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ENFSI 2022 multidisciplinary collaborative exercise: organisation and outcomes

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ABSTRACT

The use of collaborative exercises (CE) and proficiency tests (PT) as part of the governance programme for any forensic science laboratory has become commonplace and recommended by several international organisations. Traditionally these have been discipline-specific exercises testing a laboratory's ability in a single area of forensic science. However, the "real" world is normally more complex and, in many instances, forensic material must be examined for a number of different evidence types.

This article summarises the concepts, planning, design, preparation, implementation, co-ordination and evaluation of the 2022 Multidisciplinary Collaborative Exercise (2022-MdCE) covering a range of forensic disciplines, specifically DNA, fingerprint, documents and handwriting.

The exercise consisted of a questioned letter with typescript text and a signature. In addition, the letter contained a visible bloody fingermark in the area of the signature, a visible staining in the lower left-hand corner, a latent fingermark and an indented impression.

The analysis of the results showed that, in the investigation of the bloody fingermark, the priority was given to the DNA examination. Some critical issues emerged in relation to the biological (DNA)/ink sampling strategies when applied before fingermark visualisation. Another outcome of the exercise has been to demonstrate the importance of indented impressions, which have been underestimated by a significant number of participants. As setters, more in-depth studies are needed to produce consistent samples. This concerns all the disciplined involved but especially DNA and fingermarks.

Based on this exercise, it is believed that this approach to testing of forensic disciplines allows the analysis of good practice within the various scientific areas, as well as scrutinising the process and sequence of events for examining the material within a forensic laboratory in the best conservative way for all kind of evidences.

1. Introduction

In order to become accredited according to ISO/IEC 17025 [1] a laboratory shall have quality control procedures for monitoring the validity of tests and calibration undertaken. One of the actions to promote this is undertaking Proficiency tests (PT) or Collaborative Exercises (CE). So, the use of CE and PT as part of the governance programme for any forensic science laboratory has become commonplace and recommended by several international organisations [2–4]. For example, the European Network of Forensic Science Institutes (ENFSI),

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promoted the use of such tests within its working groups and developed a guideline on how to conduct PT and CE [3]. This guideline helpfully provides the following definitions: a) Proficiency Tests (PT's): tests designed to evaluate the participants' performance against pre-established criteria by means of inter-laboratory comparisons; b) Collaborative Exercises (CE's): inter-laboratory comparisons designed to address specific issues (e.g. test of an analytical method). CE's are not designed to monitor laboratory performance of analysis or interpretation, but CE's may include monitoring of laboratory performance and/or interpretation.

Traditionally, these have been discipline-specific exercises testing a laboratory's ability in a single area of forensic science. Later, some of the working groups developed their own document, like the ENFSI Fingerprint Working Group [5] or Digital Imaging Working Group. These CEs have been used by participating laboratories to benchmark themselves against other comparable organisations, and have allowed the individual disciplines to challenge themselves in particular processes.

However, the "real" world is normally more complex and, in many instances, forensic material must be examined for a number of different evidence types. The first attempt to run a multidisciplinary CE occurred in 2019 as part of the ENFSI-EU funded project "Steps Towards a European Forensic Science Area" (the STEFA Project) which covered the diverse forensic disciplines of document examination, DNA, fingerprints (both visualisation and identification) and handwriting examination. Although successful, it provided valuable insight into how to improve the interdisciplinary challenges associated with running such an exercise. To build on this, it was decided that a component of the ENFSI-EU funded project CERTAIN-FORS "Competency, Education, Research, Testing, Accreditation, and Innovation in Forensic Science" (ISFP-2020-AG-IBA-ENFSI-CERTAIN-FORS) would be to develop one multidisciplinary CE per year (in 2022 and 2023) covering at least three forensic disciplines each time. This approach to testing of forensic disciplines allows the analysis of good practice within the various scientific areas, as well as scrutinising the process and sequence of events for examining the material within a laboratory.

This article summarises the concepts, planning, design, preparation, implementation, co-ordination and evaluation of the 2022 Multidisciplinary Collaborative Exercise (2022-MdCE) covering a range of forensic disciplines, specifically DNA, fingerprint, documents and handwriting. Given the scope of the exercise, the focus will be on outcomes related to the multidisciplinary aspects and not on the single disciplines.

2. Materials and methods

2.1. Conceptualisation

The design and outcomes of the first multidisciplinary CE run within ENFSI were reviewed for possible improvements. On this basis, it was agreed among the exercise setters that a multidisciplinary CE should be set-up in such a way that the applied procedure (i.e., the sequence of forensic disciplines) is likely to affect the outcomes (i.e., the capability to recover the traces). To do this, it is crucial to create "points of contact" on the item between the different forensic trace types, which means a considered sequential recovery plan is essential to maximise evidence recovery. This should be discussed upstream ideally in consultation amongst appropriate practitioners in their field.

In this context:

- The level of difficulty of the exercise can be controlled both on these "points of contact" and on the specific traces.
- The multidisciplinary CE is not intended to allow laboratories to benchmark themselves against other laboratories in terms of the outcome of laboratory results or the strength of conclusion but is primarily concerned in determining the sequence of examinations in a laboratory. It is understood that the material may not be consistent with CEs in individual forensic disciplines and therefore care must be taken when comparing individual forensic discipline results across laboratories.

2.2. Pilot study

The *Raggruppamento Carabinieri Investigazioni Scientifiche – R.I.S. of Parma* (Italy) volunteered to prepare the samples.

The pilot study was conducted by some of the organisations in the project team, specifically RaCIS/RIS Carabinieri (Parma, Italy), RaCIS/RIS Carabinieri (Messina, Italy), the National Forensic Laboratory (Bratislava, Slovakia), the Estonian Forensic Science Institute (Tallinn, Estonia) and the University of Porto (Portugal). The test material suitability was successfully verified. It is important to note that individual laboratories did not complete the entire exercise. Instead, they focused on specific disciplines in order to verify if trace evidence could be correctly recovered and/or analysed.

The pilot study was found to be a worthwhile phase as it provided useful information that helped to establish the test feasibility and inform the item final design.

