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Best Practice Manual

for Human Forensic Biology and DNA Profiling

ENFSI-DNA-BPM-03

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ENFSI´s position on Best Practice Manuals

ENFSI wishes to promote the improvement of mutual trust by encouraging forensic harmonization through the development and use of Best Practice Manuals. Furthermore, ENFSI encourages sharing Best Practice Manuals with the whole Forensic Science Community which also includes non ENFSI Members.

Visit www.enfsi.eu/documents/bylaws for more information. It includes the ENFSI policy document Policy on Creation of Best Practice Manuals within ENFSI (code: QCC-BPM-001).

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BEST PRACTICE MANUAL FOR HUMAN FORENSIC BIOLOGY AND DNA PROFILING

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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 human forensic biology and DNA profiling. This BPM will help to deliver reliable results, maximize 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 and promote standardization.

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 the forensic field. In this series of ENFSI Best Practice Manuals the term BPM has been maintained for reasons of continuity and recognition.

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 nor addresses the requirements of the judicial systems. It is also assumed/expected that:

a) The policies, procedures and methodologies followed by the forensic service providers fulfil the requirements of relevant national legislation as applicable. This includes but is not limited to accreditation, regulatory and/or legal requirements for the processing and international exchange of genetic and other related data i.e., the nature of the item (reference or stain) and laboratory ID number through the national DNA databases[1–5].
b) The forensic service provider follows policies and procedures related to impartiality and confidentiality meaning that all of its activities are performed within a sound ethical framework addressed in the laboratory's code of conduct preferentially harmonized with the ENFSI Code of Conduct [6].

Guidance on generally accepted procedures and workflows for human forensic biology and DNA profiling is provided, starting from the collection of items from the scene of incident to the presentation of the findings in court or other authority or customer. The fundamental requirements for the generation of valid and robust results and conclusions are addressed herein and include: resources, validation, methodology, quality assurance, handling of items, initial assessment, prioritisation and sequence of examinations, interpretation, evaluation, presentation of findings and finally, health and safety aspects. The forensic unit may use this BPM to formulate its procedures, methods and relevant documentation as well as the structure of its records for reference, peer review and audit purposes.

Where relevant, reference is made to current expert guidance documents including those of the ENFSI DNA Working Group, the Scientific Working Group on DNA Analysis Methods (SWGDAM), the Forensic Science Regulator (FSR) and the DNA Commission of the International Society for Forensic Genetics (ISFG). Relevant standards, research and review articles and books are also cited for further guidance.

3. TERMS AND DEFINITIONS

For the purposes of this BPM, the relevant terms and definitions given in ENFSI documents, the ILAC G19 Modules in a Forensic Science Process, as in standards like ISO 9000, ISO 17020 and 17025 apply [7–10].

Note: General definitions related to quality are given in ISO 9000, whereas ISO 17000 gives definitions specifically related to certification and laboratory accreditation. Terms and definitions specific to forensic

sciences from ISO 21043-1:2018 Forensic Sciences Part 1: *Terms and definitions* have also been incorporated in this BPM [11].

Allelic drop-in
Additional random alleles present in a profile originating from fragmented sources and regarded as independent events.

Allelic/locus drop-out
Alleles missing from a DNA profile, so that it is partially represented.

There are circumstances in which a profile is not "complete" (occurrence of locus drop-out, i.e., investigated loci without any detected alleles present). Reasons for locus drop-outs could be for instance low template DNA, DNA degradation, PCR inhibition, and/or primer site mutations.

Allelic frequencies and relative frequencies
A frequency is the number of times the allele of interest appears in the surveyed population. The relative frequency of this allele is its frequency divided by the total number of alleles observed (i.e., twice the number of individuals surveyed).

