODC)

Frequency: twice a year

Parameters/samples: identification and quantification of the most common drugs of abuse in urine

Participation in intercomparison exercises of the Toxicology and Environmental Assessment Service

Program: Wastewater: Toxicity (GSCAR4) Organiser: Quality Services Office (GSC)

Frequency: Annual

Parameters/samples: toxicity (inhibitory substances) in waste water

Program: Ecotoxicology: Aquacheck - Group 50

Organiser: LGC Standards Frequency: twice a year

Parameters/samples: toxicity with *Daphnia magna* in effluents Program: Nutrients and other analyses: Aquacheck - Group 10

Organiser: LGC Standards Frequency: Annual

Parameters: BOD5, COD/COT, suspended solids, turbidity, non-ionic surfactants

Program: Metals: Aquacheck - Group 12

Organiser: LGC Standard Frequency: twice a year

Parameters/samples: metals in effluent matrix Program: IELAB Physico-chemical parameters

Organiser: IELAB Frequency: twice a year

Parameters/samples: ammonium, nitrates, BOD5, COD, suspended solids, fluorides, and toxicity in waste water

Program: Effluent, Waste Water, Contaminated Land and Hazardous waste - Group 3

Organiser: Laboratory Environmental Analysis Proficiency (LEAP)

Frequency: twice a year

Parameters/samples: nitrate/nitrite, ammonium, chloride sulfate, PO4, total phosphorus, total nitrogen/Kjeldahl in

aqueous matrix

Program: Quality in Water Analysis Scheme (QWAS)

Organiser: LGC Standards Frequency: twice a year

Parameters/samples: total coliforms, fecal coliforms, and fecal streptococci in waters

Program: Ignitable liquid identification

Organiser: Collaborative Testing Services (CTS)

Frequency: Annual

Parameters/samples: combustion-accelerating substances in different media

Participation in intercomparison exercises of the Histopathology Service

Program: Forensic Pathology (FR)

Organiser: College of American Pathologists (CAP)

Samples: histories and images of crime scenes, external examinations and macroscopic and microscopic images

of 6 real cases

Frequency: twice a year Parameters: final diagnosis

8.2.3. Accreditation scopes

The Quality Assurance Service of each department acts as the main point of contact with ENAC in all matters relating to the accreditation process and scope.

The Barcelona Department has two accreditation files open in accordance with the requirements of ISO 17025 standards: Accreditation No. 297/LE640 and Accreditation No. 297/ LE639, that bring together testing methods in the forensic and environmental areas respectively.

Following the scope extension audit carried out by ENAC in September 2021, two new tests of the Biology Service were included in the scope of accreditation No. 297/LE640.

Reports issued

Much of the work carried out by the Quality Assurance Service does not need to be recorded in the LIMS application, which is why only some of the activities carried out in relation to specific requests related to certain types of applications (assessments of participation in interlaboratory exercises, study of claims and complaints, internal audits carried out, etc.) are recorded in this information management system.

In this case, the total number of requests opened in the LIMS application during 2021 was 119, which generated 109 reports.

Participation in investigation projects and meetings related to quality

The Quality Assurance Service at this Department, along with the services from the other Departments, have actively participated and collaborated within the Quality Group of the Network of Official Spanish Forensic Laboratories (RLFOE), virtually attending the annual meeting.

They participated as an expert in the international project TR 16 IPA JH 03 18 (Turkey), collaborating in the following activities:

- Activity No 3.9 Activity on the Drafting of Sops for the New Methods of the EU Twinning Project on Forensic Trainings Towards Advanced Examination Methods (April 2021).
- Activity No. 3.11 Seminar of Drafting Effective Standard Operating Procedures (SOPs) of the EU Twinning Project on Forensic Trainings Towards Advanced Examination Methods (March 2021).

8.2.4. Forensic case of interest: Intercomparison Exercise on Common Drugs of Abuse in Seizures

In 2021, the Barcelona Department once again organised a new control of the Intercomparison Exercise on Common Drugs of Abuse in Seizures (DAHA), a proficiency test that allows laboratories that analyse these type of substances for public administrations to dispose of a useful and economic tool for quality assessment in the analyses they perform.

The high cost of the interlaboratory exercises in relation to drugs and the acquisition of reference materials of narcotics and psychotropic substances required for the quality assurance of this kind of analysis makes the work of drug testing laboratories difficult.

Aware of this problem and in fulfilment of its role as a reference centre, the INTCFB Quality Assurance Service has worked throughout the year in the search and selection of adequate samples that come from illegal drug seizures and the request for judicial authorisations for the use of these samples in the preparation of the exercise items.

The samples corresponding to the DAHA 1/21 control could be sent to the participating laboratories in May 2021, facilitating the self-assessment of 28 laboratories in the qualitative and quantitative analysis of 6 different drugs, substances to which reference values have been attributed, allowing the use of the surpluses as internal quality controls.

Figure 8.2.4.1



The activity concluded in July 2021 with the issuance of a results report prepared by the Quality Assurance Service with the information from the exercise, the results issued by all participants, the statistical treatment of the data and the assessment of the laboratories' performance.

In relation to the participants in the DAHA 1/21 Control:

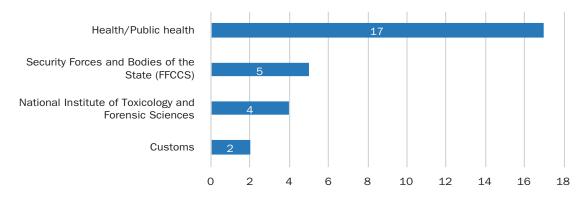
	Participating laboratories	Laboratories issuing results
Control DAHA 1-21	28	28

Ranking of the participating laboratories according to shareholding:

Public laboratories	27
Private laboratories	1

The participating laboratories according to their field of activity are listed in this figure:

Figure 8.2.4.2. Participating laboratories according to their field of activity



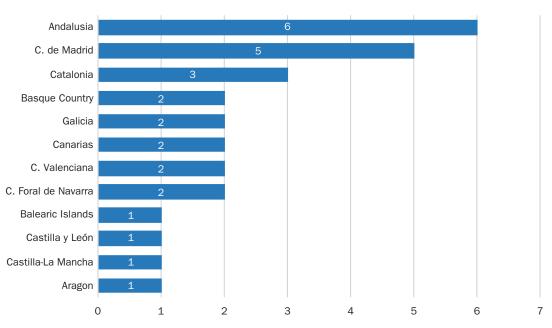


Figure 8.2.4.3. Geographical distribution of participating laboratories

The DAHA 1/2021 control consolidates the changes made in 2020 (establishment of standard target deviation, new homogeneity study, etc.). As a new development, it includes the preparation and sending of a specific and different Individual Report for each of the laboratories to each of the participants (in addition to the Results Report that is usually issued after each round), in which the results obtained by the participant are summarised, as well as trend graphs by round and substance.

8.2.5. Teaching and scientific activity

8.2.5.1. Participation in investigation projects and collaborations with other institutions

Enreig Cabanes E., Izquierdo Vigil R.

We have participated as experts in two international projects. Staff from this Service have collaborated as teachers and advisers in a range of activities that form part of the Twinning Project TR16 IPA JH 03 18 with the European Union on Forensic Trainings Towards Advance Examination Methods, managed by the FIIAPP.

- Activity no. 3.11. Seminar on Drafting Effective Standard Operating Procedures (SOPs). Twining Project TR16 IPA JH 03 18. Online. From 22 to 23 March 2021.
- Activity no. 3.9. Activity on the Drafting of SOPs for the New Methods. Twinning Project TR16 IPA JH 03 18. Asynchronous activity. From 19 to 24 April 2021.

8.2.5.2. Teaching and training activities

Education activities

Izquierdo Vigil R. Standard Operating Procedure. Part 1 – Overview and basic recommendations to drafting SOP. Twinning Project TR16 IPA JH 03 18. Online. 23 March 2021.

Practical workshop on the dissemination of the INTCF quality system. Organised by the Centre for Legal Studies (CEJ). From 21 to 28 September 2021.

INTCF. Experience in the development and implementation of the quality system of a multidisciplinary forensic laboratory. Activity: 2-TOX-2021: quality. Study of the UNE-EN ISO/IEC 17025:2017 standard. General Requirements for the Competence of Testing and Calibration Laboratories. Organised by the Training Service of the Subdirectorate General for Access and Promotion of Justice Administration Staff. Online. Madrid edition, 12 November 2021. Seville edition, 18 November 2021. Barcelona edition, 19 November 2021.

Structure of the INTCF Quality System. History. Background. Quality Policy. Objectives. Reference standards. Activity: 1-TOX-2021: quality assurance in the INTCF, quality manuals, standard operating procedures, concepts and definitions. Madrid edition. Organised by the Training Service of the Subdirectorate General for Access and Promotion of Justice Administration Staff. Online, 22 November 2021.

Structure of the INTCF Quality System. History. Background. Quality Policy. Objectives. Reference standards. Activity: 1-TOX-2021: quality assurance in the INTCF, quality manuals, standard operating procedures, concepts and definitions. Barcelona edition. Organised by the Training Service of the Subdirectorate General for Access and Promotion of Justice Administration Staff. Online, 23 November 2021.

Documentation of the INTCF quality system. Quality manual, procedures, annexes, data collection sheets. Types of procedures. Control of documentation. Activity: 1-TOX-2021: quality assurance in the INTCF, quality manuals, standard operating procedures, concepts and definitions. Barcelona edition. Organised by the Training Service of the Subdirectorate General for Access and Promotion of Justice Administration Staff. Online, 23 November 2021.

INTCF staff: personnel management to ensure competence. Goals. Activity: 1-TOX-2021: quality assurance in the INTCF, quality manuals, standard operating procedures, concepts and definitions. Barcelona edition. Organised by the Training Service of the Subdirectorate General for Access and Promotion of Justice Administration Staff. Online, 24 November 2021.

Supply management to be able to work with quality. Activity: 1-TOX-2021: quality assurance in the INTCF, quality manuals, standard operating procedures, concepts and definitions.

Barcelona edition. Organised by the Training Service of the Subdirectorate General for Access and Promotion of Justice Administration Staff. Online, 24 November 2021.

Dealing with deviations and complaints. How to overcome a non-conformity or non-conforming work? System improvements and risk analysis, why is this necessary? Activity: 1-TOX-2021: quality assurance in the INTCF, quality manuals, standard operating procedures, concepts and definitions. Barcelona edition. Organised by the Training Service of the Subdirectorate General for Access and Promotion of Justice Administration Staff. Online, 30 November 2021.

Internal audits: What are they for? How are they performed? Who performs them? Activity: 1-TOX-2021: quality assurance in the INTCF, quality manuals, standard operating procedures, concepts and definitions. Barcelona edition. Organised by the Training Service of the Subdirectorate General for Access and Promotion of Justice Administration Staff. Online, 30 November 2021.

Accreditation of a laboratory. How is this achieved? Activity: 1-TOX-2021: quality assurance in the INTCF, quality manuals, standard operating procedures, concepts and definitions. Barcelona edition. Organised by the Training Service of the Subdirectorate General for Access and Promotion of Justice Administration Staff. Online, 01 December 2021.

Enreig Cabanes E. Standard Operating Procedure. Part 2 - General & Specific Format and Types of SOPs. Twining Project TR16 IPA JH 03 18. Online, 23 March 2021.

Perea Falomir M. INTCF facilities and environmental conditions: factors and impact on the different INTCF activities. INTCF equipment. Equipment management. Activity: 1-TOX-2021: quality assurance in the INTCF, quality manuals, standard operating procedures, concepts and definitions. Barcelona edition. Organised by the Training Service of the Subdirectorate General for Access and Promotion of Justice Administration Staff. Online, 24 November 2021.

Training activities

Izquierdo Vigil R.

- Forensic detection and identification of chemical weapons. Toxicity and impact on the environment. Organised by the Centre for Legal Studies (CEJ). Online.
 4-11 November 2021.
- Interpretation of toxicological results and their influence on the expert context in which the analysis is requested. Toxicity and impact on the environment. Organised by the Centre for Legal Studies (CEJ). Online. 4-11 November 2021.
- Basic LIMS: structure, data organisation and queries. Organised by the Centre for Legal Studies (CEJ). Online. 17-24 November 2021.
- Practical workshop on validation of forensic toxicology methods. Organised by the Centre for Legal Studies (CEJ). Online. 4-7 October 2021.

Enreig Cabanes E.

- Interpretation of expert opinions in the field of legal medicine. Organised by the Centre for Legal Studies (CEJ). Online. 19-26 April 2021.
- Basic LIMS: structure, data organisation and queries. Organised by the Centre for Legal Studies (CEJ). Online. 17-24 November 2021.
- Treatment of offences against sexual freedom and integrity in the forensic laboratory. Organised by the Centre for Legal Studies (CEJ). Online. 15-21 June 2021.

Perea Falomir M.

- Update in Forensic Chemistry and Toxicology Organised by the Centre for Legal Studies (CEJ). Online. 8-16 March 2021.
- Interpretation of expert opinions in the field of legal medicine. Organised by the Centre for Legal Studies (CEJ). Online. 19-26 April 2021.
- Basic LIMS: structure, data organisation and queries. Organised by the Centre for Legal Studies (CEJ). Online. 17-24 November 2021.
- Forensic toxicology. Toxic pathology. Organised by the Centre for Legal Studies (CEJ). Online. 1-14 June 2021.
- Practical workshop on the dissemination of the INTCF quality system. Organised by the Centre for Legal Studies (CEJ). Online. 21-28 June 2021.
- Practical workshop on validation of forensic toxicology methods. Organised by the Centre for Legal Studies (CEJ). Online. 4-7 October 2021.
- Quality assurance in the forensic process. A step forward. Organised by the Centre for Legal Studies (CEJ). Online. 2-5 November 2021.

8.3. Quality Assurance Service at the Seville Department

8.3.1. Activities performed by the Service

The activities and functions that the Quality Assurance Service has carried out during 2021 are reflected in Table 8.3.1.1.