2.3. Final design

On completion of the pilot study, the final design of the CE was determined. It was agreed that the exercise would consist of a questioned letter with typescript text and a signature. In addition, the letter contained a visible bloody fingermark in the area of the signature, a visible staining in the lower left-hand corner, a latent fingermark and an indented impression. Fig. 1 shows the location of the traces deposited on the item (in red the visible traces; in blue the latent ones), where:

#1 = signature + intersection ink/toner + ink
#2 = bloody fingermark
#3 = non-biological stain
#4 = latent fingermark
#5 = indented impression

The scenario was as it follows: a man, Robert Miconi, was found at home with a gunshot to the temple. Assumed suicide. A letter was found on the desk. The Police are investigating the case. Some doubts arose from the initial information and a potentially involved person has been identified. The item has therefore been submitted to the forensic lab with the following requests:

- Are there fingerprints? If so, do they belong to the victim or to the suspect?
- Are there biological traces? If so, is it possible to obtain a DNA profile suitable for comparison or DNA databank uptake?
- Does the signature belong to the victim?
- Was the signature written before or after the typed text?
- As for the ink used to write the signature, can it be linked to the pen found on the crime scene?

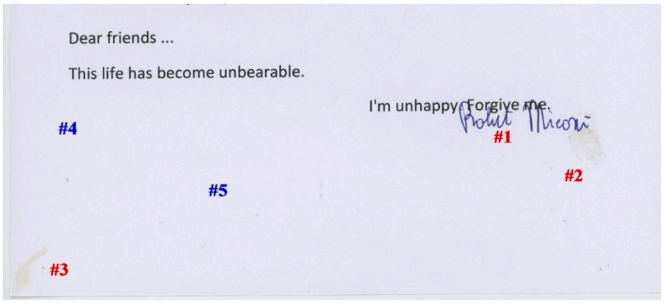


Fig. 1. The item final design.

2.3.1. Deposition of fingermarks

Two fingermarks were deposited on the item:

- #2 – a visible bloody fingermark. It was deposited by a real donor according to the following procedure. The donor's blood (collected in a tube containing an anticoagulant) was diluted 1:1 with water. An aliquot of 10 μL was then pipetted on a non-porous surface. The donor placed their finger on the drop in order to cover most of the area of interest with blood. Then, after two depletions on a different sheet, a third impression was deposited on the item.
- #4 – a latent fingermark. It was deposited by a real donor (different to the donor that provided trace #2). In this case, the donor washed their hands and was asked to wear clean powder-free nitryl gloves for 10 min before donating eccrine-enriched fingermarks. Immediately after this deposition, the donor placed another impression of the same finger on an additional sheet of paper, to act as a control. The donor did not use the same finger on all samples; thumbs, index and middle fingers of both hands were used. The organisers noted which finger was used on each sample.

2.3.2. Deposition of human biological cell material

Human biological cell material could be found in two traces:

- #2 – in the bloody fingermark that was deposited by a real donor according to the procedure explained in section 2.3.1. After the deposition, 1 μL of the original blood (not diluted) was added to an area of the fingermarks void of ridge detail to create "a red spot" (Fig. 2).
- #4 – potentially, the latent fingermark could be analysed as a "touch-DNA" trace.

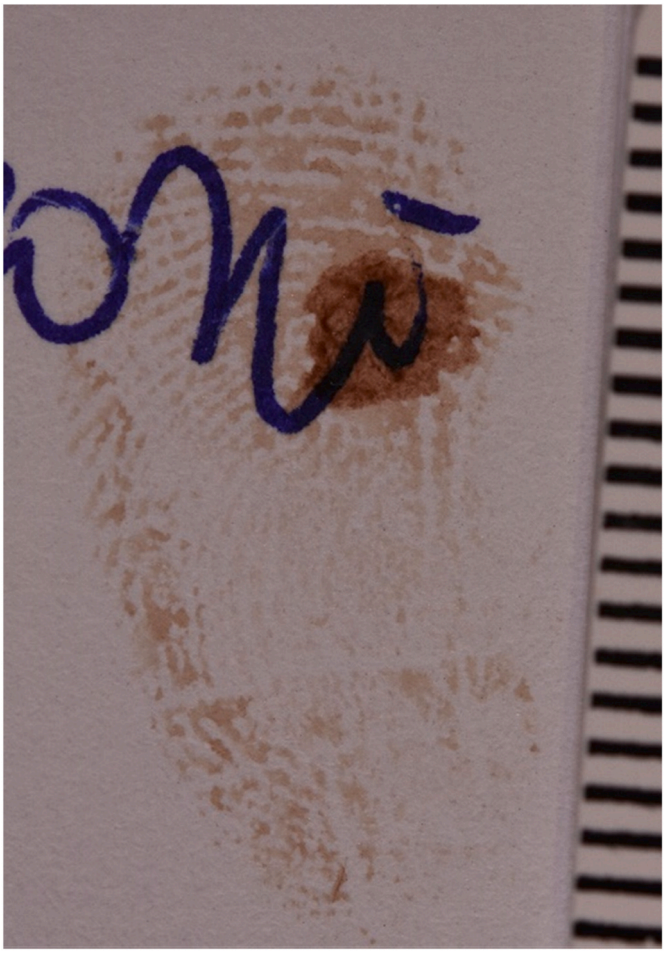


Fig. 2. The bloody fingermark – the "red spot" is evident in the upper part. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

The red stain (#3) in the lower left corner was diluted ink and did not originate from a biological source.

2.3.3. Deposition of the indented impression

Trace #5 was deposited using a Mitsubishi SXN-210 blue ballpoint pen. The questioned letter was placed between four other sheets of paper, two above and two below. The upper sheet of paper was written on using the pen. The writing consisted of a handwritten amount of money (different for each participant) in the lower-middle section of the letter (from left to right depending on the specific sample).

As a control, the sheet of paper directly underneath the questioned letter underwent an ESDA analysis to verify the presence of the indented impression. All of the deposited impressions on the control were successfully visualised. Therefore, after ESDA analysis, this handwritten number is expected to be visualised.

