Best Practice Manual for the Forensic Comparison of Soil Traces

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Further information
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BEST PRACTICE MANUAL FOR THE FORENSIC COMPARISON OF SOIL TRACES

1. AIMS

This Best Practice Manual (BPM) aims to provide a framework for procedures, quality principles, training processes and approaches to the forensic examination. This BPM can be used by Member laboratories of ENFSI and other forensic science laboratories to establish and maintain working practices in the field of forensic examination and comparison of soil that will deliver reliable results, maximise the quality of the information obtained and produce robust evidence. The use of consistent methodology and the production of more comparable results will facilitate interchange of data between laboratories.

The diversity in analytical methods for soil analyses available within this field, the huge variability of equipment available in the different laboratories and the development of new promising tools and techniques, makes it undesirable to formulate best practices or make recommendations based upon the whole set of analytical approaches adopted. The term BPM is used to reflect the scientifically accepted practices at the time of creating. The term BPM does not imply that the practices laid out in this manual are the only good practices used in this forensic field. In this series of ENFSI Practice Manuals the term BPM has been maintained for reasons of continuity and recognition.

Some recommendations for the interpretation of the analytical results and the expression of the evidential value of these will be provided. These are recommendations and cover the advantages and disadvantages of their use in forensic casework. It should be emphasised that this manual is produced to encourage the use of common systematic approaches with high forensic quality and value without limiting the use of novel analytical tools and procedures (as the local legal system allows). For that purpose, the manual gives minimum requirements for effective validation of the methods and applications to a case rather than best practices. Recommendations are provided in order to align methodologies between forensic laboratories, allowing the exchange of knowledge, expertise and databases.

2. SCOPE

This BPM is aimed at experts in the field and assumes prior knowledge in the discipline. It is not a standard operating procedure and addresses the requirements of the judicial systems in general terms only.

This document serves as a framework for the minimum requirements and recommendations for the comparison of questioned soil samples with reference samples within a forensic context. The forensic casework related to soil usually starts with either an outdoor crime scene or an exhibit with soil on it. The sampling of the crime scene ideally should start with pre-sampling planning (e.g. checking soil and geological maps, land cover, aerial images, etc.) to allow the most suitable sampling of the crime scene to take place [2].

The next step after pre-planning in the process is the examination and description of any soil traces to formulate a subsequent analytical plan. The analytical plan is dependent on the forensic question posed, the technologies available, the state of preservation of the questioned sample, the condition and amount of the questioned sample, and the likely potential evidential value of the analytical results.

This framework document includes the securing of reference samples at the crime scene and the recovery of questioned samples from items of evidence (exhibits), their examination and subsequent analysis using various methods, the interpretation of the associated data, formulation of a forensic report and presentation of findings in court.

This document does not consider soil provenancing, the investigation of soil for environmental crimes or the analysis of dust.

3. DEFINITIONS AND TERMS

For the purposes of this BPM, the relevant terms and definitions given in ENFSI documents, the ILAC G19 "Modules in Forensic science Process", as in standards like ISO 9000, ISO 17020 and 17025 apply.

Table 1: Most common definitions and terms used in this document

Agglomerate	See "Soil aggregate".
Aliquot	A representative portion of the whole soil sample. Under certain circumstances it can also be the whole sample (e.g. very small sample).
Analyst	An individual carrying out general casework examinations or analytical tests as a specialist in their field or on behalf of the expert witness.
APST WG	ENFSI Animal, Plant and Soil Traces Working Group.
Assumption	An assumption is something that is accepted as true without proof.
CE	Collaborative Exercise. Interlaboratory comparisons that are designed to address specific issues such as troubleshooting, method validation or characterisation of reference materials. CEs are not designed to monitor laboratory performance of analysis or interpretation, although CEs may include monitoring of laboratory performance and/or interpretation [1].
Certified standard	A certified standard is one that is guaranteed by the provider of the sample to be as it is specified on the sample certificate. Details are given on certificate. The provider of the certificate should be a certification body.
Client	The entity requesting the forensic soil comparison, such as the prosecutor, lawyer, police, judge, private individual or company.
Collection sample	A sample whose origin is undisputed and from reference collections, archives etc.
Control sample	Due to various definitions for the term 'control' this term is not used within this document.
Databases	Digitally available collection of data but also reference data such as maps, citable literature and related information, sample collection, geographic information system (GIS) databases, identification keys, etc.
Destructive analytical technique	An analytical technique that consumes the sample during analysis. After analysis no (sub) sample remains.
Dust	Airborne material deposited on a surface, can contain soil particles. Includes household dust.
Examiner	Employee trained in the application of current discipline specific methods with a wide variety of experience, training and background knowledge, according to their assigned roles (e.g. expert witness or analyst).

Exhibit	A physical item for examination which contains a trace, also referred to as a questioned (unknown) item or a production (e.g. spades, tools, footwear, clothing).
Expert Witness	An individual responsible in a particular case for directing, carrying out the examination of the items submitted, interpreting the findings, writing the report and providing evidence of fact and opinion for the court. The actual responsibilities can differ according to national legislature. The expert must stay within their area of expertise.
Hypothesis	The proposition established from the prosecution and/or defence positions based on the case circumstances.
Macroremains	Macroremains are large fragments or particles visible to the naked eye (or low magnification) that can be picked out by hand and generally can be analysed using different techniques than used for soil. Macroremains can be diverse in origin, for example botanical, anthropogenic or animal. (From: Archaeobotany).
Non-destructive analytical technique	An analytical technique that does not consume the sample during analysis and does not change the sample during sample preparation. After analysis the (sub)sample remains.
PT	Proficiency Tests. Tests designed to evaluate the participants' performance against pre-established criteria by means of interlaboratory comparisons. [1].
Questioned sample	Sample collected which is of an unknown or questioned origin and with unknown composition. Also, can be called evidential sample.
Reference sample / material	Sample collected with a known and undisputed origin such as a path at a crime scene or to represent an area of potential contact. Also referred to as 'known' sample, 'comparator' sample, or 'alibi' sample in a forensic context.
Room temperature	Room temperature is a comfortable ambient temperature, generally taken as about 20°C.
Scenario	A sequence of events devised either by the prosecution, defence or police.
Semi-destructive analytical technique	An analytical technique that does not consume the sample during analysis, but sample preparation either makes the sample difficult to analyse with a different technique or destroys a certain part of the sample. After analysis the prepared (sub) sample remains in this altered state.
Soil	A complex mixture of particles / fragments with living or dead organic and inorganic components, both natural and anthropogenic. The main inorganic components are naturally formed from local rock and by sedimentation processes. For forensic applications 'soil' includes all soil layers down to the natural bedrock (if any) and is not limited to the top layer.
Soil aggregate	Aggregates or agglomerates are distinct clumps of soil particles, including peds, casts or fragments. They are a combination of different soil components and are not homogenous.
Soil ped	Peds are aggregates of soil particles formed as a result of natural pedological processes.
Standard	A sample with known composition. Can be certified or uncertified.
Uncertified standard	The composition of the uncertified standard is not guaranteed by the certification body.
Volatile organic compounds	Organic compounds which are volatile (gaseous) at a low temperature.