Note 1 to entry: Assume that we have surveyed 100 unrelated persons. In that study, we counted the allele "14", 23 times, allele "18" 29 times and genotype "14, 18", 3 times.
The frequency of allele "14", in this sample of 100 persons, is therefore 23.
The relative frequency of allele "14", in this sample of 100 persons (i.e., 200 hundred alleles), is 23/200 or 11.5%.
The frequency of allele "18", in this sample of 100 persons, is therefore 29.
The relative frequency of allele "18", in this sample of 100 persons (i.e., 200 hundred alleles), is 29/200 or 14.5%.
The frequency of genotype "14, 18" for that locus, in this sample of 100 persons, is therefore 3.
The relative frequency of genotype "14, 18", in this sample of 100 persons (i.e., 200 hundred alleles), is 3/100 or 3%.
Relative frequency and match probability (sometimes known as random match probability or conditional genotype probability) are not synonyms. Indeed, we do not actually count the number of persons in the sample in order to estimate the rarity of the genotype (e.g., we do not count the number of people in the sample that have genotype 14, 18), but use a genetic model. As we do not actually count genotypes, one should not speak of a frequency or a relative frequency of a genotype, we should speak of its probability.

Allelic proportions
An allelic proportion characterises the rarity of an allele in a population of interest: it is estimated using statistical methods based on data (i.e., allelic frequencies) pertaining to a sample taken from the population of interest.

Note 1 to entry: When we survey a population, we do not study the entire population, but only a sample that we assume is representative of the whole population. We know the allelic frequency in our sample and use this result to infer something about the allelic proportion in our population in general. At a given time and at a given place, if we surveyed the entire population, we could know what the true proportion is. There is a true value for this proportion, but we cannot in practice survey the entire population. As a consequence, we estimate the allelic proportion using statistical methods that take into account sampling variation: this value will only be an estimate. Assume for example that we have surveyed 100 unrelated persons. In that study, we counted the allele "14.2", 0 times. The frequency of this allele is 0, and its relative frequency is 0%. This is the relative frequency in the surveyed population (i.e., a sample of the population for which we would like to estimate the proportion of the allele "14.2"). There are several methods to estimate the proportion of an allele. A common method is to use the following equation: $(x_i+1/k)/(2N+1)$, where x_i is the number of observations of allele i in a database, N is the number of individuals surveyed and k is the number of allele designations with non-zero observations in the surveyed sample [if there are 6 alleles observed in the surveyed population for that loci, then k=6]. Thus, in our example, the proportion of the allele "14.2" in the population of interest can be estimated as (0+1/6)/(2*100+1), thus 0.08% and not zero contrary to what was observed in the surveyed population (i.e., our sample) [12].

Analysis
Part of the examination process consisting in measuring, observing and comparing items to obtain results. The analysis process can be human-based, instrumental or combined [11].

Background DNA
DNA that is present from unknown sources and unknown activities. It can be described as 'foreign' (non-self). We don't know how or why it is there. For example:
1) DNA underneath fingernails from unknown sources/activities.
2) Non-self DNA on clothing from unknown sources/activities.
3) Non-self DNA on a surface from unknown sources/activities.

Background does not include DNA from known individuals – this is known as prevalent DNA. (See definition for prevalent DNA). The distinction is important since they are treated differently when modelled [13].

Bias (Cognitive bias)
A pattern of deviation in judgement whereby inferences about other people and situations may be drawn in an illogical fashion. These include, expectation, confirmation, contextual and motivational biases, anchoring effects or focalism (related to expectation and confirmation biases), role effects (e.g. adversarial roles) and reconstructive effects (rely on memory rather than contemporaneous notes).

DNA profile/Electropherogram/Genetic profile
A set of values (alleles) of a group of genetic markers identified in an individual's DNA by DNA profiling.

Evaluation/Evaluative opinion
FSR: An opinion on the value of the findings, based upon a pair of case specific propositions and conditioning information (framework of circumstances) that is provided for possible use as evidence in court [14].

ENFSI: Evaluative reports for use in court should be produced when two conditions are met:
1) The forensic practitioner has been asked by a mandating authority or party to examine and/or compare material (typically recovered trace material with reference material from known potential sources).
2) The forensic practitioner seeks to evaluate results with respect to particular competing propositions set by the specific case circumstances or as indicated by the mandating authority [15].