Table 8.3.1.1. Data on the activities carried out by the Service

Activities	
Development of new standard operating procedures (SOPs) and data collection sheets (DCSs), and the modification of existing ones	29 (PNT) 16 (DCS)
2. New annexes elaboration and modification of annexes versions	7
3. Training programs and staff qualification certificates	11

Activities	
4. Management of internal equipment calibrations	254
5. Management of external equipment calibrations	111
6. Assessment of participation in intercomparison exercises	27
7. Evaluation of external participant	51
8. Validation methods analysis	7
9. Internal audits	-
10. Management review of the quality system by the Direction	1
11. Records of non-conformities or non conformity works	11
12. Records of corrective actions	11
13. Records of actions to address risks and opportunities	2
14. Claims and complaints management	3

The activities in 2021 have consisted:

1 and 2. Management of system documents. As a result of the reviews of the procedures in force, a total of 29 procedures have been updated, of which 3 are general procedures and 26 are technical procedures, 4 general annexes, 3 technical annexes and 16 data collection sheets. Standard operating procedures (SOPs) are written documents that describe how an activity has to be carried out. The annexes include specific information that given its importance, constitute an independent document. Data collection sheets are used to record the activity. The SGC manages and controls the list of documents in force and the list of staff.

3. Training programs and staff qualification certificates. To ensure the quality of testing, one of the requirements of the standard is the availability of qualified staff with the appropriate skills to perform the laboratory activities entrusted to them. The training programs and all the registers derived until the obtainment of the qualification certificate are supervised by the SGC. During 2021, 11 initial training programmes were managed and reviewed for new and reassigned staff. In all cases, the corresponding Certificate of Qualification was issued.

As part of continuous quality training, the Department's staff have taken three courses organised by the Centre for Legal Studies for medical staff and two courses organised by the Ministry of Justice through the National Institute of Public Administration for laboratory technicians and laboratory assistants.

The Service has coordinated and delivered one of the three online courses, "Quality assurance in the forensic process. A step forward", aimed at all INTCF staff, forensic experts and prosecutors, organised by the CEJ. In the other courses, organised by both the CEJ and the Ministry, the Service's staff participated as lecturers.

During the year, all queries made by staff from the Services were also dealt with, especially those related to calibrations, intercomparison exercises, and the issuing of reports.

- 4 and 5. Management of internal (4) and external (5) equipment calibrations. The SGC coordinates and manages the inventory of equipment (calibration, verification and maintenance) and reference materials, standards and reference data. The Annual Equipment Calibration, Verification and Maintenance Plan and the calibrations of auxiliary laboratory equipment and physical standards are drawn up in collaboration with the staff responsible for them. External calibrations are performed by ISO 17025 accredited suppliers. For automatic pipettes with a volume equal to or less than 10 μ l, external calibration is by centralised contract. In all cases, the SGC evaluates both internal calibrations or verifications and external calibration certificates, and assesses the possible significance with the heads of the Services.
- 6. Assessment of participation in intercomparison exercises The performance of external quality checks, called intercomparison exercises, is an ISO 17025 requirement to ensure the validity of the results issued in the reports. Assessments are performed against criteria established by the suppliers of the exercise and whenever possible against criteria established by this Service, mainly taking into consideration the validation parameters. The Services of the Seville Department participated in 17 intercomparison exercises that generated 27 evaluations by the SGC. Some of them have more than one round. Each year, the SGC updates the Quality Control Activities Plan in collaboration with the Services and periodically manages participation in the different exercises that have been selected based on their needs and suitability.
- **7. Evaluation of external participants.** The Seville Department organises and coordinates the blood alcohol intercomparison exercise for different types of laboratories performing this analysis. It consists of three rounds with three samples of biological fluids (blood and plasma) and 57 participating laboratories (6 from abroad). After the results submitted by the participants, the SGC issues the corresponding report with the results of all laboratories. Once the last round is complete, it sends the corresponding certificate to each of them.
- **8. Validation studies.** In 2021, the following validations were carried out by the different Services:

Table 8.3.1.2. Validations carried out in 2021

SERVICE	Test/technique method	Status
BIOLOGY	Species determination. Cytochrome B analysis	In process
Бюсоч	Analysis of PCR products by capillary electrophoresis in the QSep-100 instrument.	In process
	Determination of cocaine, opiates, amphetamines and methadone compounds in hair samples by liquid chromatography-triple quadrupole mass spectrometry (HPLC-MS/MS).	In process
CHEMICAL	Verification of the method for the determination of ethyl alcohol in blood samples by gas chromatography with FID detector.	In process
	Determination of potassium in vitreous humour samples by flame atomic absorption spectrometry	In process
TOXICOLOGICAL VALIDATIONS AND	Determination of metals by inductive plasma atomic emission spectrometry	In process
ENVIRONMENT	Determination of anions by ion chromatography	In process

- **9 and 10. Internal audits and Quality System Review.** Both activities are of significant interest to obtain information about compliance with the requirements of the corresponding system and with the requirements of ISO 17025, and whether they are correctly implemented. No internal audit for testing was carried out in 2021.
- 11 and 12. Non-conformity (NC) or non-conforming works (TNC) records and records of corrective actions (AC). The SGC documents the mentioned actions, and carries out the cause analysis and extension analysis to determine the impact of the deviation on the Quality System. During 2021, 11 non-conformities and 11 corrective actions were opened. In all cases, the analysis of causes and extension analysis has been carried out.
- **13.** Records of actions to address risks and opportunities. In 2021, two risks were identified and opportunities for improvement established. These are now closed.
- **14. Management of claims and complaints.** In compliance with the complaints procedure of this Department (PNT-MC-005) and once it is linked in the LIMS to the corresponding issue, the signed complaint form is delivered to the SGC, which proceeds to open the request in the application, assesses whether a non-conformity should be applied and controls the closure of the same. A total of 6 complaints have been opened referring to errors detected in the reports (3) or delays in the issuance of cases (3).

8.3.2. Intercomparison exercises in which the INTCFS Services participated in 2021

Table 8.3.2.1. Intercomparison exercises in which the INTCFS Services participated in 2021

Participation in intercomparison exercises of the Biology Service

Program: Study of DNA polymorphisms in blood stains and other biological samples. Basic and advanced levels

Organiser: INTCF-GHEP-ISFG.

Samples: Blood, hair and other matrices

Frequency: Annual

Parameters: preliminary biological fluids, identification by DNA techniques

Program: GEDNAP Proficiency test

Organiser: GEDNAP-ENFSI (German Speaking Working Group of the International Society for Forensic Genetics)

Samples: blood stains and other biological fluids.

Frequency: Annual

Parameters: preliminary biological fluids, identification by DNA techniques

Participation in intercomparison exercises of the Chemistry Service

Program: Blood Alcohol Intercomparison Exercise

Organiser: INTCF-SEVILLA Samples: blood, plasma Frequency: four-monthly

Parameters: ethyl alcohol and other volatile compounds

Program: International Quality Assurance Programme (IQAP-UNODC). Determination of psychotropic substances

and narcotic drugs in consignments

Organiser: United Nations Office on Drugs and Crime (UNODC)

Samples: 4 powder samples (solids)

Frequency: twice a year

Parameters: identification and quantification of most common drugs of abuse

Participation in intercomparison exercises of the Chemistry Service

Program: International Quality Assurance Programme (IQAP-UNODC). Biological Specimens Group

Organiser: United Nations Office on Drugs and Crime (UNODC)

Samples: 4 urine samples Frequency: twice a year

Parameters: identification and quantification of most common drugs of abuse

Program: Interlaboratory Exercise on Drugs of Abuse Commonly Abused in Addictions

Organiser: INTCF-BARCELONA Samples: powder-solid (seizures)

Frequency: Annual

Parameters: qualitative and quantitative analysis of drugs of abuse and qualitative analysis of adulterants and diluents.

Program: Forensic Blood Toxicology PT-Quartz Scheme

Organiser: LGC Standards

Samples: blood (3 samples per submission)

Frequency: twice a year

Parameters: identification and quantification of substances of toxicological interest

Program: Interlaboratory control of ethylglucuronide determination in hair samples

Organiser: Society of Hair Testing (SOHT) Samples: hair (three samples per submission)

Frequency: twice a year

Parameters: identification and quantification of ethylglucuronide

Program: Toxicology Organiser: LGC Standards Samples: blood Frequency: twice a year

Parameters: identification and quantification of carboxyhaemoglobin

Program: Toxicology Organiser: LGC Standards Samples: blood Frequency: twice a year

Parameters: identification and quantification of ethanol in blood

Program: Drugs of abuse in hair testing Organiser: Society of Hair Testing Samples: hair (3 samples) Frequency: twice a year

Parameters: identification and quantification of drugs of abuse in hair

Participation in intercomparison exercises of the Toxicology and Environmental Assessment Service

Program: IELAB Physico-chemical parameters

Organiser: IELAB

Samples: environmental matrices

Frequency: one round

Parameters: ammonium, nitrates, BOD5, COD, suspended solids, fluorides and toxicity (EC50) Microtox

Program: IELAB Microbiology and Physiochemical Parameters

Organiser: IELAB Samples: seawater Frequency: one round

Parameters: total coliforms, Escherichia coli, enterococci. Ammonium, nitrates and pH

Program: Exercises in the environmental sector: Waste water

Organiser: Quality Services Office (GSC) Samples: high salinity water

Frequency: one round per year

Parameters: conductivity, ammonium, nitrate, fluoride, total phosphorus, pH, chlorides, nitrites, sulphates, phosphates,

arsenic, boron, copper, iron, nickel, and lead

Program: Exercises in the environmental sector: Waste water

Organiser: Quality Services Office (GSC)

Samples: waste water Frequency: one round per year

Parameters: Toxicity

Program: General parameters in water

Organiser: Consejería de Agricultura, Ganadería, Pesca y Desarrollo Sostenible. Regional Government of Andalusia

Samples: seawater and waste water Frequency: one round per year

Parameters: phosphates (seawater), suspended solids, total phosphorus (waste water)

Participation in intercomparison exercises of the Toxicology and Environmental Assessment Service

Program: Metals in water

Organiser: Consejería de Agricultura, Ganadería, Pesca y Desarrollo Sostenible. Regional Government of Andalusia

Samples: surface water Frequency: one round per year

Parameters: aluminium, barium, boron, cadmium, chromium, cobalt, copper, iron, lead, manganese, nickel and selenium

Program: In situ

Organiser: Consejería de Agricultura, Ganadería, Pesca y Desarrollo Sostenible. Regional Government of Andalusia

Samples: surface water Frequency: one round per year

Parameters: chlorides, phosphates, nitrates and sulphates

8.3.3 Scope of accreditation

The Quality Assurance Service acts as the main point of contact and is responsible for the quality of the Seville Department before the National Accreditation Body (ENAC).

Following the monitoring audit performed in April 2021, the Seville Department has two accreditation files opened, Accreditation No. 297/LE1833 Rev. 10, corresponding to toxicological and forensic testing (Chemistry and Biology Technical Units), and Accreditation No. 297/LE2239 Rev. 6, corresponding to environmental testing (Toxicological and Environmental Assessment Technical Unit).

8.3.4 Forensic case of interest: Blood alcohol intercomparison exercise (EIAS)

In 2021, the Seville Department organised the intercomparison exercise of blood alcohol (EIAS), with this exercise involving:

• Rounds and type of samples. This consists of three rounds of analysis, with three samples each of blood (2) and plasma (1), where participants identify and quantify the ethyl alcohol in each of the samples. In addition, in one of the rounds, a sample is spiked with another volatile. (This situation is frequently encountered in routine).

Figure 8.3.4.1





- Homogeneity and stability studies. They are performed in line with the procedures set out in Annex A of ISO 13528:2015. Statistical methods for use in proficiency testing by interlaboratory comparisons and the Association of Official Analytical Chemists (AOAC) 2016.
- Assigned value. The assigned value is defined as "value attributed to a particular property of an aptitude test element". In this study, the property is the concentration of the analyte in the test samples. The assigned values were the robust averages of the participants' results. Both the assigned value (by consensus) and its uncertainty, as well as the robust standard deviation are obtained following the procedure described in Algorithm A of the international standard ISO 13528:2015 mentioned above (Annex C of ISO 13528). The robust standard deviation is the one used for the calculation of the z-score.
- The evaluation criterion used for the quantitative results is the z-score. For its calculation, in addition to the result of the participating laboratory, the assigned value and the robust standard deviation are required.

As for the results, the distribution of the results is studied to rule out the possibility of a bimodal distribution.