2.3.4. Reference materials

The reference material consisted of:

- Ink analysis: a series of writings coming from the reference pen, specifically a Mitsubishi SX-210 blue. The paper was the same type as that used for the questioned letter.
- Handwriting: signatures/handwriting from the "victim" were scanned at a resolution of 1000 dpi and saved in jpeg format. The files were placed in an online repository reachable via a link provided to the participants. These included six samples of signatures for a total of 24 signatures, two course of business signatures and two samples of handwriting.
- Fingerprints: the reference material consisted of fingerprint/palmprint samples from the suspect and the victim. The reference material was scanned at a resolution of 1000 dpi, saved in jpeg and pdf format, and placed in the same online folder used for the handwriting reference material.

2.4. Material preparation

All the samples were prepared in the laboratories of RaCIS-RIS Carabinieri Parma (Italy) according to the following procedure:

- The signature was written by individual #X (seated and under normal lighting conditions) on standard A4 paper using a Mitsubishi SXN-210 blue pen.
- The message and the fragments of the text on the upper part of the document were printed in one run using a Xerox – WorkCentre 3335 laser printer (black and white). This means that the toner was over the ink of the signature. All of the text was printed in one run to simplify the preparation procedure of the samples. As a consequence, there are no toner particles below the signature, as would be expected in a real scenario, i.e. when a document is forged using another existing document with toner print and signature.
- The indented impression was made as detailed in section 2.3.3.
- The non-biological stain (diluted ink) was deposited on the lower left corner.
- The material was 'sterilised' using ultra-violet radiation (covering the area containing the signature ink with an aluminium foil in order to avoid possible decomposition).
- The latent fingermark was deposited by individual #Y as detailed in section 2.3.1.
- The bloody fingermark was deposited by individual #Z as detailed in section 2.3.1.

- The sheet of paper was cut so that only the part containing the farewell message was kept. Fragments of the additional "original" text (Rome 01/04/2022 – written in calibri, size 24) and a dash dot line printed in light grey could have still been visible on the final item on the top edge of the paper.

Suitable control methods to prevent DNA contamination and unwanted fingermark deposition were implemented throughout the process.

2.5. Predicted results: ground truth and expected

The test was set up knowing that there may be some (slight) variations in the material sent to different participants.

The "ground truth" reflects the process of the CE development, but does not necessarily correspond to the expected results or the consensus results. These outcomes were known to the exercise setters before the material was sent out and related more directly to the process of exercise development.

Trying to determine the expected results is extremely difficult and liable to a sizeable error margin as there are many factors to consider. This process was easier for some of the test areas than for others, and the expectations must be treated with caution.

Considering the expertise of the project team members and the relevant literature in the forensic field, the following points constitute best practice when facing such an item/sample:

- The handwriting experts should observe the item prior to other examinations to see it in its original condition.
- The bloody fingermark (trace #2) must be photographed before any other analysis in order to verify its suitability for a comparison without further chemical treatments [6].
- ESDA must be performed before applying any chemical fingermark visualisation method [7].
- ESDA must be applied in conditions of controlled relative humidity [8].
- The chemical analysis of the ink shall be performed before the application of chemical fingermark visualisation techniques.
- The sampling for chemical analysis shall be "minimally invasive" in order not to destroy any fingermark or biological traces in the area of the signature.
- It is highly advisable that the analysis of the intersection between the toner and ink shall be performed before the application of chemical fingermark visualisation techniques.
- Fingermark visualisation methods targeting the water-insoluble fraction of the mark must be applied at the end of the procedure (when the DNA part is completed) [9].

Table 1 summarises the ground truth and the expected results for each discipline [10–12].

3. Results and discussion

It was agreed by the project team that only those laboratories that carried out the full range of activities themselves (or had direct access and an agreement with a secondary laboratory to undertake aspects of the work) would be considered eligible to take part in the multidisciplinary CE.

The project team received responses from 55 laboratories, of which 50 met this criteria. Table 2 summarises the results obtained by these 50 laboratories.

Fig. 3 gives a general overview of the laboratories' accreditation status according to ISO/IEC 17025 for each forensic discipline involved. Accreditation status for specific methods within each discipline is not provided here.

Table 1
Ground truth and expected results for each discipline.

Discipline	Ground truth	Expected results
DNA	• Human blood was present in detectable levels on the bloody fingermark (trace #2). • The latent fingermark (trace #4) was not in the main scope of the test for DNA, but it could be expected that laboratories would try to obtain a DNA profile from it.	• For the bloody fingermark (trace #2), DNA profiling would result in the single source profile of the donor suitable for comparison and databank uptake. This outcome could be obtained using a targeted approach in the areas of the trace not containing papillary ridges. • For the bloody fingermark (trace #2), DNA profiling after the application of fingermark visualisation techniques may not result in a single source profile. • For the latent fingermark (trace #4) revealed by the application of fingermark visualisation techniques, sampling the whole mark may lead to the identification of a partial to complete DNA profile suitable for comparison or DNA database uptake. • For the non-biological stain (trace #3), any presumptive testing for body fluid should return negative. Obviously, it was not expected to yield any DNA profile. • Blind sampling would not yield any DNA profile or DNA database uptake.
Fingermarks (visualisation)	• The bloody fingermark (trace #2) was left by the right index of the suspect. • The latent fingermark (trace #4) was left by the victim. The exact finger depends on the specific sample received by each participant.	• The bloody fingermark (trace #2) would be photographed before any biological (DNA) sampling. • Improvements to the quality of the bloody fingermark would be attempted by applying specific methods. • The latent fingermark (trace #4) would be developed.
Fingermark (comparison)		• The bloody fingermark (trace #2) and the latent fingermark (trace #4) would be analysed. • The bloody fingermark (trace #2) would be positively associated as coming from the right index finger of the suspect. • The latent fingermark (trace #4) would be positively associated as coming from the victim (thumb, index or middle finger (right or left) depending on the specific item).
Documents	• The pen used for the signature was a Mitsubishi SXN-210 blue pen and this was different from the provided reference sample that was made with a Mitsubishi SX-210 blue pen. • The text of the letter was produced using a Xerox – WorkCentre 3335 laser printer.	• Each laboratory would be able to conclude that the toner (printed text) was over the ink (signature). • Each laboratory would be able to conclude that the signature ink on the questioned letter was different from the provided reference. • Laboratories would be able to recognise the manually cut edge and to detect the