In the evaluation of a DNA comparison, the term "value" is used which refers to the LR and "weight" which refers to the log (LR). The term "strength" is no longer used.

Evidence
The word "evidence" has a very specific legal meaning and refers to results that would be accepted by the court.

Explanation
In the context of evaluation, explanations have been recognised as intermediate considerations when exploring less formal alternatives. While they have the potential to account for given observations and can be very useful in the investigative stage, they do not qualify as formal propositions for evaluative reporting.

Extrinsic characteristics
Characteristics which encompass attributes such as the location of the trace, its size quality, quantity or relative quantity.

Factual reporting
This is the reporting of observations. No inferences/explanations (opinions) are drawn from the observations. A factual report explains what the practitioner has done and the observations obtained.

Forensic DNA expert
A person trained and experienced in forensic DNA analysis and may function as an expert witness in a court of law.

Fst
The co-ancestry coefficient in Subpopulation (S) relative to the Total (T) population: it measures the relationships among alleles of different individuals in the same subpopulation compared to alleles in different subpopulations. Fst correction is implemented in many software.

Hierarchy of propositions
The concept of a hierarchy of propositions helps scientists to focus on the key issue they can help with identifying the results they need to assess and the factors that are important for evaluation. Propositions are classified into five levels: offence, activity, source, sub-source and sub-sub-source.

- Offence – propositions that refer to the commission of a criminal offence.
- Activity – propositions about a human activity or a happening.
- Source – propositions relate to whether or not a person of interest (POI) is the source of the biological material.
- Sub-source – propositions relate to whether or not a POI is the source of the DNA, irrespective of the proportion of contributor material.
- Sub-sub-source – propositions relate to the donor of a portion of the DNA profile (i.e., a major or minor contribution).

Investigative reporting
An investigative opinion arises when explanations are generated to account for the observations. Investigative opinions (i.e., provision of an explanation) are generally made in the absence of a POI and are not meant to be used in court, as one does not assess the value of the findings.

Note 1 to entry: An example of an investigative opinion in a possible sexual assault would be explanations for the absence of sperm: an explanation may be that a condom was worn, or there was no ejaculation or that all trace of sperm was lost.

Likelihood ratio
Expression of an examiner's assessment of the ratio of the probabilities of the observations if one of two competing propositions were true versus if the other proposition were true. This is considered the remit of DNA scientists [11].

Match
A recognition that there is agreement between two sets of observations (e.g. comparison of crime-related material (unknown source) and reference material from a known source) that would be expected if the two analysed samples had come from the same source.

Mutually exclusive
Related such that each precludes the other [11].

Opinion
The expert's judgment as the result of an analysis and interpretation [11].

Person of interest (POI)
A person (e.g., a suspect, a victim, a candidate) who is considered as a potential source of material recovered in the context of a crime, a paternity or a missing person's case.

Prevalent DNA
DNA that is present from known sources/activities that includes self-DNA.

Note 1 to entry: Self-DNA is from the known individual wearing an item of clothing, for example.
Note 2 to entry: The analyst has a prior expectation of finding DNA from specific individuals. Specific examples of prevalent DNA are:
1) DNA from a person observed on swabs taken from underneath her/his own fingernails.
2) DNA from clothing taken from a known wearer.
3) DNA from a surface where there are known occupants at a premise [13].

Probability
Probability is a concept by which one can express one's uncertainty (about an event or, more generally, an unknown state of affairs).

Note 1 to entry: The laws of probability define the values that probability can take (a value between 0 [event false] and 1 [event true]) and how probabilities combine.