4. RESOURCES

4.1 Personnel

Personnel are likely to be the most important resource in any forensic application. In order to allow staff to work effectively and efficiently, everybody involved in the process must understand the nature of the tasks and competences required to perform them.

It is accepted that individual organisations will recruit forensic soil examiners in accordance with the requirements of that organisation including legal considerations and academic qualifications or field specific experience. Forensic soil examiners will be educated or trained in accordance with national rules and regulations in the application of current soil investigation methods, and will have a variety of experience, training and background knowledge, according to their assigned roles: *analysts* will carry out general casework examinations or analytical tests as a specialist in their field or on behalf of the expert witness and *expert witnesses* are responsible for directing, carrying out the examination of the items submitted, interpreting the findings, writing the report and providing evidence of fact and opinion for the court. The actual responsibilities can differ according to national legislature and depending on the individual institution the forensic examination and analysis team can consist of one or more persons.

Requirements of competence should be set for all personnel involved in the field of soil examination demonstrating their competence before being allowed to undertake any casework. The attainment of competence should be recorded. Personnel involved in soil investigation should demonstrate their competence following their quality system requirements.

It is recommended that laboratories give consideration to routine proficiency testing of all staff to ensure they retain the capability in accordance with their assigned roles. The period between routine proficiency testing is at the discretion of each laboratory, but it should be acknowledged that each proficiency test will typically only cover a small sampled proportion of the actual work undertaken by each staff member (see chapter 7).

Forensic soil examiners would be recommended to take part in appropriate workshops, seminars, conferences and research and development projects.

4.2 Equipment

The equipment should fulfil the specific requirements of the field. In general, equipment and other resources to carry out physical, chemical, biological or mineralogical soil analyses are required (see examples chapter 5.3). The specific software used should be chosen according to the applied method.

The equipment should be tested for validated methods and proven to operate properly for the method before using it in casework.

An equipment inventory should be maintained for all forensic tools (and any associated test equipment and software products) held in the forensic laboratory, recording all data (e.g. model / version, serial number, maintenance interval, location) required by the local quality system.

4.3 Reference materials (standards)

Commercial reference materials for forensic soil comparison are generally not available. For specific analyses single standards from accredited authorities are available. Additional reference materials can be obtained from specific pedological, geological and agricultural collections as well as from recognised scientific institutes working with soils.

Information about the sample soil characteristics can also be obtained from the specific geological and soil maps provided by the regional geological/soil authorities. Collections prepared by other forensic laboratories and samples collected by the forensic laboratory can be used as a standard only after validation. Technical reference materials (e.g. internal standards) to calibrate equipment, are usually provided by the equipment manufacturers.

4.4 Accommodation and environmental conditions

Laboratories used for the examination of items with soil traces should be designed to be an efficient and effective working area. Particular consideration should be given to the need for avoidance of contamination caused by the environment if appropriate (e.g. pollen and spore contamination through the air). Incompatible activities within a laboratory (e.g. questioned and reference material, different pieces of evidence) should be separated in order to prevent contamination and carry-over. Procedures to avoid contamination and monitoring contamination levels should be implemented.

4.5 Materials and Reagents

All materials and reagents used for soil examination should be of a suitable quality and fit for purpose.

5. METHODS

This chapter does not provide any Standard Operating Procedures (SOPs) but provides guidance on systematic approaches to be followed in specific examinations. It provides an outline of the minimum requirements and recommendations for the selection of appropriate analytical techniques and the preferred order of their application in forensic casework.

5.1 General aspects

The collection and securing of reference samples and questioned samples should be carried out in a methodical and systematic manner to ensure no contamination and no loss of sample material. Another reason for careful sampling is to get as much soil as possible to run as many analytical methods as is possible (or as is necessary), without obstructing analyses in other forensic fields (such as human DNA, fibres or non-human biological traces). It is also important to try not to completely exhaust all the sample material to allow for possible re-examination.

Before examination of any samples, it should first be established that there is soil present on the item (e.g. not household-dust, not snow, etc.) and enough sample should be available to carry out the subsequent analysis. After acquiring the soil samples (see chapter 8) forensic soil comparison has four stages (see figure 1):

- **(i)** Preliminary investigation and description
- **(ii)** Analysing and/or identifying components or composition
- **(iii)** Comparing results from components or compositions
- **(iv)** Interpretation and evaluation of the evidential value of the comparison