(continued on next page)

Table 1 (*continued*)

Discipline	Ground truth	Expected results
	• As described in section 5, the toner was over the signature ink. • The fragments of the printed text at the upper edge of the letter were produced using the same printer (Xerox – WorkCentre 3335 laser printer) and the upper edge of the letter was manually cut. This was not the main scope for document examination, but it could be expected that laboratories will examine this. • The indented impression of a handwritten number (different for each participant) was present in the lower middle section of the letter (from left to right depending on the specific sample).	fragments of the toner printed text on the upper edge of the letter. This trace was not the main focus of the exercise. • Laboratories would be able to detect an indented impression of the handwritten number "xx.000€" (xx = a unique number for each sample) on the lower section of the letter, as shown in Fig. 1.
Handwriting	• The signature was produced by the victim. • The indented impression was created using a deliberate disguise mechanism, produced by an individual different from the victim. In this way, no comparison was then expected.	• Laboratories would find extremely strong or strong support for the proposition that the victim wrote the questioned signature. • Laboratories would not examine or comment on the authorship of the handwriting visualised by ESDA because the handwriting is too limited to make a useful comparison with the known material.

3.1. Sequence of examinations

In the specific scenario of the exercise, two main areas in which the sequence of analyses could change have been identified: a) the bloody fingermark (trace #2), and b) the signature ink (trace #1).

3.1.1. Bloody fingermark

Irrespective of the nature of the fingermarks, ten laboratories performed the DNA analysis at the end of the sequence of examinations i.e. after chemical treatments of any kind (in this case, for fingerprint recovery and ink analysis). One of those laboratories did perform a blind sampling initially. Overall, the results from these laboratories were good, except for two where a DNA profile was not obtained. One laboratory obtained a partial profile. It is worth mentioning that one of these labs was among the few that obtained the correct DNA profile from the latent fingermark.

Focusing on the bloody fingermark, all the other laboratories performed the DNA analysis before the fingermark (chemical) visualisation methods. However, it was expected that laboratories would image the bloody mark (trace #2) prior to any DNA recovery. Unexpectedly, about 16 laboratories made the decision to sample the bloody mark (trace #2) for DNA prior to imaging the mark.

The visible bloody fingermark should have been documented in order to allow the fingerprint examiners to analyse it (in a few cases, this step was enough to correctly associate the mark). This constitutes good practice, but particular care should be taken in order to avoid potential DNA contamination. DNA and fingerprint experts must be involved during this step and subsequent biological (DNA) sampling.

For this scenario, it was considered more important to associate the fingermark with the suspect rather than confirming that the blood came from the victim. Thus, most of the laboratories performed the biological sampling within a Biological Unit. Some specific exceptions were observed:

- In four cases, the biological sampling was performed by a CSI unit.
- In two cases, a multidisciplinary section was involved.
- In two cases, the activity was carried out within the Fingermark Visualisation Department.
- In one case, the sampling was performed by a subcontractor that was an external accredited laboratory.

Finally, some laboratories (different from the above-mentioned ten laboratories) tried to analyse the bloody fingermark after chemical treatments.

3.1.2. Signature ink

The analysis of the signature ink was another important step of the process. Seventeen laboratories used an invasive technique (e.g., thin layer chromatography (TLC) [13,14], high-performance liquid chromatography (HPLC) [15–17]). From a multidisciplinary perspective, it should be noted that:

- Six of these laboratories carried out the sampling/analysis after the fingermark chemical visualisation methods.
- The remaining 11 laboratories cut out part of the signature from the letter before applying the fingermark visualisation methods. This will be further discussed in section 3.2.1.

3.1.3. Overview of the sequence of the examination process

As part of the reporting process, each participant was asked to describe the sequence of the examination processes. Bearing in mind the preliminary non-destructive activities (of any kind, e.g., presumptive tests, optical examination, picture recording, etc.) and sections 3.1.1 and 3.1.2 above, a summary of the sequences is given in Table 3. This table considers the discipline only when the related activity resulted in a subsequent analysis. For simplicity, biological (DNA) blind sampling is considered a non-destructive activity.

3.2. Multidisciplinary - discussion

3.2.1. Chemical analysis of the ink and fingermark visualisation methods

Seventeen laboratories performed a destructive chemical analysis of the ink exploiting the following methods (see Ref. [18] for a general review):

- Gas chromatography/mass spectrometry - GC/MS (one laboratory)
- Thermodesorption gas chromatography combined with mass spectrometry -GC/MS (one laboratory) [19].
- Thin-layer chromatography - TLC (nine laboratories)
- High-performance liquid chromatography - HPLC (two laboratories)
- High-performance liquid chromatography with photodiode-array detection/fluorescence detection -HPLC-DAD/FLD (two laboratories)
- Ultra-high performance liquid chromatography - UHPLC (one laboratory)
- High performance thin-layer chromatography - HPTLC (four laboratories)

Eleven laboratories cut out a section of the paper from the area containing the signature, before applying the fingermark visualisation methods. This operation allowed the analysis of the ink without the interference of the chemicals used for visualising the fingermarks. This kind of sampling merits specific attention and should be performed with consideration for the requirements of this and subsequent disciplines. Specifically, the area to be cut should be as small as possible in order to reduce the possibility to lose potential traces on the item (e.g.

Table 2
Summarisation of the results (✔ = result consistent with the ground truth (in light green when deemed particularly significant); ✖ = result not consistent with the ground truth (in grey when considered as a deviation from the expected result); **n.a.** = not analysed; **DNA** = biological examination; **FP** = fingerprint examination; **DOC** = documents examination; **HW** = handwriting examination; **(bf)** = bloody fingermark; -m = modifying, that is the sample has been changed and subsequent forensic examinations may be affected; -nm = non-modifying).