Note 2 to entry: Among forensic practitioners and other members of the judicial area at large, it is useful to view probabilities as conditioned on the information available to the individual who makes a probability assignment (i.e., all probabilities are conditional) [15].
Prior probability – initial probability or belief of the proposition being true or false before taking into consideration other findings. This is generally not considered to be the DNA scientists' remit.
Posterior probability – probability or belief of the proposition being true or false after taking into consideration other findings. This is generally not considered to be the DNA scientists' remit.
Prior or Posterior Odds – are the ratio of the probability of the proposition being true divided by the probability of it being false. This is generally not considered to be the DNA scientists' remit.

Proposition
Statement that is either true or false, the truth of which is uncertain.

Note 1 to entry: Also, sometimes referred to as hypothesis.

Sensitivity analysis
Investigation of the impact of the amount of data that are available for assessing results to explore the sensitivity of the likelihood ratios to changes to the data.

Substrate control
In the context of activity level propositions, these are specimens collected (e.g., swab) from an area on an item, close to biological material of interest where one would expect to obtain no result.

Note 1 to entry: These controls act as "blanks" or as "negative". They allow to assess the presence of background material (i.e., for reasons unrelated to the disputed activity).

Task-relevant information
Information which a forensic scientist should consider when performing a particular task.

Note 1 to entry: Examples of task-relevant information would be: what is the alternative population, what the persons of interest say in the case, what activities are alleged to have taken place, what are the timelines, if the persons have legitimate access to the objects/persons/premises of interest.
Note 2 to entry: Examples that are not task-relevant information would be other evidence that points toward the suspect, or e.g., previous convictions. These are not be requested by the forensic scientist.

Value/weight of the findings
In this document, the value of the findings refers to the likelihood ratio value. The weight of findings is defined as the log(LR). In this document the term "strength" is not used to refer to the value of the results.

4. RESOURCES

4.1 Personnel

All personnel employed in the forensic unit shall have adequate training according to their responsibilities, following a specific training programme. This training programme shall describe all methods and documentation applicable. During the training programme, the trainee shall be supervised and assessed by a qualified person. Once deemed competent, the trainee shall be authorized to perform the tasks. Competency shall be subject to ongoing monitoring. Further details for training, competency tests and monitoring of training may be found in the ENFSI Guideline for the Training of Staff in DNA Laboratories [16,17].

There is a need for the forensic community to acknowledge that helping to address activity level issues requires separate skill sets from those for evaluation of DNA comparisons considering sub-source level propositions [17]. Consequently, separate training programmes, competency testing, authorizations and peer review are required.

4.2 Equipment

4.2.1 Equipment Selection

Equipment selection and procurement should be based on documented specifications to ensure that equipment selected is appropriate for the methods of the forensic unit.

4.2.2 Equipment Inventory and Records

All laboratory equipment, computers, firmware, operating and data analysis software determined to be critical by the forensic unit should have a unique identification and be listed in an equipment inventory. All records generated from the moment of installation and henceforth, related to preventive maintenance, calibration, verification, repair, relocation, upgrade etc. should be archived in either electronic or hardcopy format for reference for the lifespan of the instrument and for a period thereafter defined by the forensic unit.

4.2.3 Equipment Verification

Prior to the use of equipment in routine work in the forensic unit, verification/internal validation in accordance with a laboratory procedure that specifies acceptance criteria, shall be completed to ensure that it is fit for the intended methods/analytical procedures. See further details in chapter 6, pertinent to validation and estimation of uncertainty of measurement and the ENFSI validation guidelines. [18,19].

4.2.4 Equipment Standard Operating Procedures

Equipment standard operating procedures (SOPs) should be available for reference to allow proper and safe operation of equipment by authorized personnel.

Equipment programmed to run specific methods should be safeguarded from inadvertent alteration of these settings through access control and verification of these settings only by authorized personnel.

Awareness of the performance limits of each instrument and the variability between the same type of instruments should be recorded in the context of the verification studies and detailed in the SOP of the equipment/software as applicable. Any identified performance drift can lead to actions after risk assessment.

4.2.5 Software and Firmware

New or updated versions of laboratory specific software including LIMS, Expert Systems, instrument software, firmware and in-house macros should be internally validated/verified for intended use prior to implementation in routine work [20].