The total number of participants varied in each round as follows:

	Number of participants	Emitting results
EIAS 1-21	57	54
EIAS 2-21	57	53
EIAS 3-21	57	55
TOTAL	171	162

The distribution by participating countries is as follows:

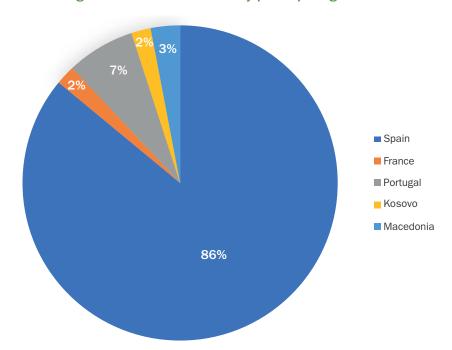


Figure 8.3.4.2. Distribution by participating countries

Analysis techniques used:

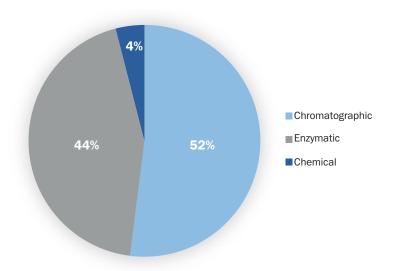


Figure 8.3.4.3. Analysis techniques used

The Spanish laboratories belong to the following autonomous communities:

0 2 6 8 10 12 14 Catalonia 13 Andalusia 9 Asturias C. de Madrid Basque Country Aragon Balearic Islands 2 Canary Islands 2 C. Valenciana 2 Galicia Castilla-La Mancha 1 Murcia C.F. Navarre

Figure 8.3.4.4. Origin of Spanish laboratories by autonomous communities

Regarding the distribution of participating institutions:

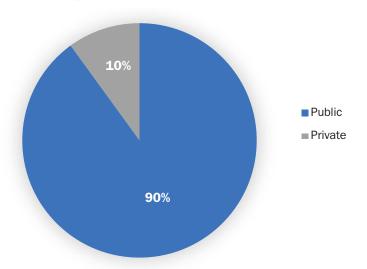


Figure 8.3.4.5. Distribution laboratories

The public laboratories are distributed as follows:

Hospitals
Legal Medicine Institutes
Public Health
University

National Institute of Toxicology and Forensic Sciences
Ministry of the Interior

3

Figure 8.3.4.6. Public laboratories

The criteria for evaluating the acceptability of the results taking into account the assigned value, the participant's value and the robust standard deviation is the calculation of the z-score so that:

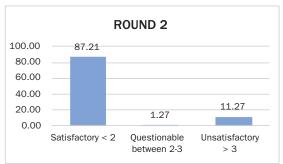
 $\begin{tabular}{ll} If z-score \le 2 & Result SATISFACTORY \\ If 2 < z-score \le 3 & Result QUESTIONABLE \\ If z-score > 3 & Result UNSATISFACTORY \\ \end{tabular}$

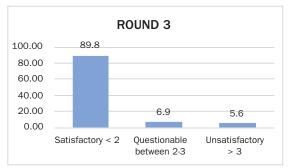
After applying the assessment criteria to each of the results obtained by the different laboratories on each of the samples, the percentages of the z-score ranges obtained in 2021 are as follows:

ROUND 1

100.00
80.00
60.00
40.00
20.00
0.00
Satisfactory < 2 Questionable between 2-3 > 3

Figure 8.3.4.7





Considerations about the exercise

Although this exercise is not yet accredited by ISO/IEC 17043, it has several characteristics that make it suitable for the intended purpose. These include:

- The samples received are in the same matrix as samples routinely analysed in the laboratory at concentrations within the working range.
- In this exercise, the organiser estimates the assigned value of the measure by consensus among the results issued by the participating laboratories using robust statistical techniques. The estimate is influenced by the number of participants.
 In this case, the number of participants in this exercise (between 50-60) ensures the statistical validity of the results.
- Robust statistical techniques are used to minimise the influence of extreme results in the calculations performed (ISO 13528:2015 Robust Analysis Algorithm A).
- Calculation of the z-score parameter, using the robust standard deviation calculated using Algorithm A as a standard deviation.
- This is currently under review by the SGC Seville Department to prepare a series of improvements, including the establishment of a target sigma.

Blood alcohol intercomparison exercise (EIAS) improvements

a) In the transport of samples

- The sample referral tubes have been changed from glass to polypropylene.
- For supply reasons, availability has been restricted to 1.5 ml tubes.
- Due to the time required for delivery, the traditionally used packaging has been replaced with a cold holding kit.
- For foreign laboratories, an external company has been used to guarantee delivery within 24 hours.
- b) Preparation of proposals for calculating a target sigma

A study of the results of previous exercises (10 years) was carried out to establish a target standard deviation by concentration ranges, using the variation coefficient between laboratories that would be expected from the participants given the characteristics of the exercise.

The objectives of this proposal were:

- Use z-scores as a fixed benchmark for the performance assessment, regardless of variations in group results from one study to the next.
- The resulting z-scores could be compared from one study to the next to demonstrate trend performance for an individual laboratory.
- Setting a realistic target standard deviation can provide a benchmark for the progressive improvement for each laboratory.

During the first half of the year, SDPAs (standard deviation for proficiency assessments) were proposed, the z-scores obtained were calculated and compared with the z-scores contained in the reports.

Having applied the criteria established by ENAC in ENAC Guide G-14 (ENAC, 2008), both in relation to the dispersion of results of participating laboratories and the uncertainty of the assigned value, the conclusion was reached that perhaps it was too permissive, as these values are always lower than those reported. This occurs mainly at ethyl alcohol concentrations of less than 0.5 g/l.

During the second half of the year, new, more restrictive criteria were proposed and the range of concentrations was extended to 6 g/l, since in the last sample analysed the value assigned was 5.8 g/l, and the same action guidelines were applied.

The new proposal was studied and the proposed criteria were found to be well adapted to the requirements of ENAC, which follows the ratio below:

 $SDPA = 0.07 \times VA^{0.5}$

Assigned value	TARGET CV	SDPA
0.1	15	0.015
0.3	12	0.036
0.5	10	0.050
1	8	0.080
2	6	0.110
3	4	0.013
4	3	0.140
5	3	0.140
6	6	0.180

8.3.5. Teaching and scientific activity

8.3.5.1. Participation in investigation projects and collaborations with other institutions

The Quality Assurance Service at this Department, along with the services from the other Departments, have actively participated and collaborated within the Quality Group of the Network of Official Spanish Forensic Laboratories (RLFOE), virtually attending the annual meeting.

On behalf of the INTCF, he is part of the working group CTN197-SC2 Forensic Services in the Spanish Standardisation Organisation (UNE). Among other activities, this group reviews and provides comments on the documents of the ISO 21043: Forensic sciences standard, which is being prepared by the different ISO/TC 272 working groups.

On behalf of the INTCF, it is in the group CTN197-SC1 Expert Services in UNE. The activity of this group has been dedicated to reviewing the UNE 197010 ICT standard.

It has participated as an expert in two international projects: collaboration with the Spanish Agency for International Development Cooperation (AECID) as part of an online training course on ISO/IEC 17025 General requirements for the conformity of testing and calibration laboratories and in the framework of the ICrime Project: Cooperation in criminal investigation in Central America to combat crime and drug trafficking at an international level AC1/ICRIME, with the face-to-face mission in El Salvador for "Follow-up on ISO Accreditation in chemistry and genetics services" (October 2020) and participation in the meeting of directors and online participation in the Central American Quality Training Meetings.

In 2021, meetings continued to be held by the QCLG-QCC Measurement Uncertainty (MU) Project (ENFSI) working group to update the current ENFSI documents related to the calculation of measurement uncertainty, in which this Service participates and which is due to end in 2022.

8.3.5.2. Contribution in scientific congresses

Maria Luisa Soria. Participation in the annual meeting of the ENFSI QCLG-QCC. 13 October 2021.

Maria Luisa Soria. Speaker: "Objectives and functioning of international networks. ENFSI" at the Meeting of Forensic Departments of SICA countries, in the framework of the ICRIME cooperation project. El Salvador, 19 October 2021.

8.3.5.3. Teaching and training activities

Education activities

Soria Sánchez, ML.

Teaching of the subject "Toxicology of Drugs of Abuse", on the degree in Criminology. "Drugs of Abuse: Legal Framework". February 2021.

Teaching of the subject "Toxicology of Drugs of Abuse", on the degree in Criminology. "Chemical submission". March 2021.

Teaching of the subject "Introduction to Forensic Sciences: Toxicology and Forensic Medicine", on the degree in Criminology. "The Chemical Toxicology Report". March 2021.

Teaching of the subject "Toxicology of Drugs of Abuse", on the degree in Criminology. "Practical cases". May 2021.

Coordinator of the online training programme "Validation, verification and transfer of methods" included in the European project "Cooperation in criminal investigation in Central America to combat crime and drug trafficking at an international level" ICRIME-LA/2017/39066". From 10 to 14 May 2021.

"Practical workshop on the dissemination of the INTCF quality system". Organised by the Centre for Legal Studies (CEJ). From 21 to 28 September 2021.

Speaker at the presentation "Standard Validation Working Procedure at the National Institute of Toxicology", included in the training activity "Practical workshop on validation of forensic toxicology methods" (CEJ). From 4 to 08 October 2021.

Speaker at the presentation "The value of the quality system. General aspects". Selective course for forensic doctors. 08 October 2021.

Coordinator of the training activity "Quality Assurance in the European Process. A step forward" included in the Continuous Training Plan for 2021 organised by the Centre for Legal Studies.

Speaker at the presentation "Standardisation of the forensic process. Background and current framework" included in the training activity "Quality Assurance in the European Process. A step forward", included in the Continuing Education Plan for 2021 organised by the Centre for Legal Studies.

Speaker at the presentation "Structure of the INTCF Quality System. History. Background. Quality Policy. Objectives. Reference standards". Activity: 1-TOX-2021: Quality assurance in the INTCF, quality manuals, standard operating procedures, concepts and definitions. Seville edition. Organised by the National Institute of Public Administration. 08 November 2021.

Speaker at the presentation "INTCF Staff: Personnel management to ensure competence. Goals. Activity": 1-TOX-2021: quality assurance in the INTCF, quality manuals, standard operating procedures, concepts and definitions. Seville edition. Organised by the National Institute of Public Administration. 08 November 2021.

Speaker at the presentation "INTCF facilities and environmental conditions: factors and impact on the different INTCF activities". Activity: 1-TOX-2021: quality assurance in the INTCF, quality manuals, standard operating procedures, concepts and definitions. Seville edition. Organised by the National Institute of Public Administration. 09 November 2021.

Speaker at the presentation "Sample management at a laboratory with a quality system". Activity: 1-TOX-2021: quality assurance in the INTCF, quality manuals, standard operating procedures, concepts and definitions. Seville edition. Organised by the National Institute of Public Administration. 10 November 2021.

Speaker at the presentation "Intercomparison exercises. How does the INTCF work? Activity: 1-TOX-2021: Quality assurance in the INTCF, quality manuals, standard operating procedures, concepts and definitions. Seville edition. Organised by the National Institute of Public Administration. 11 November 2021.

Speaker at the presentation "Internal audits: What are they for? How are they performed? Who performs them?" Activity: 1-TOX-2021: quality assurance in the INTCF, quality manuals, standard operating procedures, concepts and definitions. Seville edition. Organised by the National Institute of Public Administration. 12 November 2021.

Speaker at the presentation "Standardisation in forensic sciences. National and international organisations that support and promote quality". Course 2-TOX-2021 Quality. Study of the UNE-EN ISO/IEC 17025:2017 standard. General Requirements for the Competence of Testing and Calibration Laboratories. Madrid edition. Organised by the National Institute of Public Administration, 12 November 2021.

Speaker of the presentation "INTCF reports from a quality perspective". Activity: 1-TOX-2021: Quality assurance in the INTCF, quality manuals, standard operating procedures, concepts and definitions. Seville edition. Organised by the National Institute of Public Administration. 15 November 2021.

Speaker at the presentation "Accreditation of a laboratory. How is this achieved?" Activity: 1-TOX-2021: Quality assurance in the INTCF, quality manuals, standard operating procedures, concepts and definitions. Seville edition. Organised by the National Institute of Public Administration. 15 November 2021.

Speaker at the presentation "Standardisation in forensic sciences. National and international organisations that support and promote quality". Course 2-TOX-2021 Quality. Study of the UNE-EN ISO/IEC 17025:2017 standard. General Requirements for the Competence of Testing and Calibration Laboratories. Seville edition. Organised by the National Institute of Public Administration. 19 November 2021.

Speaker at the presentation "Standardisation in forensic sciences. National and international organisations that support and promote quality". Course 2-TOX-2021 Quality. Study of the UNE-EN ISO/IEC 17025:2017 standard. General Requirements for the Competence of Testing and Calibration Laboratories. Barcelona edition. Organised by the National Institute of Public Administration. 23 November 2021.

Speaker at the presentation "INTCF participation in national and international working groups and quality standardisation committees". Course 2-TOX-2021 Quality. Study of the UNE-EN ISO/IEC 17025:2017 standard. General Requirements for the Competence of Testing and Calibration Laboratories. Barcelona edition. Organised by the National Institute of Public Administration. 23 November 2021.

García Repetto, R.

Associate teacher at the Pablo de Olavide University of Seville in the Degree in Criminology and Double Degree in Law and Criminology.

Teacher in the Master's Degree in Criminology and Forensic Sciences. Pablo de Olavide University of Seville.

Associate teacher at the Pablo de Olavide University of Seville in the Degree in Criminology and Double Degree in Law and Criminology.

in the Master's Degree in Criminology and Forensic Sciences at the Pablo de Olavide University of Seville.

Expert in the online training programme "Calibration and maintenance of equipment" included in the European project "Cooperation in criminal investigation in Central America to combat crime and drug trafficking at an international level" ICRIME-LA/2017/39066", from 8 to 12 March 2021.

Expert in the online training programme "Validation, verification and transfer of methods" included in the European project "Cooperation in criminal investigation in Central America to combat crime and drug trafficking at an international level" ICRIME-LA/2017/39066" from 10 to 14 May 2021.

Speaker of the presentation "Standardisation in forensic laboratories" (ISO 17025) in the training activity "Quality Assurance in the European Process. A step forward", included in the Continuing Education Plan for 2021 organised by the Centre for Legal Studies.

Speaker at the presentation "Documentation of the INTCF quality system. Quality manual, procedures, annexes, data collection sheets. Types of procedures. Control of

documentation". Activity: 1-TOX-2021: quality assurance in the INTCF, quality manuals, standard operating procedures, concepts and definitions. Seville edition. Organised by the National Institute of Public Administration. 08 November 2021.

Speaker at the presentation "INTCF equipment. Equipment management. Part 1. Biology, forensic sciences and histopathology teams". Activity: 1-TOX-2021: quality assurance in the INTCF, quality manuals, standard operating procedures, concepts and definitions. Seville edition. Organised by the National Institute of Public Administration. 09 November 2021.

Speaker at the presentation "INTCF equipment. Equipment management. Part 2. Chemistry and Drugs, Assessment and General equipment". Activity: 1-TOX-2021: quality assurance in the INTCF, quality manuals, standard operating procedures, concepts and definitions. Seville edition. Organised by the National Institute of Public Administration. 09 November 2021.

Speaker at the presentation "Traceability of measurements. Patterns and reference material. Calibration and verification at the INTCF". Activity: 1-TOX-2021: quality assurance in the INTCF, quality manuals, standard operating procedures, concepts and definitions. Seville edition. Organised by the National Institute of Public Administration. 10 November 2021.