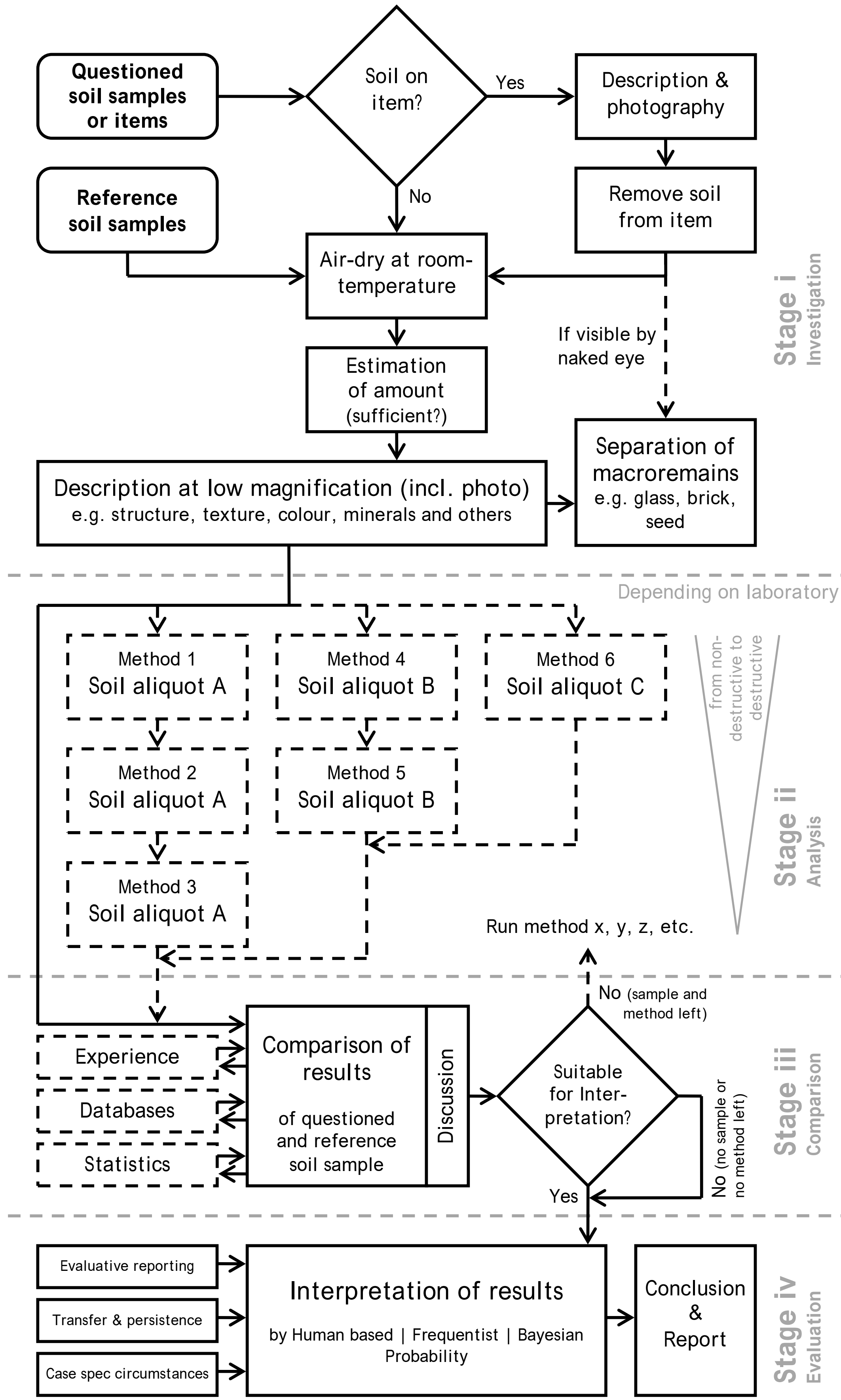

Figure 1: Flow chart describing the examination sequence

In the first stage (i) the soil sample is examined and described, commenting upon possible mixtures and/or layering and on the spatial location of the adherent soil. Remarks regarding the chain of custody and the amount of sample present should also be recorded (see chapter 9). Other types of forensically relevant material (e.g. botanical remains, glass fragments, animal or human hairs and fibres) can be present and should be recovered into separate vials for further investigation by experts in other fields. The examiner should preferably be aware of the appropriate sampling and handling procedures for other associated trace materials (e.g. hair, paint fragments, fibres, etc.). This part of the investigation should be as non-destructive as possible.

The second stage (ii) should begin with formulating an analysis strategy which should lead to appropriate answers to the forensic questions identified. The plan must consider the potential for destruction of evidential material during analysis and/or the potential need for further analysis. Where limited evidential material is available, the efficiency of the chosen techniques in the case context should be considered. If the respective technique has not been applied in forensic investigations previously a validation process is required (see chapter 6). Before selecting the appropriate techniques, all prior available information must be carefully considered.

Due to the complex nature of soil there are many different techniques possible to identify or describe the many different soil components. These techniques can either identify/describe single particles or components that are part of the soil sample (e.g. heavy minerals, clay fraction, pollen grains or microbial community) or the composition of the whole soil sample (e.g. elemental composition or organic profile analysis). Results can be quantitative (number/value) or qualitative (presence/absence). Several techniques can be used on the same samples to increase robustness of the comparison. Positive and negative controls can be used in some techniques to identify possible loss of particles/components or contamination (e.g. *Lycopodium* sp. spores in pollen analysis).

The third stage (iii) involves comparing the results obtained from the questioned sample to the results obtained from the case reference samples. The methods for comparison can be objective (using a statistical method) or subjective (using expert opinion) or a mixture of these two. Comparison methods should take into account the variation within a sample, variation within the location the reference samples were collected from and the general variation (geographical resolution) of the analytical method used. Acquiring the relevant reference samples and relevant databases are of key importance. Examiners should be aware of the potential for misleading results if non-representative databases are used. The databases used for comparison should be relevant for the geographical area under investigation and include any alternative proposition of source, such as an alibi location if possible (see chapter 12.4).

The fourth stage (iv) deals with the interpretation of the comparison of the questioned sample with the case reference samples (see chapter 12). Questions to be addressed are transfer and persistence, the presence of possible mixtures and alternative source locations (scenarios) and the evidential value of the findings.

5.2 Demands on the analytical technique

All analytical techniques must be validated (see chapter 6) before use and requirements/limits should be known. Before analysis it must be checked that the samples under investigation are within these limits (e.g. the amount of material, only clay minerals or within a certain area).

The first step in many analytical techniques is the sample preparation. The plan of analyses may include possible subsequent analyses of the same subsample and should take into account possible effects of sample preparation on any subsequent analyses.

Analytical techniques can be non-destructive, semi-destructive or destructive. Non-destructive techniques are preferable over semi-destructive techniques, which in turn are preferable over destructive techniques. Techniques which use less material can generally be applied more often to small questioned soil samples in casework. However, since soil is a complex mixture of different particles and components, there is a limit to a minimum sample size which is still representative for the whole sample and the source location. This limit is different for the different analytical techniques and should be taken into account when selecting the analytical techniques to apply.