		Trace #1 - signature			**Trace #2** – bloody fingermark		**Trace #3** – non-biological stain	**Trace #4** – latent fingermark		**Trace #5** – written amount
		Signature	**Intersection**	**Ink**	**DNA**	**Fingermark**	**Stain**	**DNA**	**Fingermark**	**Indented impression**
	Ground truth →	*Victim*	*Toner over the ink*	*Different*	*Profile A*	*Suspect*	*No DNA*	*Profile B*	*Victim*	*Yes*
Code	**Sequence of disciplines**									
MdCEY2022N01	DOC-DNA(bf)-FP-DNA-HW	✓	✓	✖	✓	✓	n.a.	✖	✓	n.a.
MdCEY2022N02	DNA-DOC/HW-FP-DOC-m	✓	✓	✖	✖	✖	✓	n.a.	✓	n.a.
MdCEY2022N03	DNA(bf)-DOC/HW-FP-DNA-DOC(m)	✓	✓	✓	✓	✓	✓	✖	✓	n.a.
MdCEY2022N04	DNA-FP-DOC/HW	✓	✓	✓	✓	✓	✓	n.a.	✓	✖
MdCEY2022N05	DNA-DOC/HW-FP	✓	✓	n.a.	✓	✓	✓	n.a.	✓	✓
MdCEY2022N08	DOC/HW-FP-DNA	✓	✓	✓	✖	✓	✓	n.a.	✓	✓
MdCEY2022N09	DOC-DNA(bf)-FP-DNA-HW	✓	✖	✓	✖	✓	✓	✖	✓	n.a.
MdCEY2022N10	HW-DNA(bf)-DOC-FP-DNA	✓	✓	✓	✓	✓	✓	✖	✓	✓
MdCEY2022N11	DNA-DOC/HW-FP	✓	✓	✖	✖	✓	✓	n.a.	✖	n.a.
MdCEY2022N13	FP-HW/DOC-DNA	✓	✓	✓	✓	✓	✓	✖	✓	n.a.
MdCEY2022N14	DNA-DOC/HW-FP	✓	✓	✓	✓	✓	✓	n.a.	✓	✓
MdCEY2022N15	HW-DNA(bf)-FP-DNA-DOC	✓	✖	✓	✖	✓	n.a.	n.a.	✓	n.a.
MdCEY2022N16	DNA-FP-DOC-HW	✓	✓	✓	✓	✓	✓	n.a.	✓	n.a.
MdCEY2022N17	DNA-DOC/HW-FP	✓	✓	inconclusive	✖	✓	n.a.	n.a.	✓	n.a.
MdCEY2022N18	DOC/HW-FP-DNA	✓	✓	inconclusive	✓	✓	✓	✓	✓	✓
MdCEY2022N19	DNA-DOC/HW-FP	✓	inconclusive	✓	✓	✓	✓	n.a.	✓	✓
MdCEY2022N20	DNA-DOC/HW-FP	✓	✓	✓	✓	✓	n.a.	n.a.	✓	✓
MdCEY2022N21	DNA-DOC/HW-FP	✓	✓	✓	✓	✓	✓	n.a.	✓	✓
MdCEY2022N22	DNA-DOC/HW-FP	✓	✓	✓	✓	✓	✓	n.a.	✓	✓
MdCEY2022N23	DOC/HW-DNA(bf)-FP-DNA	✓	✓	✓	✓	✓	✓	✖	✓	✓
MdCEY2022N24	DNA-DOC/HW-FP	✓	✓	✓	✓	✓	✓	n.a.	✖	n.a.
MdCEY2022N25	DOC/HW-FP-DNA	✓	✓	✓	✖	✓	n.a.	✖	✓	n.a.
MdCEY2022N26	DOC/HW-FP-DNA	✓	✓	✓	✓	✓	✓	✖	✓	✓
MdCEY2022N27	DNA-DOC/HW-FP	✓	✓	✓	✖	✓	✓	n.a.	✓	n.a.
MdCEY2022N28	DOC/HW-FP-DNA	✓	✓	✓	✓	✓	✓	✓	✓	✓
MdCEY2022N29	DNA-DOC/HW-FP	✓	✓	✓	✓	✓	✓	n.a.	✓	✓
MdCEY2022N30	DNA-DOC/HW-FP	✓	✓	✖	✓	✓	✓	✖	✖	n.a.
MdCEY2022N31	DOC/HW-FP-DNA	✓	✓	✓	✓	✓	✓	n.a.	✓	✓
MdCEY2022N32	DOC/HW-DNA(bf)-FP-DNA-FP(m)	✓	✓	inconclusive	✓	✓	✓	✓	✓	✓
MdCEY2022N33	DNA-DOC/HW-FP	✓	✓	✓	✓	✓	✓	n.a.	✓	n.a.
MdCEY2022N34	DNA-DOC/HW-FP-DNA-DOC(m)	✓	✓	✓	✓	✓	✓	✖	✓	✓
MdCEY2022N35	DNA-DOC/HW-FP	✓	✓	✓	✓	✓	n.a.	n.a.	✓	n.a.
MdCEY2022N36	DNA-DOC/HW-FP	✓	inconclusive	✓	✓	✓	✓	n.a.	✓	✓
MdCEY2022N38	DNA(bf)-DOC/HW-FP-DNA	✓	✓	✓	✓	✓	✓	✖	✓	✓
MdCEY2022N39	DOC/HW-FP-DNA	✓	✓	✓	✓	✓	✓	✖	✓	n.a.
MdCEY2022N40	DNA-DOC/HW-FP	✓	✓	✓	✖	✓	✓	n.a.	✓	n.a.
MdCEY2022N41	DNA-DOC/HW-FP	✓	✓	✓	✓	✓	✓	n.a.	✓	n.a.
MdCEY2022N42	DNA(bf)-FP-DOC(nm)-DNA-DOC/HW	✓	✓	✓	✓	✓	✓	n.a.	✓	✓
MdCEY2022N43	DOC/HW-DNA(bf)-FP-DNA-DOC(m)	✓	✓	✓	✓	✓	n.a.	✖	✓	✓
MdCEY2022N44	DNA-DOC/HW-FP	✓	✓	✓	✓	✓	✖	n.a.	✓	n.a.
MdCEY2022N45	DNA-DOC/HW-DNA-FP-DNA	inconclusive	✖	✓	✖	✓	✓	n.a.	✓	n.a.
MdCEY2022N46	DNA(bf)-DOC/HW-FP-DNA-FP(m)	✓	✓	✓	✓	✓	✓	n.a.	✓	✓
MdCEY2022N47	DOC/HW-FP-DNA	✓	✓	✓	✓	✓	✓	✖	✓	✓
MdCEY2022N48	DNA-FP-DOC/HW	✖	inconclusive	inconclusive	✓	✓	n.a.	n.a.	✖	n.a.
MdCEY2022N49	DNA-DOC/HW-FP	✓	✓	✓	✓	✓	✓	n.a.	✓	n.a.
MdCEY2022N50	DNA-DOC/HW-FP	✓	✓	✖	✓	✖	✓	n.a.	✓	n.a.
MdCEY2022N52	DOC/HW-FP-DNA	✓	✓	✓	✓	✓	n.a.	✖	✓	✓
MdCEY2022N53	DOC/HW-DNA(bf)-FP-DNA	✖	✓	✓	✓	✓	✓	✖	✖	n.a.
MdCEY2022N56	DNA-FP-DOC/HW	✓	✓	✓	✓	✓	✓	n.a.	✓	n.a.
MdCEY2022N57	DNA(bf)-DOC/HW-FP-DNA	✓	inconclusive	✓	✖	✓	✓	n.a.	✓	✖
Consistent results		47 (94%)	43 (86%)	40 (80%)	39 (78%)	48 (96%)	40 (80%)	3 (6%)	45 (90%)	23 (46%)
Inconsistent results		2 (4%)	3 (6%)	5 (10%)	11 (22%)	2 (4%)	1 (2%)	16 (32%)	5 (10%)	2 (4%)
Not analysed		0	0	1 (2%)	0	0	9 (18%)	31 (61%)	0	25 (50%)
Inconclusive results		1 (2%)	4 (8%)	4	0 (8%)	0	0	0	0	0