Speaker at the presentation "Methods of analysis. What is needed according to the INTCF quality system". Activity: 1-TOX-2021: quality assurance in the INTCF, quality manuals, standard operating procedures, concepts and definitions. Seville edition. Organised by the National Institute of Public Administration. 11 November 2021.

Speaker at the presentation "Dealing with deviations and complaints. How to overcome a non-conformity or non-conforming work? System improvements and risk analysis: why is this necessary? Activity: 1-TOX-2021: quality assurance in the INTCF, quality manuals, standard operating procedures, concepts and definitions. Seville edition. Organised by the National Institute of Public Administration. 12 November 2021.

Speaker at the presentation "Supply management to be able to work with quality". Activity: 1-TOX-2021: quality assurance in the INTCF, quality manuals, standard operating procedures, concepts and definitions. Seville edition. Organised by the National Institute of Public Administration. 15 November 2021.

Training activities

Soria Sánchez ML and García Repetto R

Course organised by the Centre for Legal Studies as part of the Continuous Training Plan for 2021, "The INTCF Forensic Sciences Service: fields of action, analytical possibilities". Online, from 22 to 26 March 2021.

Course organised by the Centre for Legal Studies as part of the Continuous Training Plan for 2021, "Detection and identification of chemical weapons in the forensic field. Toxicity and impact on the environment". Online, from 4 to 11 May 2021.

Course organised by the Centre for Legal Studies as part of the Continuous Training Plan for 2021, "Interpretation of toxicological results and their influence according to the expert context in which the analysis is requested". Online, from 10 to 17 May 2021.

Course organised by the Centre for Legal Studies as part of the Continuous Training Plan for 2021, "Basic LIMS: structure, data organisation and queries". Online, from 17 to 24 May 2021.

Course organised by the Centre for Legal Studies as part of the Continuous Training Plan for 2021, "New research tools in the field of forensic genetics". Online, from 21 to 28 June 2021.

Course organised by the Centre for Legal Studies as part of the Continuous Training Plan for 2021, "Practical workshop on the dissemination of the INTCF quality system". Online, from 21 to 28 September 2021.

Course organised by the Centre for Legal Studies as part of the Continuous Training Plan for 2021, "Practical workshop on validation of methods in Forensic Toxicology". Online, from 4 to 8 October 2021.

Course organised by the Centre for Legal Studies as part of the Continuous Training Plan for 2021, "Quality Assurance in the Forensic Process: a step forward". Online, from 2 to 5 November 2021.

8.4. Quality Assurance at the La Laguna Delegation

8.4.1. Activities carried out at the Delegation

- Management of system documents. Preparation /modification of SOPs, DCSs and annexes.
- Staff training. Training programmes and qualification certificates.
- · Compliance with equipment calibration, verification and maintenance programmes.
- Assessment of participation in intercomparison exercises
- Record and monitoring of incidents, nonconformities and corrective actions.
- Record and monitoring of improvement actions and actions to address risks and opportunities.
- Management of claims and complaints.

8.4.2 Validations performed in 2021

Table 8.4.2.1.

SERVICE	Test/technique method	Status
BIOLOGY	Test method/technique: in-house validation of amplification, electrophoresis and genotyping procedures using the commercial GlobalFilerTM kit for the 3500 HID 8-capillary genetic analyser and GeneMapper ID for forensic-specific analysis software.	In process
	Test method/technique: validation of GC-FID equipment with automated headspace injector for ethyl alcohol determination	In process
CHEMICAL	Test method/technique: analysis of drugs of abuse commonly found in seizures (cannabis, amphetamine, methamphetamine, MDMA, ketamine, cocaine and heroin).	In process

8.4.3. Intercomparison exercises in which the Delegation's Services participated

Table 8.4.3.1.

Dartinination	in intor	nomnarican	OVORDICAC O	st tha L	Piology Corving
- Fai ilcibation	ı III IIILEI	JUHDAHSUH	exercises o	ливс	Biology Service

Program: Analysis of DNA polymorphism in blood stains and other biological samples

Organised by: INTCF-GHEP-ISFG Frequency: Annual

Parameters: forensic and kinship genetics and preliminary studies in blood, hair and other matrices

Participation in intercomparison exercises of the Chemistry Service

Program: Blood Alcohol Intercomparison Exercise

Organiser: INTCF-SEVILLA Samples: blood, plasma Frequency: four-monthly

Parameters: ethyl alcohol and other volatile compounds

Program: Interlaboratory Exercise on Drugs of Abuse Commonly Abused in Addictions

Organiser: INTCF-BARCELONA Samples: powder-solid (seizures)

Frequency: Annual

Parameters: qualitative and quantitative analysis of drugs of abuse and qualitative analysis of adulterants and diluents.

Program: Forensic Blood Toxicology Proficiency Testing (Quartz)

Organiser: LGC Frequency: quarterly

Parameters/samples: drugs of abuse, psychotropic drugs and synthetic cannabinoids in blood and urine

8.4.4. Accreditation scopes

The La Laguna Delegation has an accreditation file open pursuant to the requirements of the ISO 17025 Standard: Accreditation No. 297/LE1852, which brings together various test methods in the forensic area.



9. Toxicology
Information Service



The Toxicological Information Service (SIT) was created by Decree 1789/1967 of 13 July 1967 and began operating in February 1971. Article 5 of this Decree included the function of dealing with court requests related to poisonings, as well as with enquiries from the public following poisoning. It first worked out of the old building at the Faculty of Pharmacy, now the Royal Academy of Pharmacy, with 13 forensic doctors, 2 administrative assistants and 1 judicial agent as its initial staff.

Over the years, knowledge of the toxicity of the chemicals causing poisoning that were appearing on the market increased. Thus, SIT's activity grew exponentially as a result of the agreements reached with different agencies and legislative changes, which resulted in SIT's enquiry telephone number appearing on all packaging of cleaning products, on the package leaflets of medicines and on agrochemical products available to users.

Since 2009 the SIT has been located at the Las Rozas de Madrid Business Park, at the Department of Madrid and as part of the National Institute of Toxicology and Forensic Sciences (INTCF). It consists of 33 professionals, details of which will be provided in the section on its staff.

The SIT offers a public service that is unique in Spain. As a body at the disposal of the Administration of Justice, the SIT acts as a technical advisory body for magistrates, judges, prosecutors and Institutes of Legal Medicine and Forensic Doctors, and, in case of any request for information on toxicological matters, the SIT's medical staff prepare the corresponding expert reports and opinions pursuant to the court request.

It also functions as an anti-toxicology centre in itself, with medical staff providing uninterrupted and continuous telephone assistance to intoxicated patients for toxicological consultations. This is made possible by its staff's tireless efforts to constantly update its Documentation Section on chemicals included in its self-assembled database.

It therefore acts as the national centre receiving the qualitative and quantitative formulations sent by the chemical industry to the SIT and, at the same time, as the centre providing the initial health response to the intoxicated subject over the telephone, which is widely distributed to the entire population: 915 620 420.

Finally, among its targets as an anti-toxicity centre, the following are worth particular mention: to reduce the number of poisonings by establishing the relevant preventive measures, to improve its own toxicological monitoring tasks through a new model to be established, to implement its new SIT platform in relation to better management and exploitation of the data collected on poisonings, and the full development of the European harmonisation project led by the European Chemicals Agency (ECHA), the latter being especially the responsibility of the SIT's Documentation staff.

2021 saw the Service celebrate its 50th anniversary. Initially, the SIT developed its functions as staff at the service of the Courts of Justice, extending over time to those offered to society itself in the event of poisoning, similar to the anti-poison centres that were set up in neighbouring countries in the past.

Over the year of the year, different activities related to this anniversary have been undertaken, which will be detailed in this document, with special emphasis on the 50th Anniversary celebration held at the INTCF ceremony hall.

As was the case the previous year, telephone redirections from the base telephone platform to the 20 company mobiles assigned to its medical staff and teleworking has remained in place, as well as individual computer equipment for assisting users consulting our Service, which has hardly been affected. Worth mention is the coverage of sick leave by means of shifts added to all available personnel, and reference should be made to the human factor as the best resource that this Service has had, on account of its attitude, availability and efforts.

In this sense, the fact that for yet another year, various factors have persisted, such as the health problems caused by the SARS-CoV-2 virus or the sick leave suffered by a third of the Service's staff, together with the social, emotional and psychological aspects affecting the user population, emphasises the permanent work carried out by the medical, documentation and administrative staff at the SIT, having continued to offer a unique public service for yet another year of the pandemic, as an added value to be highlighted for its entire staff.

The SIT would like to thank the Directorate General of the Public Justice Service for making it possible to hold the 50th Anniversary celebrations of the Toxicology Information Service. We would also like to thank the staff from the Communications Office at the Ministry of Justice for organising the necessary infrastructure to show society the Service's trajectory over these fifty years of service to the public.

Finally, it is also necessary to recognise the time and efforts dedicated to the Service by staff from different areas of the Ministry of Justice. The Subdirectorate General for the Promotion and Innovation of Digital Justice Services, as well as the Subdirectorate General for the Quality of Digital Services, Cybersecurity and Operations, both belonging to the Directorate General for the Digital Transformation of the Administration of Justice, are worth particular mention. Both have contributed to the IT and technological evolution of the SIT with the development of the new platform Fichas SIT as a new toxicological registration model, as well as the Datalab tool in relation to the exploitation of the IT data collected by our Service. The staff at the Security Unit also deserves recognition for their work in developing regulations and adapting the SIT to the National Security Scheme.

SIT CONTACT DETAILS

Ordinary post: Calle José de Echegaray, 4. 28230 - Las Rozas de Madrid.

E-mail: intcf.sit@justicia.es (Toxicology Information)

intcf.doc@justicia.es (Documentation Section)

Twitter: @INTCFjusticia

Website: https://www.mjusticia.gob.es/es/ministerio/organismos-entidades/instituto-nacional/servicios/servicio-informacion

Telephone: 91 562 04 20

9.1. SIT workforce

The service is staffed by 33 people, all of whom are civil servants at the Justice Administration and who carry out different functions in line with their duties, corresponding to 7 civil servants from the General Bodies of Procedural and Administrative Procedures, 7 doctors from the Forensic Corps and 13 doctors from the Medical Corps for the over-the-phone management of Toxicology Information, with the corresponding university degree in Medicine and Surgery, and 4 doctors belonging to the Documentation Section with a university degree in Pharmacy and also in Medicine and Surgery, managed by a head of section, with all the staff being coordinated by a head of service.

Table 9.1.1. Toxicology Information Service staff

	Toxicology Information Service	
	INTCF	
Head of the Service	1	
Documentation Section Head	1	
Facultatives	17	
Medical examiners	7	
Clerical staff	7	

9.2. General activities

Below is a description of the activities carried out by all SIT staff during 2021.

Medical staff

Telephone enquiries handled

All requesting calls are attended both to citizens without specific health training (915 620 420) and to health personnel from health centres, hospitals and hospital and out-of-hospital emergency services (914 112 676 and for the exclusive use of said personnel).

A total of 75,677 telephone enquiries were answered last year, a slight drop on the annual average of 80,000 enquiries in recent years. This decrease is believed to be a positive consequence of the press releases, advice and communications issued by SIT during the previous year on the adverse health effects of the overuse of cleaning products intended to eliminate the SARS-CoV-2 pathogen by users.

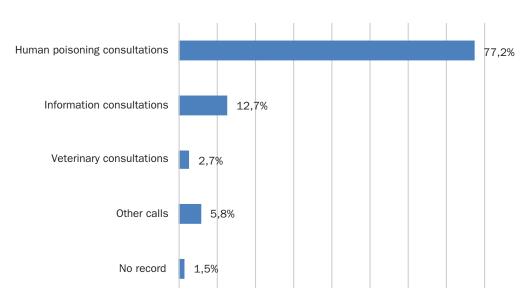


Figure 9.2.1. Distribution of consultations answered by SIT medical staff in 2021

According to the telephone enquiries recorded (Figure 9.2.1), there are four groups or types of enquiries:

• Human poisoning consultations: 58,448 (77,2%).

Cases registered following the detection of toxic exposure or actual poisoning in humans by any potentially toxic substance or product.

• Veterinary consultations: 2,081 (2,7%).

Records related to the poisoning of animals by any product or substance.

• Information consultations: 9,600 (12,7%).

Consultations requesting information, particularly in relation to health, and without exposure to toxics.

• Other calls: 4,391 (5,8%).

Part of the records of these last groups of consultations demonstrates the important work of health professionals in dealing with consultations for information or due to adverse circumstances, including loneliness, from the population. It is socially important to have a telephone line for people who simply need emotional contact, psychological help or social outreach, especially during the pandemic.

• No record: 1,162 (1,5%).

Reports issued by the medical staff of the SIT during 2021

132 toxicology reports were prepared and issued during 2021.

Their classification is determined depending on the request or case in question, catalogued as M-21, ME-21 or IC-21 reports (Figure 9.2.2).

70 63 61 60 50 40 - "M" reports "ME" reports 30 "IC" reports 20 8 10 0 "M" reports "ME" reports "IC" reports

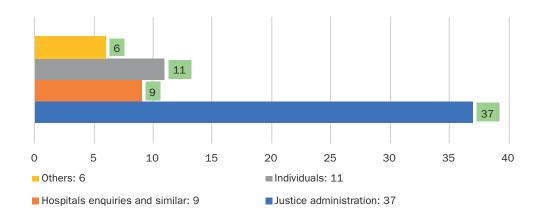
Figure 9.2.2. Distribution of reports issued by the medical staff of the SIT during 2021

In the case of M-21 reports, these are opinions issued after a detailed study of an issue requested, either by the Justice Administration or by other institutions, as shown in the attached graph (Figure 9.2.3).

A total of 63 reports were drawn up and sent following requests mainly from the Justice Administration in response to expert requests for courts and tribunals, where a detailed study of the request is required. These requests are assigned consecutively and drawn up by the SIT doctors. Based on those seeking information, there are four different groups:

- · From the Justice Administration. 37.
- · From private individuals: 11.
- · From healthcare workers: 9.
- · Other requests: 6.