Relevant and representative databases, published data, knowledge and/or experience are necessary to be able to interpret the results of an analytical technique. If these are not available, the technique should only be used with extreme caution and limitations declared. When comparing the questioned sample to the reference samples the amount of material used for the analytical technique should be similar for all samples, to prevent stochastic effects that would make interpretation more difficult. Thus, the sample size for analysis is determined by the smallest sample available (usually the questioned sample but not always).

5.3 Examples of commonly used analytical techniques

Apart from comparing any recovered macroremains the following analytical techniques can be considered for comparison of soil samples (Table 1). To increase the robustness of the soil comparison, several techniques can be used. Preferably techniques analysing different component groups (general characteristics, mineral/elemental components, biological components, living organisms) should be combined as the components are relatively independent from each other. Anthropogenic materials can be present in the sample and should be considered separately.

Table 2: Examples of analytical techniques which can be considered for comparison of soil samples

General characteristics	**Inorganic components**	**Dead organic components**	**Living organic components**
Stereomicroscopy Colour (e.g. spectro-photometer, Munsell) pH (H2O) Organic matter content (e.g. loss on ignition) Particle size distribution etc.	XRD ED-XRF or µXRF Spectrometry (e.g. ICP-MS, ICP-OES) Microscopic analyses of (heavy) minerals SEM-EDX etc.	Microscopic analyses of plant remains (e.g. pollen, phytoliths, diatoms) Gas Chromatography (e.g. Plant wax markers) etc.	Non-human DNA analyses Microscopic analyses of diatoms, mites, etc. etc.

5.4 Peer Review

Peer reviewing of data should be organised by the laboratory quality system. All peer review must be documented in the case record.

6. VALIDATION AND ESTIMATION OF UNCERTAINTY OF MEASUREMENT

6.1 Validation

The minimum requirements for considering method validation can be found in the ENFSI document "Guidelines for the single laboratory Validation of Instrumental and Human Based Methods in Forensic Science" [3]. Each method has to be validated individually according to the lab specific SOPs of that method. A few examples can be found in the ENFSI document [4].

Validation in general should focus on the process of comparison of soil traces and does not have to extend to evaluating the significance of soil trace comparison results. Some important factors to be considered include: minimum and maximum sample size, sampling and subsampling method, precision (repeatability, reproducibility), bias of part of the method (matrix or substrate effects, accuracy and specificity), working range (limit of detection/sensitivity, linearity), robustness (environmental susceptibility) and competency of the examiner.

6.2 Estimation of uncertainty of measurement

Soil is a complex mixture of particles and fragments of different sizes and composition which differs both horizontally and vertically and over time. It can therefore vary within centimetres to meters in distance. The whole scene of a crime cannot be taken to the laboratory for analysis, but reference samples can be collected from the site to best represent that site. The way that the sampling is carried out and the amount of soil collected is therefore crucial (see chapter 9). Since soil is not homogenous there is a minimum sample size for each method or soil component. Below that minimum size the reference sample is not representative for the site. This type of uncertainty should also be taken into account during validation.

Possible selective transfer or persistence of questioned soil traces found on items (e.g. shoe sole, spade, etc.) should be considered as the different components of soil do not behave in the same way on the surface of items (e.g. clay minerals stick better than gravel on a spade). Generally, the composition of the questioned soil sample can be changed during transfer or persistence from the original source contact location. For example, grain size distribution can change during the period of time of transport (e.g. on a shoe sole) and a mixture of soil material from locations other than the scene of the crime at a later stage may occur. These effects must be taken into account when selecting and validating the method for comparison, so this source of uncertainty should be minimised and known for the applied method. Individual tests have to be carried out to best match conditions at the time of soil transfer.

One way to estimate and evaluate the uncertainties of measurement is to work with multiple reference samples per site and multiple (sub-) samples of the questioned sample in order to evaluate the intra- versus the inter-variability of a site, as well as the variability of the questioned samples. It is recommended to always indicate the variability of the analysed samples in the case notes.

7. QUALITY ASSURANCE

7.1 Proficiency Testing/Collaborative Exercises

Proficiency tests (PTs) and collaborative exercises (CEs) should be used to test and assure the quality of processes and methods for forensic comparison of soil, noting that such tests are usually within the scope of laboratory investigations. A list of currently available PT/CE schemes as put together by the QCC is available at the ENFSI Secretariat and via the ENFSI website. "Guidance on the conduct of proficiency tests and collaborative exercises within ENFSI" [1] provides information for the ENFSI Expert Working Groups (EWGs) on how to organise effective proficiency tests (PTs) and collaborative exercises (CEs) for their members.

There are no accredited European proficiency tests currently available for forensic soil investigation covering the whole process addressed within this BPM. However, there are commercially accredited tests available at this time and the APST WG is producing PTs and CEs on a regular basis. Soil examiners are advised that they should take part in relevant PTs and CEs that cover all accredited procedures and methods that are used for the comparison of soil traces in casework. This can be an internal or an external test. If there are no PTs and CEs available in the field of expertise of the forensic soil examiner, depending on the available resources, they can create a blind trial themselves. PTs and CEs could form a part of the maintenance of an examiner's competence. Within existing methods, these tests are used to identify potential sources of error, produce corrective measures and optimise the potential for improvement of any quality management system. The quality measures are designed to demonstrate the accuracy and precision of results and the limits of detection of the analytical method tested.

Proficiency testing serves not only to evaluate the chosen method but also to assess the performance of the laboratory and their ability to reach appropriate conclusions following the application of the analytical method in question.

Participating laboratories are asked to retain an adequate quantity of the test sample for repeat analysis/ testing should there be any disagreement over the characteristics of the sample or concerns regarding contamination prior to the sample being received by the analyst/investigator.

Accredited laboratories according to ISO 17025 are required to perform PTs and CEs periodically on a regular basis following their own regulatory requirements. They must document their policy which states the frequency of PTs and CEs they will participate in.

Participants in the tests should follow the laboratory standard procedures for the casework. They should not give the test any special treatment that would not be given in the same circumstances to routine casework.

The performance in PTs and CEs must be reviewed by the organisation in a timely manner and any anomalous results investigated and addressed with appropriate actions according to the quality system in the organisation. Any results not in accordance with the expected outcome should be recorded in the local quality system so that corrective measures are introduced as soon as possible.