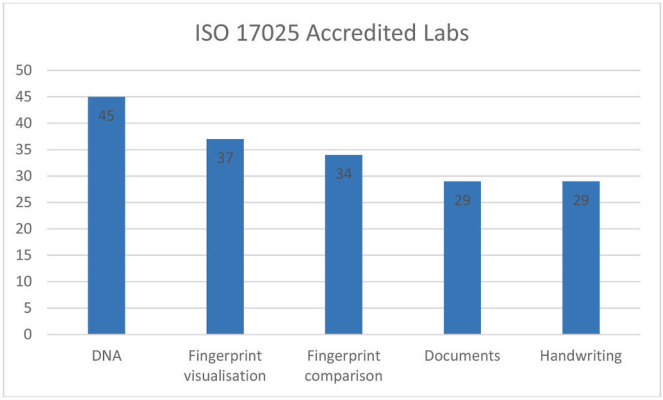


Fig. 3. Laboratories' accreditation status according to ISO/IEC 17025.

Table 3
Examination sequences. (DNA = biological examination; FP = fingerprint examination; DOC = documents examination; HW = handwriting examination; (bf) = bloody fingermark; -m = modifying, that is the sample has been changed and subsequent forensic examinations may be affected; -nm = non-modifying).

Sequence	Examination sequence	No of laboratories
A	**DOC → DNA(bf) → FP → DNA → HW**	7
	Sequences deemed similar:	
	HW→ DNA(bf)→ FP → DNA → DOC	
	DOC/HW → DNA(bf) → FP → DNA → DOC-m	
	DOC/HW→ DNA(bf) → FP→ DNA→ FP-m	
	DOC/HW→ DNA(bf) → FP→ DNA	
B	**DNA → DOC/HW → FP**	28
	Sequences deemed similar:	
	DNA → DOC/HW → FP → DOC-m	
	HW → DNA(bf) → DOC → FP → DNA	
	DNA(bf) → DOC/HW → FP → DNA → DOC-m	
	DNA → DOC/HW → DNA → FP → DNA	
	DNA(bf) → DOC/HW → FP → DNA → FP-m	
C	**DNA → FP → DOC/HW**	5
	Sequences deemed similar:	
	DNA(bf) → FP → DOC(nm) → DNA → DOC/HW	
	DNA → FP → DOC/HW → DNA	
D	**DOC/HW → FP → DNA**	10
	Sequences deemed similar:	
	FP → DOC/HW → DNA	

fingermarks). Fig. 4 shows the sampling approaches from nine laboratories (the images are not available for two labs). It is clear that different approaches were taken with the methods from laboratories #A, #C, #D and #F (named as such in Fig. 3) being the preferable ones.

Six laboratories performed a chemical analysis of the ink after the application of fingermark visualisation methods. All of these laboratories were capable to discriminate the two inks, except for one lab that concluded "*the ink samples from the pen found on the crime scene and the ink used to write the signature, contain a likely identical pigment*" (strong support for the proposition that the signature was written with the pen found at the crime scene).

These outcomes have demonstrated that the applied sequence (fingermark visualisation → ink analysis) still allowed (in this case) for a correct interpretation of the data. However, it is undeniable that in applying such a sequence, a series of analytical and interpretative measures must be implemented to avoid misinterpretations.

3.2.2. Sampling of potential biological traces and fingermark visualisation

One laboratory reported the observation of a luminescent area of interest during the preliminary fluorescence examination exploiting Forensic Light Sources (FLS – details not available). The identified area coincided with the position of the latent fingermark. A small part of this latent stain was cut. Despite the cutting area being small, this activity could potentially affect the visibility of the trace (the nature of which was not established). Indeed, after the application of the fingermark visualisation methods, some ridges were lost (Fig. 5).

It is recognised that, in this specific case, the lost information did not affect the evaluation of the fingermark (judged "not of value"), but this was more luck than judgement and a risky approach to take.