Figure 9.2.3. Distribution of M-21 reports produced by SIT medical staff in 2021



In relation to ME-21 Reports, this information is sent by e-mail at an early stage as they are requests from the public and do not require detailed toxicological assessment.

A total of 61 reports were issued and drawn up following requests mainly from users, with the Head of the Service replying by e-mail promptly to the request from the public, in general.

IC-21 Reports answer the health professional's requests in relation to toxicology cases, sending requested complementary information by email.

Eight reports were prepared and sent as responses to requests from health professionals, where the response related to a toxicological case requiring additional information to that previously exchanged via e-mail and over the phone was sent.

Activities carried out by the SIT Documentation Section during 2021

Its main activity is related to the project promoted by the European Union and led by the European Chemicals Agency (ECHA) for the notification of chemical substances, in order to obtain the most complete toxicology information available and to be able to provide the best possible health response to intoxicated patients.

As part of this project, the ECHA set up different working groups, in which the Documentation Section has been actively collaborating through Webex and the issuing of reports, with a view to participating in the updating and maintenance of the specific software application for the preparation of notifications of hazardous chemical mixtures, and of the ECHA European portal, which allows the corresponding submission to the Member States where the chemical mixture is placed on the market.

During 2021, the ECHA scheduled several meetings with representatives of both Member States and all industrial sectors concerned to identify gaps and areas for improvement in the procedure implemented. This has involved the merger of various working groups that had been active over the past few years in different areas (IT, validation rules, etc.) into a general group, which included members from all the previous sectors to harmonise and channel relevant issues that arose in the different areas, with the meetings becoming more distant and the process now considered to be one of maintenance rather than development, as it was in the years prior to its entry into force.

The working groups in which this Section has collaborated in 2021 are as follows:

- Validation Rules (VR) WG: working group that reviews the validation rules for notifications processed by the chemical industry through the ECHA portal. Furthermore, the inclusion of new validation rules has been considered when situations were detected that had not been initially contemplated and required intervention.
- PCN Stakeholders Group: this year, the ECHA decided to create this new working group, incorporating both people and issues that were previously dealt with by separate and independent working groups, which were relevant during the development stage of

both the procedure and the tools. At the start of 2021, the ECHA determined the transition of these groups to a maintenance mode, which is the reason for this merger.

The following teams and contents are incorporated into this new working group:

- IT tools WG: working group initially conceived for the development and review of the IT application developed by ECHA as well as for the preparation of the export file in a harmonised format for the entire European Union (PCN format).
- Guidance WG: working group for the adaptation of the ECHA Guidelines to the legal regulations (Annex VIII of the CLP Regulation and Article 25 of the CLP Regulation itself).
- Database WG: working group for the design and review of the ECHA's central database.
- Expert Group for the final review of the Guidelines (PEG), working group nominated by the Competent Authority of CARACAL (in Spain, the Ministry of Health).

On 1 January 2021, the harmonised notification of hazardous chemical mixtures came into force, whereby companies notify the bodies appointed in each Member State where their products are marketed, with the INTCF being the designated body for Spain. A transitional period until 1 January 2024 has been established for submitting notifications for industrial use only, although these can also be notified through the harmonised route in advance.

This notification is processed pursuant to the provisions of Annex VIII of the CLP Regulation (classification, labelling and packaging of substances and mixtures), which has involved significant efforts and joint work with the team of the Subdirectorate General for Territorial Cooperation and Coordination, as well as with the Directorate General for the Digital Transformation of the Administration of Justice, to adapt the computer systems to this new notification system.

As part of this harmonisation process, a European notification portal, developed by ECHA, has been developed, which receives notifications from chemical companies and forwards them to the Member State where these mixtures are placed on the market.

An important aspect of the harmonised notification process is that it allows companies to notify their products with certain quality warnings, as long as they strictly comply with what is reflected in the legislation in force. The designated body in each Member State may, if it deems so appropriate, request further information to enable its poison control centre to provide the relevant health response. Almost half of the notifications received during 2021 for Spain (113,687) include warnings that could become relevant for designated bodies. It is the role of the Documentation Section to ask the company for any additional data it deems necessary to obtain better and more information that will enable the Poison Control Centre to provide a more appropriate response to a possible case of poisoning or accident involving one of these products.

This has led to the development of different tools at a national level to detect the most critical or relevant situations with a view to prioritising the actions to be taken in each case.

The requirements for the information notified, as well as its submission format and any information related to this harmonised notification procedure (legislation, guidance, format, Q&A, etc.), are available to both the designated bodies and industry, and any other relevant parties that require it, on the website developed by ECHA (https://poisoncentres.echa.europa.eu/es/).

During 2021, the Documentation Section has worked intensively with national authorities (Ministry of Health, REACH-CLP Portal, etc.), as well as with the chemical industry and its different associations, for a better understanding and improvement of this procedure and its implementation at a national level.

Reports produced by the SIT Documentation Section

In 2021, 1,952 reports were issued in response to requests for information from companies in the chemical sector.

Most of the reports handled came from Catalonia (23.3%), the Community of Madrid (18.0%) and the Community of Valencia (11.2%) (Figure 9.2.4).

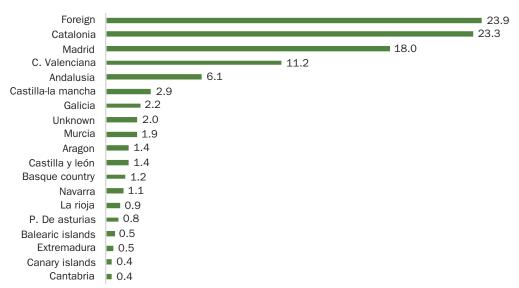


Figure 9.2.4. Distribution of information requests registered in the Documentation Section (in %) according to their geographical origin

Also worth mention were the requests for information from other EU Member States or third countries, raising their queries in relation to the procedure established in Spain for the notifications of mixtures classified as dangerous that are marketed in our country, with 23.9% of the requests for information registered in the Documentation Section, throughout 2021. Most of these consultations came from Germany (27.0%), Italy (15.8%), France (9.2%) and the United Kingdom (8.4%) (Figure 9.2.5).

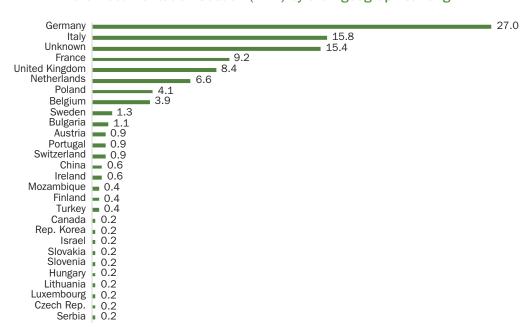


Figure 9.2.5. Distribution of information requests from foreign companies registered in the Documentation Section (in %) by their geographical origin

Notifications to the INTCF

Details concerning the update in Spain of the procedure for the notification of chemical substances and mixtures to the INTCF were set out in Order JUS/288/2021, of 25 March, regulating the procedure for the notification of chemical substances and mixtures to the INTCF, published in the Official State Gazette on 27 March 2021.

Over the course of the year, the national and harmonised notification procedures for chemical mixtures to the INTCF were combined, while at the same time active efforts were made to ensure that notifications made to the INTCF by either route were available to SIT's doctors so that they could provide the appropriate health response immediately, with all the information available on the mixture in the INTCF database.

The Documentation Section, in addition to the 1,952 requests for information reflected in the previous section, registered 104,374 submissions, either through the national procedure (4,629 submissions through the Company Registration System (CRS); 4.4%) or through the harmonised procedure (99,745 notifications made through the ECHA; 95.6% of the notifications received), from the 2,306 companies authorised to make notifications of the products they market in Spain.

In these 104,374 submissions, a total of 114,230 products were notified during 2021, as, in cases of notifications made under the national procedure, more than one product is allowed to be included in each submission.

Of the 2,306 companies that have reported products to the INTCF, 911 (39.5%) are Spanish, while 1,395 (60.5%) are foreign companies who market products in Spain.

Harmonised procedure

During 2021, 262,382 notifications were registered through ECHA's European portal for redirection to the INTCF. The following aspects are worth particular mention:

The final recipient of the mixture is mainly the end consumer, with 110,125 notifications, while 202,604 are mixtures for professional use and 186,064 for industrial use. In many cases, the same mixture or product can share different uses.

A total of 212,510 notifications were initial and unique, i.e. mixtures being notified to the INTCF for the first time, while 26,728 were updates to the information submitted on non-relevant aspects, in no case affecting the composition of the mixture. There were 23,144 updates that included a change in composition.

In total, 98.1% of the notifications received through the ECHA portal, processed via the harmonised procedure, include information on a single hazardous classified chemical mixture, which can be marketed in our country under several different trade names (standard notifications). Only 3,908 notifications are notifications of non-classified hazardous chemicals or chemical mixtures, which are notified to the INTCF by industry on a voluntary basis (voluntary notifications). Furthermore, 1,110 notifications concern hazardous chemical mixtures for industrial use only, in which the company includes only the relevant information from the safety data sheets, but includes an emergency telephone number (24 hours a day in Spanish) to request complete information on the mixture, which will be available to the INTCF if necessary (limited notifications).

Of the total number of products notified for Spain through ECHA's harmonised portal during 2021, 71,683 correspond to products notified by a Spanish legal person. It is also worth noting that 81,857 products were notified by Italian legal entities and 46,838 by German legal entities.

Of the products notified to ECHA, 90,914 products were processed in 2021 and included in the FICHAS SIT database, with all the information sent by the company, in order for the INTCF, through the SIT's doctors, to provide the appropriate health response. Of the products available in FICHAS SIT over the course of the year, 9,274 products (10.2%) were notified by companies whose legal person is located in Spain and the rest, 81,640 chemical mixtures (89.8%), in other Member States such as Italy (49.3%), Germany (12.6%) or France (12.6%) (Figure 9.2.6).

Germany
12.6%

France 12.6%

Belgium 1.7%
Portugal 1.6%
United Kingdom 0.3%
Finland 0.1%
Austria 0.2%

Spain 10.2%

Figure 9.2.6. Geographical distribution of the legal entity notifying chemical mixtures to the INTCF under the harmonised procedure

National notification

Over the course of the year, the national and harmonised notification procedures for chemical mixtures to the INTCF have been combined, with a view to avoiding the adaptation period of the harmonised notification procedure leading to a loss of information and doctors not being able to provide the health response because they could not include the information of the chemical mixture in the INTCF database. Many companies, despite having notified under the harmonised procedure, were also notifying under the national procedure. Furthermore, some companies who market products for industrial use only opted for national notification, as according to Order JUS/288/2021 of 25 March, this remains in force and available until 1 January 2024 for mixtures for industrial use only.

Among the products notified through the national procedure, only 7.6% came from companies located in other Member States, as these companies mainly used the harmonised notification via ECHA, while 92.4% were Spanish companies, accustomed to using the national procedure (via SRE).

The Autonomous Communities that reported the highest proportion through this national procedure are Catalonia (24.5%), Community of Madrid (22.8%), Community of Valencia (15.2%) and La Rioja (12.1%) (Figure 9.2.7).

Catalonia Madrid 22.8 C. Valenciana 15.2 La Rioja 12.1 Andalusia 7.3 Castilla-La Mancha 4.7 Basque Country 2.8 Galicia 2.8 Murcia P. de Asturias Aragon == Castilla y León = 1.0 Extremadura 0.8 Cantabria - 0.8 Navarra 0.6 Canary Islands 0.5 Balearic Islands 0.0 0.0 5.0 10.0 15.0 20.0 25.0 30.0

Figure 9.2.7. Distribution by Autonomous Communities of Spanish companies that have notified under the national procedure (via SRE)

In 80.3% of cases, the notifications made following this procedure were new products that had not been notified previously; 12.2% were formula modifications of previously notified products for which the composition had been changed; and 0.4% were updates of non-relevant data, such as label design, package size, etc. Worth particular note is that 7.1% of notifications were for product discontinuation, a much higher percentage than in previous years, probably because they had been notified under the harmonised procedure and the company notified the cancellation of the previous notification so that there was only one piece of information on its product in the INTCF database (Figure 9.2.8).

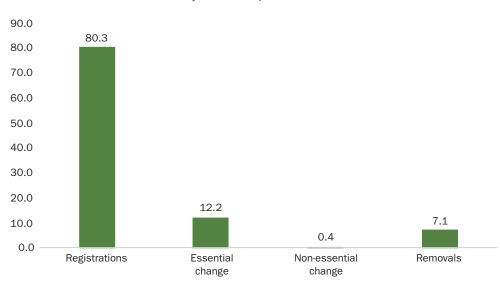


Figure 9.2.8. Distribution by type of notification to the INTCF in 2021, by national procedure

Administrative staff

The tasks carried out by the administrative staff, mainly belonging to the Procedural and Administrative Processing Corps, are of essential importance to the daily activities of the SIT. Work is distributed either focussing on the administrative management of the requests received and their corresponding referral, or aimed at transcribing and preparing the response models, both for medical and documentation staff.

9.3. Description of a case of interest with media coverage

On 10 June, at the INTCF ceremony hall, the SIT's 50th anniversary celebrations took place, under the slogan "Fifty years of uninterrupted telephone assistance to those suffering from intoxications".

The event was opened by the then Minister of Justice Juan Carlos Campo, and was also attended by relevant ministerial representatives. It *streamed* to offer the entire population a closer look at such a significant event. As part of two round tables, the different activities that the SIT has carried out and continues to offer were presented by representatives of different public and private institutions of recognised prestige.

Over the course of the 50th Anniversary celebrations, the institutional and social importance of this Service was made clear, and the focus was on the preventive approach carried out by the Service through the toxicological surveillance measures established over the years as a public service for the population and in relation to its new project in this regard. Its most recent activities during the SARS-CoV-2 pandemic were highlighted as was the development of the future IT platform for the SIT, as well as the project on the notification of chemical substances to generate the most comprehensive toxicology information available to provide the optimal health response to intoxicated patients.