7.2 Quality Controls

Quality controls (e.g. standards, positive or negative controls) to the methods applied should be listed in each particular operating protocol of the respective procedure. Examples include the addition of soil standard which can be run with every batch of samples to ensure no drift in analytical methods over time. Peer reviewing (see chapter 5.4) should be organised.

7.3 Data Collection for control, monitoring and trend analysis

Data collection should be according to the regulatory requirements and quality assurance system of each laboratory.

8. HANDLING ITEMS

8.1 General

Within this document 'handling items' relates to the physical collection and seizure, protection, transportation and storage of exhibits for subsequent analysis either at the scene or in the laboratory. The handling of items in the laboratory will be briefly mentioned in the later part of this section (see also chapters 5 and 9). Health and Safety considerations are reported in chapter 14. The conditions of relevance in your particular country and for the particular scene or samples should be checked to be aware of the potential risks. Any work carried out will be to meet the requirements of the clients and with actions selected based on an assessment of information available at the time which may be subject to change. The collection, packaging and handling of samples for soil requires the same standards as the collection of other evidence types. The integrity of the item (security of seal and bag or box) and the traceability (chain of custody) are of utmost importance and are required in all case investigations.

8.2 At the scene

The decision for a Scene of Crime Examiner or a forensic soil examiner to attend any given crime scene will be made in reference to the police force and organisations own attendance criteria and related policies. For more information please refer to ENFSI Scenes of Crime BPM [5].

The examiner should be aware of and understand the laboratory procedures concerning preparation and assessment at the scene. The examiner should remain in compliance with their local legislature relating to search, sampling, seizure and recovery of items of evidence.

8.2.1 Examination of the Scene

Crime scene examination is a systematic process which aims to record the scene as it is first encountered, identify and collect all physical evidence that might be relevant to the case under investigation. Careful and chronological documentation of the handling, examination and analysis of evidentiary materials is very important. Every step taken in the process must be documented to ensure traceability and continuity of evidence from the crime scene to the courtroom (chain of custody).

It is likely that there will be a requirement to work with other forensic specialists, e.g. experts in DNA, fingerprints etc. and the sequence of forensic work should follow an agreed predefined sequence that is dependent on the priority given by the person in charge of the scene. This will depend upon the relative importance of the pertinent evidence types and the destructive nature of the examinations.

8.2.2 Avoidance of contamination

It is recommended that the suspect and potential witnesses are kept away from both the scene and any potential exhibits. When handling evidence at any stage of an investigation, potential contamination must be avoided. To avoid contamination sampling materials should be cleaned between sampling and it is recommended to change gloves regularly to prevent contamination/cross contamination between samples. If possible, the use of disposable consumables is recommended.

8.2.3 Sampling the scene and wider reference sample locations

The scene should be secured by the appropriate personnel prior to sampling. Once an appropriate sampling strategy has been agreed upon with the person in charge of the scene and the secure access path has been defined, sampling can start.

When collecting samples, examiners should wear non-powdered gloves, use sterile non-porous sampling tools (e.g. flat trowel to collect surface contact soil samples) and place samples in an appropriate container to keep the soil aggregates intact if possible.

An adequate number of reference samples should be collected to represent the locations of potential contact (in general more samples will be required if the location is heterogeneous (such as a planted garden border) rather than at a homogeneous location (such as a sown managed grassland) see chapter 9). Also, the activity which might have taken place at the crime scene should be taken into account (e.g. different samples should be taken when sampling a grave site with different soil layers than when sampling surface contact point locations for footwear or tyre marks) and areas which look like they have recently been disturbed should be prioritised for sampling.

If the soil trace is on a surface that cannot be moved (such as on a floor or wall) it is recommended that the section be cut out (if possible) to remove the section and place it into an evidence bag. In such cases, reference samples should also be collected from unstained areas of the background material (such as carpet, laminate, wallpaper, etc). Whenever possible the soil should be recovered as single source contact sample, such as aggregates, soil peds and individual layers of material which have a comparable appearance such as in colour, texture, etc. The collection of material of similar appearance will therefore maximise the chance that they will have originated from a single contact source.

8.2.4 Preservation and packaging

Soil samples that are wet at the time of collection should be air dried at room temperature prior to storage if possible. Each laboratory will have their own temperature limits set to fulfil the requirements of any subsequent test that is carried out prior to drying of samples. If a soil is dry it should be collected into a marked unused container and sealed within a labelled evidence bag. If the sample has a large amount of vegetation within the sample, it should be stored in a paper (or breathable) evidence bag. However, if the latter is used, care has to be given to avoid potential cross contamination with volatile organic compounds.

Contemporaneous records shall be made at the time of seizure, or as near is practically possible, of items recovered from the scene, or person, or exhibit and describing the location from where the item was recovered. These records should be entered into the case file in a way that is compliant with your local legal system.

8.2.5 Labelling and documentation

It is advisable to obtain photographs of each sample location (taking a wide angle shot and a close up shot with scale and a sample location identifier) including the vegetation and its general surroundings. Consideration should also be given to producing a sketch or map of the scene, marking the sampling locations and recording all relevant information in context.

The recording should be carried out in a methodological and logical manner to ensure all areas are captured thoroughly and should be continued throughout the examination as necessary. The legal status and use of labels can vary, although sealed items should always be labelled with a unique identifier at the time and date of seizure (with this information recorded on the container/bag). The minimum details that should be recorded and be directly and unequivocally attributed to each package are:

- A unique identifying reference or case identification
- The date and time that the material was seized
- A brief description of the material (e.g. surface soil) and specific location from where the material has been seized (e.g. beneath a large birch tree, at the side of a body)
- The name of the person/identifier code and organisation name responsible for collecting and packaging the material

Documentation with the relevant information (including a list of samples collected) should accompany any samples submitted to the laboratory [6].

8.2.6 Transport

Recovered material should be handled as little as possible and packaged at the earliest opportunity. It should be transported to the laboratory as soon as possible. Care should be taken with the transportation of samples. In particular the item should be carefully packaged to avoid loss, fragmentation or movement of any adhering debris/soil from the recovered items.

8.3 <u>In the laboratory</u>

Prior to receiving samples in the laboratory any potential risk of contamination should be assessed to establish whether there was any chance of transfer of soil between individuals, scenes, or items. The risk of cross-contamination must always be considered and minimised at all stages of the examination. All the individual items must be properly handled during recovery and packaged appropriately. If there are any doubts, the client should be notified before any further work is started. A note should be made if any container is cracked, broken or if material is lost.