All changes in the firmware of robotic systems that can influence the performance of the instrument/software should be recorded \and validated where necessary.

4.2.6 Equipment Calibration and Preventive Maintenance

Documented calibration and preventive maintenance/service programmes for equipment should be in place at the forensic unit specific to the type of equipment/instrument, its running capacity, performance history and manufacturer recommendations in order to generate valid results.

The calibration procedure applied to each instrument should be validated, documented, controlled and follow the relevant standard/guide or accredited procedure. Applicable, certified reference materials traceable to national or international standards and/or quality controls should be used in order to ensure that the instrument operates in accordance with the required specifications (e.g. *range of operation, resolution, accuracy, precision*). The specifications of the forensic unit protocols/test methods should be taken into consideration in the calibration protocol (calibration target) of the equipment (e.g., *temperature, centrifugation speeds, weight, pipetting volumes, spectral calibration, spatial calibration).*

Calibration and preventive maintenance should be performed by qualified field service engineers contracted from accredited calibration laboratories or instrument manufacturers/authorized suppliers or trained and authorized forensic unit personnel as applicable.

4.3 Reference Materials and Reference Data

Reference material and reference data have many applications in the forensic unit such as in test method verifications/validations, equipment calibrations, determination of test sample characteristics and in the determination of frequency/rarity of genetic data (autosomal & Y, STR & SNPs, mitochondrial DNA haplotypes/haplogroups).

Reference material or reference data should be:

- Certified Reference Material (CRM) traceable to national or international standards accompanied with their uncertainty of measurement and certificate of analysis, or
- Material or data obtained from known sources. Reference materials or data from known sources should be verified.

Where available, reference materials should be purchased from suppliers accredited in accordance with the relevant standard: ISO 17034 General Requirements for the Competence of Reference Material Producers [21].

4.3.1 Certified Reference Materials (CRMs) for Equipment Calibrations

Certified reference materials are to be used for the calibrations of critical equipment.

4.3.2 Certified and Known Reference Materials for Verification/Internal Validation Studies

Reference material, as defined in sub-section 4.3, of known characteristics, such as the quality, amount and profile or sequence shall be used to validate a method.

4.3.3 Certified and Known Reference Materials for the Assignment of Values to Samples

For DNA quantitation, quantitation standards of known, certified quantity should be used to construct a calibration curve for the estimation of DNA concentration and where required, female to male contribution/ratio. A DNA of known concentration/quantity can also be used as a positive control of the quantitation batch.

For DNA profiling, reference materials (i.e., allelic ladders and internal lane/size standards) shall be used for STR PCR fragment sizing and allele designation. The panels, bins and stutter text files allowing the automatic assignment of alleles through the data analysis software provided by the STR system manufacturers can be used. In addition, probabilistic genotyping software can be used. The internal lane/size standard for each kit is either provided by the manufacturer or created according to the fragment sizes and dye colour as instructed by the kit manufacturer.

4.3.4 International Standards – European Standard Set (ESS) Loci

STR systems which include the European Standard Set (ESS) of Loci indicated below are recommended for STR typing for national DNA databases and European/Interpol DNA Data exchange. [These are the 12 ESS markers ESS Loci: *D3S1358, VWA, D8S1179, D21S11, D18S51, HUMTH01, FGA, D1S1656, D2S441, D10S1248, D12S391, D22S1045* as presented in: Council Resolution of 30 November 2009 on the exchange of DNA analysis results]. However, many countries use a larger set of loci such as the CODIS Core Loci (Combined DNA Index System which is the United States national DNA database created and maintained by the Federal Bureau of Investigation [22].

4.3.5 Population Frequency Data of STR Alleles, SNPs and Haplotype Frequency Data

Biostatistical evaluation relies on population frequency data for the markers analysed. They are therefore indispensable data for this purpose. Reliable, quality controlled, updated sources are openly available for consultation.