Another way of commemorating the SIT's 50th anniversary were the ten releases made via social media, in the form of information modules, presenting the institutional usefulness of the SIT to the public, as well as the toxicological effects caused by different types of products and substances.

9.4. Teaching and scientific activity

9.4.1. Participation in investigation projects and collaborations with other institutions

Investigation projects

"Detergent capsules – accidentology project (laundry, dishwasher and others)". Collaborating entities: International Association for Soaps, Detergents and Maintenance Products (AISE), Toxicological Information Service, and other European Anti-toxin Centres. Period of execution: 2012-2021.

Brief summary of objectives: Retrospective (2012-2016) and prospective (2017-2021) toxicological surveillance study of accidental exposure to detergents in capsule form (laundry, dishwasher and others). The SIT forwards case numbers of patients exposed to such cleaning products and in said commercial format.

The SIT reports data with the aim of establishing improvements in the properties of the packaging and design of commercially marketed containers to make the use of these products safer, especially aimed at the paediatric population due to their particularly attractive format. It also includes an estimate of the severity depending on each toxic exposure in the data sent.

Institutional partnerships

Special mention must be made of the collaboration agreement agreed with the Spanish Agency for Medicines and Health Products (AEMPS), through Provision 11895 of the Official State Gazette No. 169 of 2021 and the Resolution of 12 July 2021, for the exchange of information through the SIT and its Veterinary Department on consultations or intoxications in humans and animals of veterinary medicines and medicines for human use when administered to animals.

Other collaborations

- Member State Communicators' network meeting (ECHA). Communications in COVID-19 pandemic and REACH restrictions.
- · Appointed Bodies and Poison Centres (ECHA). Consultation Plan and Questionnaires.
- Working group on the Appointed Bodies and Poison Centres (ECHA). Portal Notifications database.
- EuroCigua. Grupo de trabajo de Intoxicaciones por ciguatera.
- Centro Coordinador de Alertas y Emergencias Sanitarias (CCAES). Ministerio de Sanidad. Notifications of health alerts.
- Dirección General de Protección Civil y Emergencias. Toxicological data sheets for chemicals. European Seveso III Directive in relation to chemical accidents.
- Confederación Española de Consumidores y Usuarios (CECU). Production of question-and-answer worksheets for children of different ages.
- Comisión Asesora del Organismo Notificado. Agencia Española de Medicamentos y Productos Sanitarios (AEMPS).
- Comité Científico de Productos Sanitarios. Agencia Española de Medicamentos y Productos Sanitarios (AEMPS).
- Departamento de Medicamentos Veterinarios. Agencia Española de Medicamentos y Productos Sanitarios (AEMPS).

- · Comisión Nacional para el uso forense del ADN. Ministry of Justice.
- Comisión Interministerial para la Ordenación Alimentaria (CIOA). Agencia Española de Seguridad Alimentaria y Nutrición (AESAN).
- · Grupo Interministerial de Coordinación de Asuntos REACH y CLP.
- Working Group on Poisons Centre Activities & European Regulatory Issues. European Association of Poison Centres and Clinical Toxicologists (EAPCCT). Surveys.
- Meeting of the CARACAL Sub-group on ATPs to CLP European Commission, Brussels.
- · Guidance WG on Poison Centres. ECHA.
- · Partner Expert Group (PEG). ECHA.
- · International Association for Soaps, Detergents and Maintenance Products (AISE).
- · Federación Empresarial de la Industria Química Española (FEIQUE).
- · Asociación Española de Fabricantes de Pinturas y Tintas de Imprimir (ASEFAPI).
- · Asociación de Empresas de Detergentes y Productos de Limpieza (ADELMA).
- · Federación Empresarial Catalana del Sector Químico (FEDEQUIM).
- · Instituto Tecnológico del Plástico (AIMPLAS).
- · Asociación Nacional de Perfumería y Cosmética (STANPA).
- Asociación Empresarial Española de la Industria de Sanidad y Nutrición Animal (VETERINDUSTRIA)
- · Asociación Empresarial para la Salud, Nutrición y Bienestar animal (ADIPREM).
- · Asociación Española del Comercio Químico (AECQ).
- Asociación Química y Medioambiental del Sector Químico de la Comunidad Valenciana (QUIMACOVA).
- · Asociación Empresarial para la Protección de las Plantas (AEPLA).
- · Instituto Toxicológico de la Defensa. Hospital Gómez Ulla. Ministerio de Defensa.
- Instituto Nacional de Salud y Seguridad en el Trabajo (INSST).
- · Instituto Geológico y Minero de España (IGME).
- · ATROX Group.

9.4.2. Attendance at congresses

Conejo JL. 41st Congress of the European Association of Poisons Centres and Clinical Toxicologists, organised by the European Association of Poisons Centres and Clinical Toxicologists (EAPCCT). Online, from 25 to 28 May.

Las Heras, P. 41st Congress of the European Association of Poisons Centres and Clinical Toxicologists, organised by the European Association of Poisons Centres and Clinical Toxicologists (EAPCCT). Online, from 25 to 28 May.

León, A., Martínez, R., Juanas, T. 41st Congress of the European Association of Poisons Centres and Clinical Toxicologists, organised by the European Association of Poisons Centres and Clinical Toxicology (EAPCCT). Online, 27 May.

9.4.3. Education activities

Doctors, forensic experts and SIT doctors. Internal tutoring of the specific training and coaching programme for the incorporation into the SIT of A. Jábega, V. M. Solsona and M. Martí.

Agudo, J. Internal tutor of the specific training programme for the incorporation into the SIT of M. N. Fernández and S. Jato.

Conejo, J.L. Speaker on "First aid and initial care of the victim". INTCF ceremony hall. 16 June.

Conejo, J.L. Speaker on "The SIT. Characteristics, functions and institutional usefulness". Supervised internships for the 30th graduating class of the National Corps of Forensic Doctors. INTCF. 7 September.

Conejo, J.L. Speaker on "Advice, diagnosis and prevention of accidents due to poisoning with everyday products", as part of the conference "Your training is safety. Product safety for children and adolescents". Ministry of Consumer Affairs, Madrid, 3 December.

Conejo, J.L. Speaker on "Consequences for health derived from the misuse of industrial gases", as part of the conference "More than laughter, risk". Official School of Doctors, Valencia, 10 December.

Oliva, S. de la. Speaker at the scientific session "Update on the treatment of paracetamol poisoning". INTCF. 21 December.

Larrotcha, C. Internal tutor of the specific training and coaching programme for the incorporation into the SIT of C. Hernández and M. Hermosín.

Martínez Arrieta, R. Speaker on "Update of the notification of hazardous mixtures to the INTCF", online technical workshop: REACH and CLP regulations in the sector. Organised by the Asociación de Investigación de Materiales Plásticos y Conexas (AIMPLAS). February.

Martínez Arrieta, R. Speaker on "New developments in harmonised notification to the INTCF", conference on the Notification Procedure for Toxicological Data Sheets. Asociación Española de Fabricantes de Pinturas y Tintas de Imprimir (ASEFAPI). April.

Martínez Arrieta, R. Speaker on "New INTCF notification procedure", for the 2021 Virtual Regulatory Update course. Organised by the Asociación Nacional de Perfumería y Cosmética (STANPA). April.

Martínez Arrieta, R. Speaker on "Basic principles in risk assessment" at the 11th edition of the Safety Assessment and Cosmetic Product Information File. Organised by the Faculty of Pharmacy of the San Pablo CEU University, National Association of Perfumery and Cosmetics (STANPA), Official College of Pharmacists of Madrid (COFM), Spanish Association of Industry Pharmacists (AEFI), Spanish Society of Cosmetic Chemists. Online (2 hours), June.

Martínez Arrieta R. Speaker at "Main factors in the safety assessment of cosmetics according to SCCSS recommendations". At the 11th edition of the Safety Assessment and Cosmetic Product Information File. Organised by the Faculty of Pharmacy of the San Pablo CEU University, National Association of Perfumery and Cosmetics (STANPA), Official College of Pharmacists of Madrid (COFM), Spanish Association of Industry Pharmacists (AEFI), Spanish Society of Cosmetic Chemists. Online (2 hours), June.

Martínez Arrieta R. Speaker on "Systemic toxicity of cosmetics today. The vision from a poisons centre". At the 11th edition of the Safety Assessment and Cosmetic Product Information File. Organised by the Faculty of Pharmacy of the San Pablo CEU University, National Association of Perfumery and Cosmetics (STANPA), Official College of Pharmacists of Madrid (COFM), Spanish Association of Industry Pharmacists (AEFI), Spanish Society of Cosmetic Chemists. Online (1.5 hours), June.

Martínez, R. Speaker on "Notifications to the SIT of hazardous chemical mixtures". Supervised internships for the 30th graduating class of the National Corps of Forensic Doctors. INTCF. 7 September.

Mencías, E. Speaker on "Intervention in attacks with neurotoxic agents". 27th National Firefighters' Congress. Murcia, 16-18 November.

Muñoz, T. Internal tutor of the specific training and coaching programme for the incorporation of M. N. Fernández into the SIT.

Oliva, S de la. Internal tutor for the specific training and coaching programme for the incorporation into the SIT of M. N. Fernández, C. Hernández and M. Hermosín.

Parra, C.I. Internal tutor of the specific training and coaching programme for the incorporation into the SIT of S. Jato.

Trompeta, B.I. Internal tutor for the specific training and coaching programme for the incorporation into the SIT of C. Hernández.

9.4.4. Training activities

Las Heras, P. "Update in Forensic Chemistry and Toxicology". Continuous Training Plan. CEJ. 10 hours. 8-16 March.

Las Heras, P. "The INTCF Forensic Sciences Service: Fields of action, analytical possibilities". Continuous Training Plan. CEJ. 10 hours. 22-26 March.

Las Heras, P. "Interpretation of expert opinions in the field of forensic medicine". Continuous Training Plan. CEJ. 10 hours. 19-27 April.

Las Heras, P. "Forensic detection and identification of chemical weapons. Toxicity and impact on the environment". Continuous Training Plan. CEJ. 10 hours. 4-11 May.

Las Heras P. "Interpretation of toxicological results and their influence on the expert context in which the analysis is requested". Continuous Training Plan. CEJ. 10-14 May.

Las Heras, P. "Pesticides in Forensic Toxicology". Continuous Training Plan. CEJ. 10 hours. 31 May-7 June.

Las Heras, P. "Practical workshop on the dissemination of the INTCF quality system". Continuous Training Plan. CEJ. 10 hours. 21-28 September.

Las Heras, P. "Practical workshop on validation of forensic toxicology methods". Continuous Training Plan. CEJ. 10 hours. 4-8 October.

Las Heras, P. "ECHA Safer Chemicals Conference 2021" European Chemicals Agency (ECHA) Online Training Scheme. 8 October.

Las Heras, P. "Databases of criminal interest: Operational and legal aspects". Continuous Training Plan. CEJ. 10 hours. 13-20 October.

Las Heras, P. "The quality guarantee in the forensic process. A step forward.". Continuous Training Plan. CEJ. 10 hours. 2-5 November.

Las Heras, P. "IUCLID Webinar: IUCLID 6 'October 2021' release". European Chemicals Agency (ECHA) online training scheme. 10 November.

Las Heras, P. "12th BfR User Conference Product Notifications". Organised by the Federal Institute for Risk Assessment (BfR, Germany). 15 November.

Las Heras, P. "Multidisciplinary forensic intervention in multi-victim incidents", Continuous Training Plan, CEJ. 10 hours. 15-22 November.

Las Heras, P. "Forensic medicine and public health". Continuous Training Plan. CEJ. 10 hours, 18-25 November.

Las Heras, P. "PCN Webinar: PCN: explaining the new changes and functionalities". European Chemicals Agency (ECHA) online training scheme. 24 November.

Leon, A. "Update in Forensic Chemistry and Toxicology". Continuous Training Plan. CEJ. 10 hours. 8-16 March.

Leon, A. "The INTCF Forensic Sciences Service: Fields of action, analytical possibilities". Continuous Training Plan. CEJ. 10 hours. 22-26 March.

Leon, A. "Interpretation of expert opinions in the field of forensic medicine". Continuous Training Plan. CEJ. 10 hours. 19-27 April.

Leon, A. "Interpretation of toxicological results and their influence on the expert context in which the analysis is requested". Continuous Training Plan. CEJ. 10-14 May.

Leon, A. "Pesticides in forensic toxicology". Continuous Training Plan. CEJ. 10 hours. 31 May-7 June.

Leon, A. "Forensic toxicology. Toxic pathology". Continuous Training Plan. CEJ. 10 hours. 7-14 June.

Leon, A. "New research tools in the field of Forensic Genetics". Continuous Training Plan. CEJ. 10 hours. 21-28 June.

Leon, A. "Practical workshop on the dissemination of the INTCF quality system". Continuous Training Plan. CEJ. 10 hours. 21-28 September.

Leon, A. "Practical workshop on validation of forensic toxicology methods". Continuous Training Plan. CEJ. 10 hours. 4-8 October.

Leon, A. "Quality assurance in the forensic process". A step forward.". Continuous Training Plan. CEJ. 10 hours. 2-5 November.

Leon, A. "Forensic medicine and public health". Continuous Training Plan. CEJ. 10 hours. 18-25 November.

Martínez Arrieta, R. "Forensic detection and identification of chemical weapons. Toxicity and impact on the environment". Continuous Training Plan. CEJ. 10 hours. 4-11 May.

Martínez Arrieta, R. "Basic LIMS: structure, data organisation and queries". Continuous Training Plan. CEJ. 10 hours. 17-24 May.

Martínez Arrieta, R. "Treatment of offences against sexual freedom and integrity in the forensic laboratory". Continuous Training Plan. CEJ. 10 hours. 14-21 June.

Martínez Arrieta, R. "New research tools in the field of forensic genetics". Continuous Training Plan. CEJ. 10 hours. 21-28 June.