8.3.1 Generalities and anti-contamination precautions

A high level of cleanliness in the laboratory is strongly recommended. Laboratory benches should be regularly wiped down with a cleaning agent prior to use and also after sample handling or the analytical procedure is finished. If using any product to clean surfaces, evaluation of any potential risk of this product affecting any further analysis should be considered. In order to minimise cross-contamination and not to avoid the loss of any traces, exhibits could be placed on a smooth surface on the benches for handling. Special laboratory design should be considered to avoid cross-contamination when handling and analysing samples for non-human DNA analysis.

A unique identification code label for each sample and each sub-sample/ sub-exhibit should be recorded at the laboratory at every step of the analytical process. Handling and analysing samples in a workflow system using facilities with separate areas is recommended.

8.3.2 Receipt and handling

Identifying information about samples should accompany the samples received. Upon receipt, reference samples, questioned samples and exhibits should be kept separate, retaining the integrity of the containers' seals until further analyses, in order to minimise the risk of cross-contamination. If the samples are not dried before arriving at the laboratory, then samples should be air dried as soon as possible. Samples should be kept in temperature and humidity conditions which would prevent the growth of microorganisms. If DNA analyses are going to be performed and a longer storage is required, sub-samples could be frozen. Whenever possible, an untreated sub-sample (aliquot) should be kept for a second test or further analyses.

8.3.3 Sampling of Items

Consideration should be given as to the order of examination/analysis of all relevant forensic traces. In general, human biological traces should be recovered before the soil traces are, all under controlled laboratory conditions. Whenever possible the soil should be recovered as single source contact samples, such as aggregates, soil peds and individual layers of material which have a comparable appearance such as in colour, texture. Separate areas, facilities and procedures should be designated for the treatment of distinct components within soil such as non-human DNA analysis, fibres and hair, etc. (contact the individual specialist and see the relevant BPM).

8.3.4 Recovery

The remainder of the samples should be retained, packed up and sealed when all the analyses are completed.

8.3.5 Storage and return of samples

The remainder of the sample and any sub-samples should be stored following the criteria of the chain of custody and the local legal system.

9. INITIAL ASSESSMENT

The significance and evidential value of soil evidence relies upon the correct detection, recovery and careful handling of the evidence and the appropriate choice of the sequence of examinations. Ultimately the success of soil examination, analysis and interpretation of the evidential value of results is largely dependent on the initial case assessment and forensic examination strategy which should be constructed by the examiner.

Any potential action (e.g. sieving, grinding, chemical treatment), change in environmental conditions (e.g. heating, freezing or cold storage) or natural processes (e.g. decomposition, degradation) which may affect the quality and quantity of the components in the soil must be taken into consideration when selecting the methods to be used for the analysis, the sequence of analyses and subsequent interpretation of results.

The client's questions must be clearly defined to the examiner at the start of the examination and recorded. If there is a lack of clarity, the questions have to be further discussed, clarified and noted. Information about the circumstances of the case is usually necessary to assess any potential limitations of the proposed examination and their potential effect on the perceived outcome. Factors which should be considered include: whether the laboratory has the capacity to carry out the work, the resources and expertise to carry out the required tasks, potential effects and requirements of other forensic investigations on the same pieces of evidence, the order or priority of types of evidence and any time constraints.

In relation to soil, transfer and persistence is an important consideration as is the issue of mixing and any potential fractionation of the soil. Information such as what is suspected or known to have occurred, before, during and after the key event, the persons involved, the weather conditions at the time, the items involved and the sequence of events and time frames involved, including those associated with the recovery and storage of items submitted for examination should be provided.

9.1 Assessment at the scene

When working at a scene to collect reference soil samples for comparison with questioned samples the examiner should be aware of the potential local variation in geology, vegetation and land use and try to collect representative samples incorporating as much of this variation as is possible. It is recommended that multiple samples are collected at each location. Depending on the type of activity (i.e. digging or walking) and the diversity of the soil/vegetation at the crime scene, soil samples should be taken individually from multiple points at the surface and/or at different soil depths/soil layers/horizons.

Correct sampling of reference soils is crucial and has to be carried out by well trained personnel (or the examiner himself) as the likely point of contact can vary with activity, distance (a few meters) and depth (a few centimetres). The examiner should be aware of this multi-dimensional consideration.

It is recommended to sample the places at a crime scene where transfer of soil to a vehicle, object or person is most likely to have taken place, such as muddy patches of soil, tracks and where there is any evidence of grave filling and probable routes (paths, roads) of the perpetrator in and out of the scene. This should be guided by any available intelligence such as witness accounts, phone or surveillance camera records, etc. If the crime scene can only be sampled for soil at some time after the crime was committed, the examiner should be aware that the vegetation and the soil composition could have been influenced by the time elapsed, the previous investigation (for example holes filled with sand or cut branches), subsequent activity and influence of any intervening weather. It is suggested that information is obtained about the location and environmental conditions to help in the evaluation of the results. If not all the necessary samples can be taken during the initial crime scene investigation, the most transient contact locations (such as footwear and tyre marks) should be sampled first while any marks are still evident.

9.2 Assessment in the laboratory

During the initial visual inspection in the laboratory it is important to carefully examine the questioned soil sample for any signs of a possible mixture (i.e. a sample containing soil from more than one origin) such as different soil aggregates, colours, soil layers or layers of plants. If there are signs of a possible mixture, consideration should be given to subsampling to recover discrete aggregates (i.e. a single source part of the sample). For example, this can be done by taking multiple subsamples or by recovering a new separate sample from the questioned item. The amount of questioned sample should be checked against the minimum sample size for the used methods at this stage (see chapter 6). If the reference samples show signs of being a possible mixture it should be checked how the sampling was carried out. Any signs of poor sampling, inadequate packaging, handling issues, or poor storage or transfer during transport should be noted and taken into consideration before any subsequent analyses are done. If a major issue such as breach of the packaging is observed, the client should immediately be informed and the value of any subsequent analyses should be considered.

10. PRIORITISATION AND SEQUENCE OF EXAMINATIONS

The prioritisation and sequence of examinations is determined by the two-overall deductive reasonings in forensic science: (I) *work from the general to the specific* and (II) *from non-destructive to destructive*. If the exhibit itself is undergoing multidisciplinary investigation, all relevant experts should determine the most appropriate sequence of examinations as based on each individual case.