3.2.3. DNA profiles: mixed and extraneous profiles

Two laboratories obtained mixed DNA profiles from blind sampling and from the bloody fingermark (a partial major DNA profile after the application of fingermark visualisation methods), respectively. Another two laboratories obtained a single DNA profile from blind sampling (not directly from the letter but from an additional virgin sheet of paper as a base on ESDA device, which was in contact with the suicide note) and the latent fingermark (a partial profile), respectively. These DNA profiles were checked against the elimination database of the laboratory that prepared the samples and the results were negative. Therefore, these organisations were recommended to verify if an internal contamination occurred.

3.2.4. Extraneous fingermarks

Four laboratories developed and noted additional contact marks, some of which may contain friction ridge detail on the item that were different from those deposited by the organiser. These were unexpected. Examples are given in Fig. 6.

There are three possibilities for how the extra contact marks were generated:

1. They were deposited during the manufacturing process. It is believed that this can happen, despite automated processes.
2. They were deposited during the sample preparation stage. All of the sheets used in the exercise were from a newly opened ream of paper. Strict protocols were followed to ensure extraneous marks were not deposited.
3. They were deposited during examination. This will be dependent upon the laboratories procedures.

At any of these stages, it is most likely that the item was touched with un-gloved hands, but it is possible to deposit ridge details through the glove [20].

One lab visualised two extra areas of ridge (captured as one mark) with ninhydrin that was not previously visualised with indandione. These were identified as one originating from the victim and one from the suspect. The exercise setters were unable to confirm if this was the case as close up images (specifically requested) were not provided.

The extra mark developed by one lab using physical developer (PD) is shown in Fig. 7. Although it could be a contact mark, the quality of the available image is not high enough to appreciate any ridge detail.

Finally, in a further case, contact marks were noted on some entirety images and this could be caused through handling (even with a gloved hand). The extent of this observation is unknown as not all laboratories provided entirety images after each process.

3.2.5. DNA profiling from the bloody fingermark after the use of chemical visualisation methods

In section 3.1.1, the performance of the ten laboratories that performed the DNA analysis at the end of the sequence of examinations is discussed.

For the bloody fingermark, seven laboratories tried to obtain a DNA profile also after the application of fingermark visualisation methods. Three of these laboratories were able to obtain a single DNA profile (two by swabbing, one by cutting) and one laboratory recovered a partial mixed profile judged as suitable only for exclusion (by cutting). The other four laboratories did not obtain a DNA profile.

These outcomes should not be surprising considering that the original quantity of DNA in the bloody fingermark could have been affected

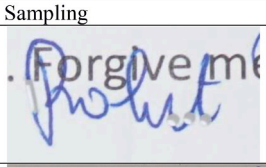
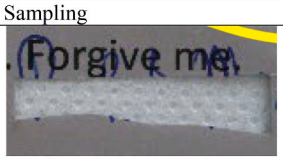
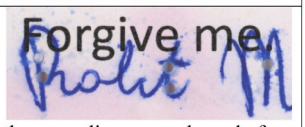
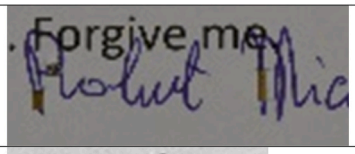
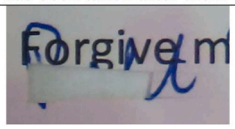
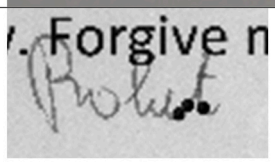
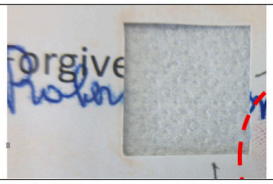
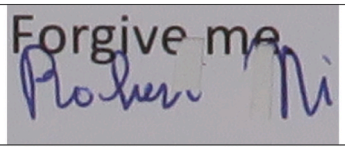
Laboratory	Sampling	Laboratory	Sampling
#A		#E	
#B		#F	 the sampling was done before fingermark visualisation methods but the available picture has been taken after them
#C		#G	
#D		#H	
#I			

Fig. 4. Sampling of the ink before fingermark visualisation methods.

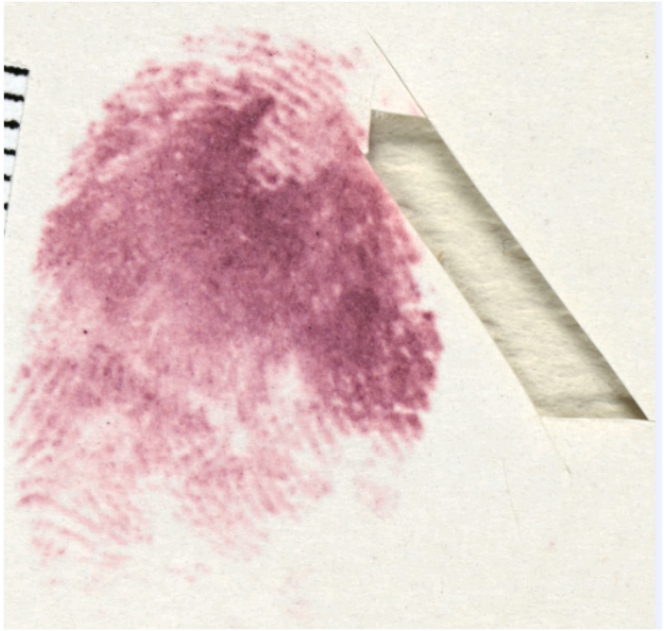


Fig. 5. Sampling of a potential biological trace before fingermark visualisation methods.

by the applied methods, thus resulting in lower chances for DNA profiling [9].

3.2.6. *ESDA: unexpected outcomes*

Two laboratories performed ESDA and were unable to visualise the indented impression. This was unexpected considering that all the control samples resulted in a positive outcome.