4.3.5.1 Population Frequency Data – Autosomal STR Alleles

Recommendation: the use of validated, quality assured population allele frequency data e.g., STRidER (**STR**s for **Id**entity **E**NFSI **R**eference Database) [23].

STRidER is the ENFSI open access population STR frequency database. These STR population frequencies can be directly downloaded (https://strider.online/) for their use in biostatistical evaluations [23].

STRidER is recommended due to the diverse population availability, allele nomenclature compliant with the guidelines published by the DNA Commission of the International Society for Forensic Genetics [24] Privacy regulations are applied so that donors of samples are anonymised. Strict QC measures are taken to validate the population frequency submissions. In addition, it is recommended that data for those bio-geographical populations not yet available should be submitted to create a comprehensive database.

4.3.5.2 Population Frequency Data – Y Haplotypes and SNPs

Recommendation: YHRD (**Y C**hromosome **H**aplotype **R**eference **D**atabase (http://yhrd.org) [25].

YHRD is an open-access resource which is recommended due to the diverse world population availability*;* privacy regulations are applied; QC measures are taken to validate the population

haplotype submissions through the “Data File Validator” tool. Recommendations for the interpretation of Y-STR results and haplotype frequency estimation using YHRD have been published by the DNA Commission of the International Society for Forensic Genetics.

4.3.5.3 Population Frequency Data – Mitochondrial DNA Haplotypes and SNPs

Recommendation: EMPOP/EDNAP mitochondrial DNA population database (http://empop.org) [26].

EMPOP is an open access mitochondrial DNA sequence variation reference database encompassing data from diverse world populations. It is equipped with various statistical tools to perform both sequence determination and probability of the sequences in different populations [26].

As with the previous international databases**,** EMPOP is recommended due to the diverse world population availability*;* privacy regulations are applied; QC measures are taken to validate the population haplotype submissions. Recommendations for mtDNA typing and haplotype frequency estimation have been published by the DNA Commission of the International Society for Forensic Genetics [27].

4.4 Facilities and Environmental Conditions

Suitable facilities and environmental conditions are requirements for:

- general safety of personnel
- proper and safe equipment operation
- safeguarding the work performed
- safeguarding consumables, item integrity and data

4.4.1 General Safety Requirements for Personnel

General safety requirements should be in place according to national requirements. In particular, biohazard, chemical and physical safety precautions should be taken into account (see chapter 14).

4.4.2 Requirements for Proper and Safe Equipment Operation

Requirements such as the following should be taken into account: appropriate bench and floor space for equipment installation, electrical power supply, lighting, internet connections, software, hardware operation, air quality, air flow and air pressure where relevant. Temperature and humidity control and monitoring should be taken into consideration for the proper and safe operation of equipment within the forensic unit as applicable (see also sub-section 4.2).

4.4.3 Requirements for Safeguarding the Work Performed

Requirements for the work performed include the design of the facility such as layout, building materials and laboratory benches, which should allow for easy cleaning to minimise the risk of contamination.

The provision for compartmentalisation, in order to accommodate/separate incompatible activities for example Pre-PCR from Post-PCR, high yield DNA containing items (e.g., buccal swabs) from low yield (trace DNA swabs, dry skeletal elements), should be taken into account. Positive air pressure or an airlock space between pre-PCR and other laboratories is also an important measure that should be taken for DNA contamination prevention.

An environmental monitoring programme to monitor the cleaning to minimise DNA contamination from the working environment with appropriate corrective actions where environmental contamination is detected should be in place. The ENFSI Guideline for DNA Contamination Prevention [28] and FSR-G-208, [29] may be consulted for further details.

4.4.4 Requirements for Safeguarding Consumables, Item Integrity and Data

Reagents shall be stored under the appropriate conditions and monitoring of these conditions should be recorded.

Items and DNA extracted from the latter shall be protected from degradation by storage in appropriate storage conditions and monitoring of these conditions should be recorded. Items and DNA samples shall be protected through security measures such as access control/ surveillance to prevent unauthorised access and should be accompanied by a chain of custody (recorded traceability system) during transfer through the forensic unit.