An overarching sequence of examinations for a questioned sample is provided in Chapter 5 (Figure 1). Deviations from the general sequence and prioritisation of examinations are possible, depending on the individual case question and sample material present.

The number and types of analytical methods which are applicable to the investigation depends on the resources available (personnel, equipment etc., see chapter 4), the amount and type of sample material recovered from the item, the quality of the sample, the quantity of sample needed for each analysis and whether the sample can be reused for other methods after the analysis. In general, destructive methods leading to a complete loss of the subsample should be avoided. If the case factors are limiting the investigation the examiner should consider:

- What is the chance of obtaining informative results from the different examinations?
- What would be the evidential value of the results?
- Which method is most likely to answer the questions from the client?

The sequence of examinations should be re-evaluated at all stages of examinations regarding the results of any previous analyses. Investigations can be stopped or modified at any stage of a pre-defined sequence of examination if sufficient answer to the question(s) of the client is provided by the results obtained, or if no additional evidential value or relevant piece of information can be expected to be available, or if the client does not require any further investigations.

11. RECONSTRUCTION

In some situations, it might be necessary to carry out experimentation or tests to evaluate any findings and assess the probabilities that events could have happened in a certain way. Examples include transfer onto fabric, splashes from puddles onto vehicles or clothing and selective persistence across a range of surfaces. They can be designed as simulations, reconstruction exercises or field experiments. All aspects of these experiments must be thoroughly documented, particularly in relation to any possible alterations or destruction of original pieces of evidence. Consideration must also be given to the possible limitations of the experimentation such as different weather conditions, fabric age, condition, fabric type etc. It might be necessary to use statistical tools to evaluate the number of replicates necessary to obtain a meaningful answer. Where replicate items are used (e.g. footwear) they should closely reflect the make, model and condition of the original questioned item.

The setup of experimentation should be designed to be as similar as possible to the original conditions of the case. The relevance and importance of each parameter must be evaluated, and it might require to have volunteers of the same size or bodyweight as the people suspected to have been involved in the crime, to re-enact the scenario, taking environmental conditions into account (e.g. rain, drought, mist, seasons, or even particular times of the day). For example, temporal requirements are important where pollen, diatom, DNA or botanical analysis are involved. In addition, if the situation relates to a cold case investigation, consideration should be given to the situations and conditions existing at the date and time the crime was committed.

If the traces are found on pieces of evidence (shoes, textile material, vehicles, etc.) it is recommended that the original piece of evidence is not used to make the tests, but instead that equivalent objects are bought or collected for the purposes of the test. When the piece of evidence cannot be reasonably sourced (vehicles, doors, etc.) a solution could be to make a specially designed device or mock-up.

If it is impossible to avoid working with the original piece of evidence, it might be necessary to evaluate a strategy with the investigators and/or other forensic laboratories, in order to ensure that the piece of evidence will be used in the best way to achieve the maximum evidential value for the case, and that the impact of possible destruction of the object is kept as low as possible. For example, it might be useful to perform conservative hair, fibre, GSR or DNA sampling prior to the reconstruction experiment involving the soil trace. Splitting of a part of the piece of evidence might be considered. For example, GSR analysis could be carried out on the right side of the front of a shirt, and the soil analysis could be carried out on the left side on the front of the same shirt. It is recommended that, if possible, separate analysis of questioned items are not split by front/back as the position of the trace stain on the item could be important for ascertaining likely place/manner of contact. Where any original evidence has to be altered by experimentation (e.g. a cut-out section of a pair of trousers) this must be discussed with and authorised by the relevant authorities prior to starting work and clearly documented and disclosed in the report.

When it is decided that the experimentation has to replicate the actual crime scene, to avoid modifying the crime scene, it is preferable to find an equivalent scene, or to evaluate if it could be performed very close to the initial crime scene instead of being carried out at the actual site of the crime.

Considering that the results from the reconstruction experimentation will be presented in a report and/or to the court, it is important to consider how the results would be interpreted and communicated early on during the design of the experimentation.

12. EVALUATION AND INTERPRETATION

12.1 Introduction

Due to the complexity of soil and the potential range of methods that can be applied to soil investigation, the forensic soil examiner is required to interpret: i) the individual analytical result and ii) the combination of all findings to answer the questions received from the client. A single analytical result taken in isolation is generally of limited value to answer the questions of the client. Comparison, evaluation and interpretation add value to the output of raw analytical results (see chapter 5). Evaluation and interpretation of the findings should be directed at answering the initial question(s) of the client and should be based on a clearly defined hypothesis - or a set of hypotheses if several interpretations are proposed.

12.2 Evidential value

The evidential value of the results of the examination and analysis of the questioned samples depends on various factors and the examiner should document and be able to explain why and how the evidential value was obtained. Those factors are e.g. size/volume of the trace, rarity of particles, mixtures, sensitivity to contamination, selective transfer and persistence and a complex combination of those factors.

While the recovery/collection of questioned samples is often the role of the examiner, the choice and sampling of reference samples is often performed by external personnel (crime scene investigators, etc.). In that situation, it might be useful to request access to the crime scene or review the crime scene documentation in order to increase the contextual value of the reference samples.

The evidential value of a sample depends on a range of factors including the type of analysis chosen by a particular laboratory and the analysis plan (see chapter 5). Laboratories should therefore make sure, through validation procedures, that their analysis flow is able to, with a known degree of uncertainty, separate samples that are different and associate samples that are similar. This is particularly important in certain legal systems, as the examiner might be asked to attend court to describe these assertions.

12.3 Discriminating power

The evaluation of the findings and subsequent interpretation(s) must take into account the limits and discriminating power of the analytical methods. The aim of this careful evaluation is to avoid reporting more than the methods are validated for.

Each method has its own limits and different discriminating power (e.g. low, moderate, high). The combination of several techniques - often the case in soil analysis - can provide a consistent picture. The methods to be combined should be relatively independent from each other (e.g. organic and inorganic analyses) to maximise the discriminatory power. It is the role of the examiner to then combine the results to reach a final conclusion.