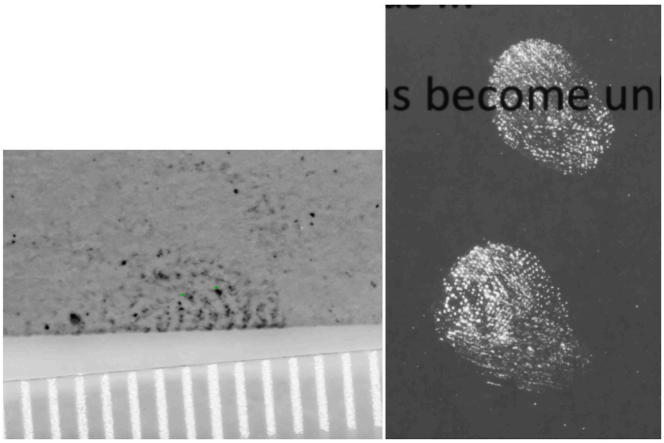


Fig. 6. Illustration of three extra-fingermarks developed on the questioned letter by participants.

The result obtained from one of these labs can be explained by looking at the followed procedure. Indeed, this laboratory applied the ESDA after chemical fingermark visualisation methods. This does not constitute best practice [7]. For the other lab, given the absence of the specific images and of details about the method, it is not possible to further discuss this outcome. As explained, a control was available. Therefore, both laboratories were recommended to verify the followed procedure and the result obtained at that time, in order to try to understand what happened.

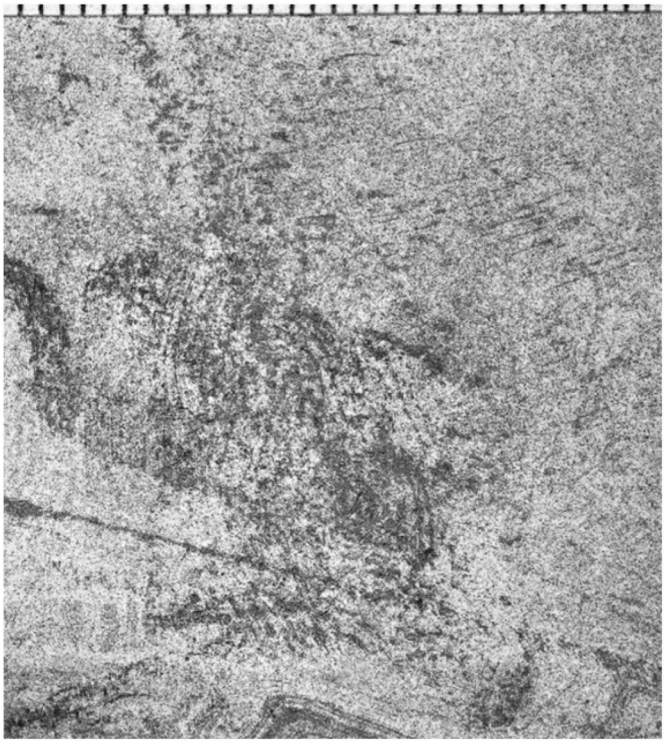


Fig. 7. Extra-mark developed on the document by means of Physical Developer (PD).

3.2.7. ESDA and fingermarks

It has been well established that ESDA is capable of developing fingermarks [21]. This happened also in this exercise. Two laboratories visualised the two deposited fingermarks by means of ESDA (Fig. 8). This outcome was important for the following reasons:

- It could guide the following analyses bearing in mind the location of the developed traces.
- The quantity and quality of the visualised ridge details could be of help during the fingermark comparison step.

4. Conclusions

The 2022 Multidisciplinary Collaborative Exercise gives the possibility for participants to compare the processes and sequences used by the forensic laboratories across Europe.

Although different approaches emerged, overall the results obtained by the vast majority of participants are in line with the expectations of the test organisers. However, some specific areas for improvement within individual organisations have been identified. From a multidisciplinary perspective, the following summary is provided in relation to the traces deposited on the item:

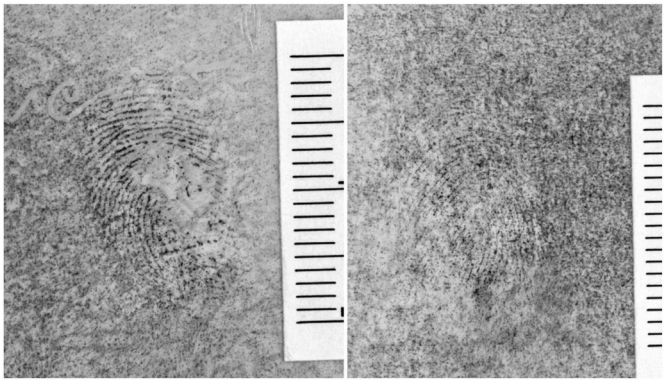


Fig. 8. Bloody fingermark (on the left) and latent fingermark (on the right) developed by means of ESDA by a participant.

- Some critical issues emerged in relation to the biological (DNA)/ink sampling strategies when applied before fingermark visualisation.
- For the bloody fingermark, a general priority was given to the DNA analysis. In most of the cases the biological (DNA) sampling was done in agreement with the fingerprint experts and this explains why it was focused on the red spot. It is worth mentioning that some laboratories reported a joint collaboration/discussion before the examination started and this is exactly in the spirit of a multidisciplinary CE.
- Several laboratories sampled both the blood in the area not containing ridge detail ("red spot") and the entire fingermark (after chemical methods) in order to check if different DNA profiles would be present. It is believed that this constitutes best practice, but particular care must be given to the visualisation/documentation of the fingermark and to contamination prevention. For future exercises, it may be useful to provide a bloody mark where there are no areas for obvious DNA recovery without damage to ridge detail.
- For the latent fingermark, some of the laboratories sampled it as a biological trace for DNA analysis and most of them were not able to obtain a DNA profile, probably due to the eccrine nature of the trace. Considering that almost all identified the fingermark as coming from the victim, it could be debated whether it was actually necessary to perform the DNA profiling as well.
- Indented impressions could provide useful information; in this CE, the importance of such trace has been underestimated by a significant part of the participants.

As setters, more in-depth studies are needed to produce consistent samples. This concerns all the disciplined involved but especially DNA and fingermarks.

Disclaimer

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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