Mencías, E. "Forensic detection and identification of chemical weapons". Toxicity and impact on the environment". LSA. Madrid. 4-11 May.

Mencías, E. "Interpretation of toxicological results and their influence on the expert context in which the analysis is requested". LSA. Madrid. 10-14 May.

Mencías, E. "Forensic toxicology. Toxic pathology". LSA. Madrid. 7-14 June.

Mencías, E. "V Jornada d'antídots. Societat Catalana de Farmàcia Clínica". Barcelona. 29 September.

Oliva, S. de la, "Virtual course on intoxications caused by venomous animals". Chihuahua Health Services (Mexico). 8 hours. 8-10 March.

Oliva, S. de la. "The INTCF Forensic Sciences Service: Fields of action, analytical possibilities". Continuous Training Plan. CEJ. 10 hours. 22-26 March.

Oliva, S. de la. "Interpretation of expert opinions issued by the National Institute of Toxicology and Forensic Sciences". Continuous Training Plan. CEJ. 10 hours. 19-27 April.

Oliva, S. de la. "Detection and identification of chemical weapons in forensics. Toxicity and impact on the environment". Continuous Training Plan. CEJ. 10 hours. 4-11 May.

Oliva, S. de la. "Comprehensive forensic perspective of suicide". Continuous Training Plan. CEJ. 10 hours. 5-12 May.

Oliva, S. de la. "Interpretation of toxicological results and their influence on the expert context in which the analysis is requested". Continuous Training Plan. CEJ. 10-17 May.

Oliva, S. de la. "Pesticides in forensic toxicology". Continuous Training Plan. CEJ. 10 hours. 31 May-7 June.

Oliva, S. de la. "Forensic toxicology. Toxic pathology". Continuous Training Plan. CEJ. 10 hours. 7-14 June.

Trompeta, B.I. "Update in Forensic Chemistry and Toxicology". Continuous Training Plan. CEJ. 10 hours. 8-16 March.

Trompeta, B.I. "Interpretation of Expert Opinions issued by the INTCF". Continuous Training Plan. CEJ. 10 hours. 19-27 April.

Trompeta, B.I. "Pesticides in forensic toxicology". Continuous Training Plan. CEJ. 10 hours. 31 May-7 June.

Trompeta, B.I. "Comprehensive approach to the institutionalised elderly patient". ICOMEM. 20 hours. March.

9.4.5. Participation in working group meetings

Conejo JL. Member State Communicators' network meeting (ECHA). Communications in COVID-19 pandemic and REACH restrictions. 3 February and 28 October.

Conejo JL. ECHA - PCN Stakeholders group, Dossier viewer Appointed Bodies and Poison Centres. Online, 1 March.

Conejo JL. EAPCCT - Submission survey. Consultation Plan and Questionnaires. 21 July.

Conejo JL. International Association for Soaps, Detergents and Maintenance Products (AISE). Referral of toxicological surveillance study of accidental exposure from detergents in capsule form. 2 April and 2 November.

Conejo JL. National Institute for Safety and Health at Work (INSST). 7 October and 19 November.

Conejo JL, Martínez R. Analysis and risks. Security Area at the Ministry of Justice and the National Cryptologic Centre (CCN-CERT). 17 November and 12 December.

Lazaro, I. ECHA - Validation rules Working Group. 20 January.

Las Heras P, Martínez R. ECHA - Validation rules Working Group. Online. 4 March, 21 April, 24 June. 29 November.

Las Heras P, Martínez R. ECHA - PCN Stakeholders Group. Online. 8 March, 17 June, 27 September.

Las Heras P. ECHA - PCN Stakeholders group. For the "Presentation dossier viewer AB/PC". Online. 1 March.

Martínez R, Las Heras P. REACH-CLP Interministerial Commission. 9 March and 26 November.

Martínez, R. Notified Organism Committee and Spanish Agency for Medicines and Health Products (AEMPS). 21 January, 12 April and 12 May.

Martínez, R. Interministerial Committee for Food Organisation (CIOA). 21 January and 25 June.

9.4.6. Dissemination activities

The following collaborations between the SIT and the Communication Office of the Ministry of Justice through different interviews with the media and broadcasts on social channels are worth particular mention:

- 19 April. Chemical submission interview on TeleMadrid (https://www.telemadrid.es/programas/madrid-directo-om/Aumento-sumision-quimica-violaciones-rob os-9-2333556658-20210419082428.html).
- 19 April. Chemical submission interview on Onda Madrid (https://media-telemadrid.secure.footprint.net/SUMISIONQUIMICA_20210419185100_ TMDAUD20210419_0037.mp3).
- 10 June. 50th anniversary of the SIT, #50AñosCuidandoDeTi, streamed live (https://twitter.com/INTCFjusticia).
- 10 June. Minister Campo highlights the work of the SIT in the fight against hoaxes.
- 14 June. "50th anniversary of the SIT" releases, Twitter, in the form of information modules.
- 23 June. Interview in *Diario Norte de Castilla*. Toxicology Information Service.

- 25 June. #50AniversarioSIT campaign. YouTube videos (https://www.youtube.com/c/MinisteriodeJusticiadeEspa%C3%B1a/videos).
- 16 July. Collaboration agreement between the Ministry of Justice and the AEMPS (https://twitter.com/INTCFjusticia/status/1415939248998522887?s=20; https://t.co/9coX6nhmM1).
- 27 July. Interview with the TVE News Team. Toxic effects of ethylene oxide (https://www.rtve.es/play/videos/telediario/15-horas-27-07-21/6022203/; https://www.rtve.es/play/videos/telediario-2/21-horas-27-07-2021/6022809/).
- 23 September. Information on toxic elements in ash and toxic gases released by the volcano on La Palma, Canary Islands. Advice to the population.
- 28 September. Interview with the TVE News Team. Prevention recommendations in relation to toxic gases.
- 29 September. Information on toxic effects after exposure to boron trichloride.
 Recommendations and protective measures.
- 11 October. Toxicological information on the associated use of cocaine and atropine. Toxic effects.
- 26 October. Interview for Equipo de Investigación at La Sexta TV. Toxic effects of ayahuasca.
- 28 October. 50th anniversary SIT.
- November. 50th anniversary SIT (https://twitter.com/justiciagob/ status/1455110415306969092?s=20 https://t.co/0HCNMdyxGN).
- November. 50th anniversary SIT (https://twitter.com/INTCFjusticia/status/1455086088238903296?s=20; https://t.co/vWoQ5R0a1t).
- 3 November. Information on arsenic and aluminium exposures from the volcano on La Palma, Canary Islands. Initial protocol.
- 8 November. 27th National Firefighters' Congress. The National Institute of Toxicology and Forensic Sciences collaborates with the 27th National Firefighters' Congress,
 ASELF (https://twitter.com/INTCFjusticia/status/1457774008347234310?s=20).
- 10 November. Dissemination campaign with the ECHA (https://twitter.com/ INTCFjusticia/status/1458512150675312641?s=20).
- 12 November. European campaign on the UFI code (https://twitter.com/ INTCFjusticia/status/1459059709877624834?s=20).

- 5 December. Information on tattoo inks (https://twitter.com/INTCFjusticia/status/1467427006765834244?s=20).
- 13 December. Dissemination on social media "Más que risa, riesgo" (https://www.youtube.com/watch?v=jzN21v-x5Q8).
- 21 December. Dissemination on social media "Tu formación es seguridad" (https://www.instagram.com/cecuconsumo/reel/CXghQq2F0pB/?utm_medium=share_sheet).



10. Other INTCF
Units in support
of forensic activity

According to article 13 of INTCF Regulation, it counts for operation with the necessary support staff that is established in the job relationships of work. It is to perform technical and administrative functions of economic management, of personnel, building, informatic and communication systems and other similar ones.

In the following headings, the units are described, whose functions are essential to the good functioning of the expert Services of the INTCF.

10.1. Sample and Waste Management Area

Each INTCF Department counts with a Sample and Waste Management Area. The mission is to manage the samples from their arrival to the laboratory until their distribution to the Services. It is to ensure the safekeeping of post-analysis samples.

This unit staff on the different INTCF sites during 2021 is shown in Table 10.1.1.

	INTCF-MADRID	INTCF-BARCELONA	INTCF-SEVILLE	INTCF-LA LAGUNA
Facultatives	2	2	1	1
Specialist technicians	6	-	1	1
Laboratory assistants	4	8	7	-
Clerical staff	5	-	-	-

Table 10.1.1. Sample and waste management staff during 2021

According to these functions, the work developed in this area consists fundamentally in:

- Reception of analysis requests and samples in the INTCF. Upon the arrival of a shipment of samples or a request for analysis, they will proceed to the register in the LIMS application, collecting all the data, including the chain of custody inside the laboratory.
- Analysis and samples request acceptance. Each collection of samples corresponding
 to the same judicial procedure must comply with the collection, transfer, and custody
 standards that ensure compliance. The INTCF established them in Order
 JUS/1291/2010 of 13 May.

Reasons for rejection of requests for analyses and/or samples by the INTCF must be fully justified.

- Coolers and packages opening. Each cooler or package will be opened individually to follow the security rules that guarantee the sample protection and the operator.
- · Cases and samples identification. Labeling.
- Adequacy and storage previous to the analysis. These actions must not expose in danger the sample integrity. The most common are: individualisation in separate containers, drying of clothes, the addition of formalin, repackaging of sharp objects

or poorly protected weapons, etc. The previous analysis storage will be done in optimal conditions to the sample type and their preservatives.

- Assignation and distribution to the Services. Cases and samples will be assigned and
 distributed to the different Services taking into account the type of analysis requested.
 The judicial priority (cases with prisoners, fast trials, etc.), the analysis priority
 (microbiological studies, volatiles, wounds, biochemical, etc.), the amount of sample
 sent, the preservatives used, whether more than one Service intervenes on the
 same sample, etc.
- Post-analysis custody. Once the analysis is finished, the samples will be maintained, labelled, and classified in a chamber located for that purpose.
- · Return/sample destruction and residues management.

Figure 10.1.1. Different units of the Sample and Waste Management Area at the INTCF Madrid Department



10.2. Library specialised in toxicology and forensic sciences

10.2.1. Mission

The main objective of the INTCF library is to bring together, manage, disseminate, facilitate, boost, and foment the most complete, precise, and helpful information in relation to the areas covered by the institution, whether at present or retrospectively, aimed at staff from the Ministry of Justice with a view to raising awareness about the most recent progress and research in the field of toxicology and forensic sciences and to attend to all the information needs generated by these activities.

10.2.2. Vision

The library aims to be proactive in the management and dissemination of toxicology and forensic sciences information with the use of new information and communication technology, thus contributing to the improvement of the quality of its services.

10.2.3. Two key services

- Selective Dissemination of Information (DSI) provides regular information on the new online content of periodic publications by e-mail to interested users who have signed up to this service:
 - Alerts adapted to the user's profile of journals subscribed and not subscribed by the institution.
 - Bibliographical references of significant titles either by subject matter or authorship.
- The Document Obtainment Service (SOD) is based on obtaining articles, chapters, and external books through the interlibrary loan at hospitals and universities at the request of different users.

10.2.4. Other services

- Face-to-face and personalised training for bibliographic searches aimed at INTCF staff at the Madrid Department who require it.
- Sending updated material on literature searches and workspace from the Pubmed and Ebsco Discovery Service search engines to the INTCF professionals, forensic doctors and other employees from the Institutes of Legal Medicine and Forensic Sciences (IMLCF).
- **Bibliographic searches** in collaboration with the professional to redirect the results to the desired website.

10.2.5. EBSCO Discovery Service

It represents a great technological step forward to be able to contract the services of EBSCO to remain on par with other Spanish institutions. The platform provides direct and immediate access to both contracted journal articles and e-books purchased on a permanent basis.

This platform is accessible to both INTCF and IMLCF staff, transferred and non-transferred. The INTCF Library is the administrator of this platform and manages user subscriptions and possible incidents.

Titles of scientific journals accessible in 2021 as part of the EBSCO Discovery Service:

- · Accreditation and Quality Assurance
- AFTE Journal
- · American Journal of Biological Anthropology
- American Journal of Forensic medicine and Pathology
- · Australian Journal of Forensic Sciences
- · Cardiovascular Pathology
- · Clinical Toxicology
- Drug Testing and Analysis
- Forensic Science International
- Forensic Science Medicine and Pathology
- · International Journal of Legal Medicine
- · Journal of Analytical Toxicology
- · Journal of Forensic & Legal Medicine
- · Journal of Forensic Sciences
- · Medicina Clínica

These three new e-books have been available on the platform since 2021:

- Forensic Entomology: The Utility of Arthropods in Legal Investigations, by Jason H. Byrd and Jeffery K. Tomberlin, CRC Press, 3rd edition, 2020.
- Practical Forensic Microscopy Wiley Online Books, by Barbara P. Wheeler, Wiley, 2nd edition, 2021.
- Statistics and Probability in Forensic Anthropology, by Zuzana Obertova, Alistair Stewart and Cristina Cattaneo, Academic Press, 2020.

10.2.6. Sections

The Library divides its holdings into:

• consultation room: a space for current monographs and another for periodicals; free access for INTCF staff and for other professionals, with prior authorisation.

The monographs and journals available can be consulted via e-mail at intcf.mad-biblioteca@justicia.es;

- · repository: monographs prior to ca. 1990; and
- museum: older collection catalogued by the Collective Catalogue of Historical Heritage (CCPB) free of charge, pursuant to Law 16/1985, of 25 June, on Spanish Historical Heritage, under the Ministry of Culture and Sport. (The institution's collection can be consulted at the following address: http://catalogos.mecd.es/CCPB/ccpbopac/).

The consultation of all the institution's repositories can be made by performing an advanced search in the "copy" data field with the following word: M-R-INTCF.

Upon request and with prior authorisation, you can also consult the institution's files from 1887-1950, which are of incalculable historical value. These offer a historical view of Spanish society and the court proceedings filed by individuals and at the request of the public prosecutor.

10.2.7. Statistics

10.2.7.1. Number of requests

During 2021, the INTCF Library received 2,011 bibliography requests.