12.4 Background information

The evaluation of the results obtained generally requires access to background information. It is recommended that the examiner gathers the necessary background information during the initial assessment phase (chapter 9). Background information is necessary to evaluate the results for the different scenarios provided or to explain results obtained from the analyses outside of the expected variation.

It is recommended that the examiner documents the background information provided and the reasoning made based on these. Such considerations will help in re-formulating/reviewing the client's questions. Examiners should be aware of potential cognitive bias and take steps to minimise this.

12.5 Reference data and databases

Forensic soil analysis encompasses a wide variety of sciences and techniques depending on the elements the examiner decides to focus on (mineralogy, botany, palynology, diatomology, microbiology, analytical chemistry, genetics, etc.). Databases exist for most of the characteristics and can provide added value to the evaluation and interpretation of the results (e.g. by giving information on diagnostic criteria of taxonomic groups or on the distribution or abundance of components and properties of the characteristics measured). Databases in this document are not only defined as a digitally available collection of data but also reference data such as maps, citable literature and related information, sample collection, geographic information system (GIS) databases, identification keys, etc.

It is recommended that relevant data is used for each case - for example, it is important to include data from the same geographical area as the case under investigation. Digital database collections are continually being developed and updated so it is recommended that the examiner stays up to date with current developments and contributes to the available digital databases. The date of access and version numbers of databases used should be noted in the case files.

12.6 Interpretation of results and conclusions

Any assumptions made during interpretation should be clearly defined. It is recommended that if you are asked to evaluate a scenario that it is described carefully and completely. The wording used should be precise, unequivocal, documented and as informative as possible.

When appropriate and relevant databases are available, a Bayesian approach should be considered in order to formulate the results in terms of probabilities, with regards to specific scenarios or hypotheses [6].

If the available background information changes or the assumptions made are no longer valid, although the examination results reported remain the same, the conclusion and opinion of the examiner could change. This should be immediately communicated to the client.

13. PRESENTATION OF EVIDENCE

The overriding duty of those providing expert testimony is to the court and to the administration of justice. As such, evidence should be provided with honesty, integrity, objectivity and impartiality. Evidence can be presented to the court either orally or in writing. Presentation of evidence should clearly state the results of any evaluation and interpretation of the examination. Written reports should include all the relevant information in a clear, concise, structured and unambiguous manner as required by the relevant legal process. Written reports must be peer reviewed. Expert-witnesses should resist responding to questions that take them outside their field of expertise unless specifically directed by the court, and even then, a declaration as to the limitations of their expertise should be made.

The examiners' findings and associated opinion are usually required in a written format either as a report or as a statement. These can be used by the investigator, the police, the prosecutor, the defence or the court. Communication of any findings should be in a language that is clear and unambiguous and uses easily understood language. If scientific terms are used then they should be explained in the report or included in a glossary of terms.

As forensic soil comparison is only occasionally presented in court as evidence it is advised to add information in the report on the strengths and weaknesses of soil evidence. In addition, an important difference between soil and man-made materials (such as glass or fibres) is that soil is a complex mixture of different particles and complete 'matches' between reference and trace samples are not found. For this reason, it is advised to avoid the use of the term 'match' in forensic soil comparison. Any wording used in forming an opinion should be carefully considered. It is recommended that the report clearly communicates the evidential value of the results to the client. Verbal scales can be used for this and should be clearly defined in the report.

14. HEALTH AND SAFETY

14.1 Risks

Soil can pose several potential risks for humans, as soil can carry multiple types of hazards able to cause injuries, diseases or death to humans, although this is very unusual. Soils can potentially contain biohazards, chemicals or radioactive particles. These can pose a risk for examiners through skin injuries, ingestion or inhalation. These risks should also be considered when sampling soils from potentially contaminated areas.

Biohazards include exposure to pathogens naturally found in soils or introduced in soils through contamination by faeces, fluids or decomposing human or animal remains. The different geographic origin of soils should be considered in terms of preventing potential biohazards, as certain diseases are endemic in some countries. As a consequence of inadequate packaging and storage, biohazards can also arise: a humid soil contained in a plastic bag can allow the growth of multiple microorganisms.

14.2 Protection

When there are indications of potential hazards, protective gloves, lab coats, filter masks (e.g. FFP3 protective mask) and goggles should be worn. A protection against inhalation begins with wearing face protection but can also be enhanced through avoiding or controlling the production of dust and aerosols as much as possible (e.g. by working in a fume hood). In terms of severity, the most serious risk may come from aerial exposure to highly toxic chemicals, highly infective agents or radioactive particles contained in the soil. If possible, check with the provider of the soil samples if there is a known possible risk. If there is a risk of suspected agents being present, specialists in these specific matters should be consulted, and if necessary, adapted decontamination protocols should be applied. Particular attention is required when analysing soils from locations involved in either accidental or terrorist biothreat events. Examiners should consider obtaining appropriate vaccinations to help protect themselves.

The risk of chronic exposure to soil should be taken seriously, as accumulation of low-risk agents over time can cause problems in the long term, through allergies, chronic contaminations, intoxication or even poisoning.

From sampling to storage, all procedures should avoid any kind of contamination and cross-contamination of pathogens between samples or cases. Adequate sampling and packaging are crucial to avoid issues of contamination and it is recommended to note possible hazards of the sample on the outside of the package. The storage area should be set up to avoid ingress of moisture and should be kept at a low temperature to best conserve samples and avoid the potential growth of microorganisms.

15. REFERENCES

[1] ENFSI QCC-PT-001, 2014, Guidance on the conduct of proficiency tests and collaborative exercises within ENFSI, version 001, 27/06/2014

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[3] ENFSI QCC-VAL-002, 2014, Guidelines for the single laboratory Validation of Instrumental and Human Based Methods in Forensic Science, version 001, 10/11/2014

[4] ENFSI QCC-VAL-002, 2014, Guidelines for the single laboratory Validation of Instrumental and Human Based Methods in Forensic Science - Examples, version 001, 10/11/2014

[5] ENFSI SoC BPM, 2012, Scenes of Crime Best Practices Manual, version 001, December 2012

[6] ENFSI guideline, 2015, ENFSI guideline for evaluative reporting in forensic science, Strengthening the Evaluation of Forensic Results across Europe (STEOFRAE), version 003, 08/03/2015

16. AMENDMENTS AGAINST PREVIOUS VERSION

Not applicable (first version).

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