The required management, technical and physical measures should be taken to prevent loss, corruption or theft of data such as the use of access control to authorized personnel only and digital data transfer should be confirmed with recipients.

4.5 Materials and Reagents

4.5.1 Required Quality of Materials and Reagents

The quality of consumables and reagents used at each stage of the forensic examination should be fit for purpose. DNA grade consumables and reagents conforming to the requirements in the ISO 18385 standard should be used where relevant for forensic DNA analysis methods [30]. For consumables that are not specified as DNA grade, then a representative sample from the lot number received should be verified prior to use in routine casework by the forensic unit through a documented procedure.

4.5.2 Materials and Reagents Inventory

Purchasing procedures should be in place to ensure that required materials and reagents are available to allow for examinations. For critical reagents as defined by the laboratory, information regarding lot numbers, purchase order numbers, expiration dates, storage conditions and storage locations should be kept. For critical reagents additional requirements may be in place as determined by the laboratory (e.g., testing prior to use).

5. METHODS

5.1 Selection

Selection should be based on available appropriate methods which have undergone developmental validation by the manufacturer or the laboratory. Methods to be routinely used shall be internally validated before they are applied for routine casework and should undergo proficiency testing as outlined in sub-chapter 7.1.

Legislation, casework requirements such as the types of items to be tested and the information sought by the customers and health and safety regulations should be considered in the selection of the method.

Table 1 lists the methods/activities, the purpose of the methods and the materials isolated and/or information provided through their application. The order of presentation in the table does not necessarily dictate the order in which the methods should or are applied. The order and indeed selection of method(s) used is the responsibility of the forensic unit.

Table 1: Overview of methods applied in DNA laboratories in case examination

Activity/Method	Purpose of Methods (as applicable)	Material Isolated &/or Information Provided
Search for traces	Visual/alternate light sources /UV/IR. Chemicals used to visualize body fluids e.g Luminol (or analogues) for blood.	Location of stains/areas of interest for the collection of biological material.
Recovery of traces	Techniques used for optimum recovery which include, swabbing, cutting and tape lifting.	Sampling for all downstream testing including sufficient material for repeat analysis if possible.
Characterization of biological material	Chemical/immunological/histological /nucleic acid-based methods* for biological fluids from items, traces, extracts.	Presumptive, probabilistic or indicative (for RNA) characterization of the nature of the biological fluid. When considering mRNA, co-extraction will be required.
DNA extraction	Isolation of DNA for downstream analysis.	DNA from forensic items and reference samples for profiling including sufficient volume/quantity for repeat analysis if possible.
DNA quantification	Estimation of DNA quantity for downstream analysis.	DNA concentration, presence of inhibitors, level of degradation, presence of male DNA.
Autosomal STR analysis	STR profiling that meets the minimum recommended STR loci (ESS loci) requirements [31].	STR profile, single source or mixture.
Y-STR analysis	Y-STR profiling that meets the minimum recommended Y-STR markers [25].	Y-STR profile, single source or mixture.
Mitochondrial DNA (mtDNA) analysis	mtDNA profiling [27]	mtDNA sequence
Simultaneous or distinct determination of autosomal STR, Y-STR mitochondrial and phenotypic, and biogeographic SNPs using massively parallel sequencing (MPS) including assessment of methylation for age determination*.	Methods to produce sequence data for STR, mitochondrial and SNPs by simultaneous or distinct sequencing. The requirements for STR, Y-STR profiles and mitochondrial DNA sequencing apply.	As for STR, Y-STR and mtDNA analysis above. SNP profiles to estimate hair colour, eye colour, age and ancestry.
Rapid DNA analysis single device*	The requirements for STR profiles apply [32–35]	STR profile
DNA database search	Comparison of a queried DNA profile with the database [36]	Candidate profiles for further investigation
Statistical evaluation	Commercial or open access software validated to compute likelihoods, and/or LRs, for pairs of mutually exclusive hypotheses given, population frequency data, Fst, drop-in/drop-out rate and in some software packages conditioned on the number of contributors.	LR given a pair of mutually exclusive propositions (see chapter 12).
Evaluation of findings given activity level propositions	Assess the value of the findings in the context of two propositions and case information where relevant resources are available [13,15].	LR given a pair of mutually exclusive activity level propositions. (see chapter 12).