10.2.7.2. Origin of petitions

In relation to the activity carried out, note that the library receives requests from all the Departments and Delegations of the INTCF, IMLCF, and hospital libraries throughout Spain.

The difference in the handling of the two types of requests is that INTCF and IMLCF requesters are full users and can therefore use the SOD, whereas hospital libraries can only request from the institution's own collection.

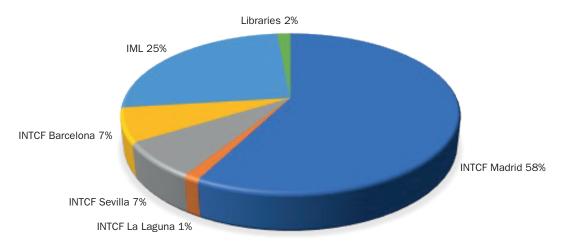


Figure 10.2.7.2.1. Origin of petitions

RESULTS

Low volume of requests from hospital libraries in the autonomous communities on account of the fact that they enjoy acceptable budget allocations at the Ministry of Health, requesting exclusively what is within the toxicological field.

Low volume of requests from the Departments and the INTCF Delegation compared to the results of the Madrid Department and the IMLCFs.

10.2.7.3. Type of requests made

The different types of requests received by the Library, current or retrospective, are obtained both from the institution's own collection and from interlibrary collaboration.

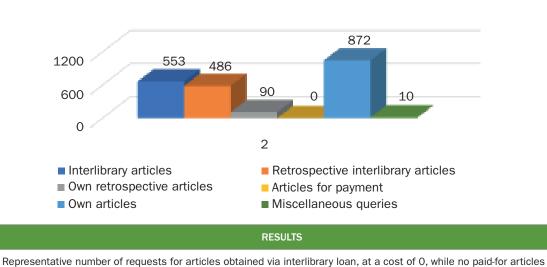


Figure 10.2.7.3.1. Types of requests

Retrospective interlibrary articles are articles obtained through other libraries published prior to 2021.

10.3. Supply Management Unit

had to be resorted to.

The Supply Management Unit at the INTCF generally performs three activities. Firstly, it applies the system established in the public sector contracts law to draw down the budgetary credit for the centralised acquisition of the necessary service for the correct execution of the analytical activity in all the INTCF laboratories. In second place, controls the non-budgetary fund provisions, for current services of a periodic or repetitive nature, made through cash advances from the Justice Territorial Management of Central Organs. In third place, controls and processes the INTCF's accrued income to provide a non-free analytical service performed in the INTCF laboratories.

The activity of this unit is done at the Madrid Department with the support of practitioners in the rest of the sites. This unit's staff at the different INTCF sites during 2021 is reflected in Table 10.3.1.

Table 10.3.1. Supply Management Unit Staff during 2021

	INTCF-MADRID	INTCF-BARCELONA	INTCF-SEVILLA	INTCF-LA LAGUNA	
Facultatives	1*	-	-	-	
Specialist technicians	1	-	-	-	
Laboratory assistants	2	-	-	-	
Clerical staff	1	1	1	1	
Non civil servant personnel	2				
* Until February 2021					

This unit activity is done respecting the classification collected on the 20 January 2014 Resolution, of the General Directorate of Budgets, to be able to:

- Request the service and process the payment order for the invoices of chapter 2, "Current expenses in goods and services", necessary for the exercise of the INTCF activities, and that do not increase public assets. This refers mainly to recurring expenses that cannot be included in the inventory. Such as repairs and preventive maintenance of analytical equipment, calibration of equipment, balances and pipettes, supply of gases, material, and laboratory consumables, services for participation in interlaboratory exercises for quality control, among other unexpected expenses during the budget year.
- To request the investment and process the payment order for the invoices of chapter 6, "Real investments", which comprises the expenses to be incurred in the acquisition of goods of inventory nature, necessary for the operational functioning of the services. They include those new investments that increase the public capital stock and those intended to replace deteriorated assets so that they can continue to be used to fulfill the purpose for which they were intended.
- Process and control the payment vouchers (Model 069) of judicial proceedings, companies, police stations, penitentiary centres, and INTCF agreements with other agencies through the computer application SIC3 of the State Budget Administration. With the ultimate aim of requesting the generation of revenue credit according to the economic classification of the revenue budget, chapter 3, "Fees, public prices and other revenues", for the provision of an analytical service not free of charge.

10.4. Occupational Risk Prevention Service

The Occupational Risk Prevention Service (SPRL) from the INTCF is a technical body whose mission is to proportionate the necessary assessment, support, and coordination to implement a managing system to prevent occupational hazards, complying with the

regulation. The aim is none other than the improvement and safeguard of security and health from more than five hundred public employees of the institution. They are distributed among the departments of Madrid, Barcelona, Seville, and La Laguna.

Ubicated in the Madrid INTCF site since its implementation in 2017, the SPRL has two senior technicians to prevent occupational hazards. One of them is the head of the service. As explained before, the service manages the preventive activity according to the attributions given in the art. 37 of the Prevention Services Regulations approved by Royal Decree 39/1997, of 17 January. Covering the work security, the industrial hygiene, the ergonomy, the applied psychology, except for the vigilance and health control of the workers and those other preventive activities which, due to their volume or the technical requirements necessary for their execution, have to be outsourced to one or more external prevention services because the company's resources are not sufficient.

Among the SPRL's support, the tasks are the design, implementation, and application of an occupational risk prevention plan that enables the integration of prevention in the organisation, the evaluation of risk factors that may affect the employee's security, the preventive activity planning, the determination of the priorities in preventive measures, the employees training, the first aids, the emergency plans, and the health vigilance related to risks derived from work.

The activity developed in the SPRL is not easily programmable. The same will depend to a great extent on the different needs and vicissitudes of the moment. During 2021, the actions carried out can be summarised as follows:

- Updating and review of prevention plans and procedures.
- Follow-up of the risk evaluations in the different INTCF sites.
- Execution level control of the preventive activity planification.
- Evaluation of the work conditions and environment.
- Knowledge of the work incidents, investigating the causes, and making subsequent preventive recommendations.
- Overview of the occupational accident rate.
- Remission of information cards about the employees' risks.
- Elaboration of reports destined for the worker's communication with the SPRL or the personal situations.
- Training for the workers like those destined for emergency action or new workers.
- Employees' health vigilance: periodical medical examination, initial for incorporation or reincorporation to the job, occupational risk assessment during pregnancy, breastfeeding or for health reasons, and the administration of vaccines to workers exposed to biological risks.

- Concerning the workers especially sensible, their job vacancy adaptation, breastfeeding, or health causes.
- Coordination of business activities as planned in Article 24 of LPRL and the Royal Decree 171/2004, of 8 November.
- Information to the workers, thus by SPRL initiative or by the request that they do, the service responsible, the prevention delegates or union representatives when they are the ones who detect a need in this regard.
- Assessment reports destined to the INTCF directors and other Administration authorities.
- The advice of installation facilities, equipment acquisition, or protection material.
- · Participation in the Security and Health Committees.
- · Collaboration with the Labour Inspection and Social Security.
- Elaboration and management of the SPRL documentation.
- Communication with the companies and other institutions, especially with the Coordination of Occupational Risks.
- Collaboration with the Occupational Risk Coordination Unit and communication of all incidents of INTCF staff related to SARS-CoV-19.

In July 2021, the position of the head of the Occupational Risk Prevention Service became vacant when the incumbent requested a transfer; therefore, the prevention work of INTCF staff has been coordinated by the Central Unit for the Coordination of Occupational Risks of the Ministry of Justice until this position is filled.

10.5. Secretarial staff

It is the unit in charge of the management and administrative processing of the expert reports generated by INTCF Services. Since the publication of the Royal Decree 1065/2015, of 27 November, about electronic communications in the Ministry of Justice (LexNet), the platform is used as a secure exchange of information to communicate the INTCF with the different judicial bodies and other legal operators. However, despite that, it is a safe information method using the cryptographic technique. It ensures the writing of presentations, documents, and reception of communications, its emission dates, the provision, and reception or access to the content. It ensures the content of the communications and the identification of the sender and recipient of the same. We are still obliged to use traditional mail, since that some Autonomous Communities have not implemented it in their territory.

Another function that deserves attention is the archiving of files and their management. The finished files that have been completed are kept in the different archives that the INTCF has set up. The rest of the years are in an external file managed by the company contracted by the Ministry of Justice. With the advent of zero paper and the consequent implementation of digital archiving, this problem will be corrected.

Finally, it should be noted that during 2021, a judicial assistant was recruited as a reinforcement, exclusively dedicated to the management of videoconferencing systems.

The staffing of the secretariat team at the different INTCF sites during 2021 is reflected in Table 10.5.1.

Table 10.5.1. Staff of the INTCF Secretariat team during 2021

	INTCF-MADRID	INTCF-BARCELONA	INTCF-SEVILLA	INTCF-LA LAGUNA	
Secretariat Staff Manager	1	1 *	1*	-	
Procedural Manager	1	-	-	-	
Procedural processor	2	11	9	3	
Judicial assistance	3	3	3	1	
* Procedure Manager					

10.6. Informatics System Section

It makes important functions managing the information generated by the analytical services such as the integration of the various INTCF databases and maintenance of the applications developed, the elaboration of reports and consultations of data, the office-based production of the Institute's annual activity report, and to assure of the security of the automatised files from the Institute. Besides that, the INTCF receives support from the Directorate General of Digital Transformation of the Ministry of Justice, specifically from the LIMS group-related consultations, incidences, and developments from the LIMS system of the INTCF.

The staffing of the IT Systems Section at the different INTCF sites during 2021 is shown in Table 10.6.1.

Table 10.6.1. Staff of the Informatics System Section during 2021

	INTCF-MADRID	INTCF-BARCELONA	INTCF-SEVILLA	INTCF-LA LAGUNA
Head of the IT Systems Section	1	1	1	-

Annex I. Methodology used in obtaining the data and glossary of indicators used in the statistical data

The statistical data of the current report has been extracted from the information management system of the laboratory that the INTCF uses (LIMS system: Laboratory Information Management System) and from the databases of the Toxicological Information Service. The global data from the Departments and Services have been extracted with the consultation tool (Dashboard) on a specific date. There may be variations in data in subsequent queries due to occasional request openings.

Hereunder there is a brief explanation of the indicators used for the elaboration of this report:

- Number of cases registered. Lists the cases, court cases for the most part, for which an order has been made to send samples to the INTCF for their analysis by the corresponding Service or Services, between 01/01/2021 and 31/12/2021.
- Number of requests generated. Measures the volume of analyses or study requests which determine the issue of a report between 01/01/2021 and 31/12/2021.
- Number of samples received. Lists the objects, substances, samples or parts registered by each Service between 01/01/2021 and 31/12/2021.
- Number of samples analysed. Lists the objects, substances, samples or parts analysed by each Service between 01/01/2021 and 31/12/2021.
- Number of analyses performed. Counts all the analytic tests performed on the samples of each INTCF Service between 01/01/2021 and 31/12/2021.
- Number of reports emitted. After all the analysis and the register of results, an expert report is emitted to the required institution. This item lists the volume of reports issued by each Department and/or Service between 01/01/2021 and 31/12/2021.
- Number of company notifications. Relates the amount of received information in the Institute and managed by the SIT, through the Documentation Section, about the composition of toxic products commercialised, through toxicological cards to Law 8/2010, 31 March, laying down the system of penalties provided for in the Regulations (EC) concerning the registration, evaluation, authorisation, and restriction of chemical substances and mixtures (REACH), and on the classification, labelling, and packaging of substances and mixtures (CLP), which amends it. The toxicological data sheet preparation is carried out in compliance with the order JUS/909/2017.

 Toxicological consultations by telephone. Lists the number of consultations on poisoning and exposure to toxic substances. It is made by telephone by citizens and professionals between 01/01/2021 and 31/12/2021.

Annex II. Regulations applicable to the National Institute of Toxicology and Forensic Sciences (chronological order)

Order JUS/288/2021, or 25 March, regulating the procedure of notification of substances and chemical mixture to the National Toxicology and Forensic Sciences.

Royal Decree 63/2015, of 6 February, modifying Royal Decree 862/1998, 8 May, approving the Regulation of the Toxicology Institute, the Royal Decree 386/1996, 1 March, approving the Regulation of the Institutes of Forensic Medicine, and Royal Decree 1451/2005, 7 December, approving the Regulations for the entry, provision of jobs and professional pro-motion of civil servants in the service of the Justice Administration.

Order JUS/836/2013, procedure for notification of additions, deletions, and modifications of toxicological data sheets to the INTCF's SIT chemical register.

Order JUS/2267/2010, 30 July, modifying Order JUS/1294/2003, 30 April, determining the files with personal data of the department and public organisms (Official State Gazette No. 208, 27 August 2010).

Order JUS/1291/2010, 13 May, Rules for the preparation and submission of samples for analysis by the Institute of Toxicology (Official State Gazette No. 122, 19 May).

Order JUS/215/2010, 27 January, modifying Order, 24 February 1999, fixing the amount of the public prices for services provided by the Institute of Toxicology (Official State Gazette No. 33, 6 February 2010).

Order JUS/3403/2009, 17 November, approving the list of jobs of the INTCF (Official State Gazette No. 304, 18 December 2009).

Royal Decree 32/2009, 16 January, approving the National Protocol for Forensic Medical and Scientific Police action in events with multiple victims (Official State Gazette No. 32, 6 February 2009).

Royal Decree 1977/2008, 28 November, regulating the composition and functions of the National Commission for the forensic use of DNA.

Royal Decree 1451/2005, 7 December, approving the Rules of Entry, Job Provision, and Professional Promotion of Civil Servants in the Service of the Justice Administration (Official State Gazette No. 309, 27 December 2005).

Royal Decree 862/1998, 8 May, publishes the Organic Regulations of the National Institute of Toxicology and Forensic Sciences (Official State Gazette No. 134, 5 June 1998).

Organic Law 6/1985, date 1 July, on the Judiciary.