* At the time of writing this BPM, these methods are not commonly used in routine casework.

DNA comparison workflow can be structured into the following steps:
1) Acquisition of data.
2) Quality assessment.
3) Comparison of DNA profiles (with known POI(s) or National DNA database).
4) Evaluation and verification of results, covered in chapter 12.

5.1.1 National DNA Databases (NDNADB), Missing Persons' DNA Databases

National DNA databases are established and operate according to national legislation and contain entries of unidentified DNA profiles and DNA profiles of persons in compliance with national law. In addition, DNA profiles of missing persons, unidentified human remains (UHRs) and relatives of missing persons can be registered in a national DNA database, in compliance with national legislation in order to locate missing persons and contribute to identifying human remains. These profiles are searched to look for potential candidates. Recommendations for the operation of the NDNADB are provided in the ENFSI guideline on DNA Database Management [36] and includes recommendations for the formulation of profile inclusion and deletion criteria, matching rules, international DNA data exchange, legislation, personnel, quality control, auditing and software requirements etc.

5.2 Peer Review

The forensic unit should have a documented procedure(s) for the peer review of critical information and findings in the process of item analysis. This procedure should include the method for reconciliation and remedy for diverging opinions. The peer review is defined as the evaluation of the reports, examinations, notes, data and findings by others competent in the same field to assess that there is an appropriate and sufficient basis for the conclusions and/or opinions. Peer review methods are used to maintain quality standards, improve performance, and provide credibility.

6. VALIDATION AND ESTIMATION OF UNCERTAINTY OF MEASUREMENT

Uncertainty of measurement does not apply in forensic genetics as it does not affect the value of the findings. Indeed, in forensic genetics, the estimation of quantities of DNA, size or lengths is not the key issue for the court, contrary to what would be done, for example, in forensic chemistry. In the latter case, the quantity of cocaine for example contained in an item seized from a suspect is a key issue for the court. This is not the case for quantities or sizes measured in forensic genetics. Therefore, in forensic genetics, we will refer to potential sources of variation instead.

However, for example, the value of the results of DNA comparisons is very relevant to the court, more specifically, how probable it is to observe the DNA results if the DNA is from the person of interest, or not. Because probabilities do not exist *per se* (i.e., probabilities are a state of mind and not of nature), there is no need to give uncertainty on probabilities. As Lindley mentions in his book 'Understanding uncertainty': "*According to the attitude adopted in this book, it is nonsense for you to have a belief about your beliefs…*" (p. 115) [37].

6.1 Validation

For new and updated methods, kits, instruments and/or software, internal validation (or verification) shall be performed prior to implementation for casework in the forensic unit. A documented validation plan/procedure should be followed specific to the method, kit, instrument and/or software under validation which should address the acceptance criteria based on the ENFSI Validation guidelines [18,19].

A risk assessment should be performed in order to design and implement the relevant control measures to mitigate the potential risk(s) that may be identified. Available standards may be followed for risk assessment [38,39].

6.1.1 Prerequisites for Validation

- competent personnel
- calibrated instruments
- appropriate environmental conditions
- minimum number of relevant test samples including replicates
- reference materials and statistical methods and reference data to be used

6.1.2 Post Validation Requirements

- Implementation post validation shall include the training of staff [16] and the implementation of the SOP.

6.2 Potential Sources of Variation: Variables/Factors That Impact Upon the Value of the Likelihood Ratio

The likelihood ratio (LR) is a measure of the value of the forensic findings when two alternate propositions are considered. An LR is defined in terms of the ratio of two conditional probabilities: (i) the probability of the findings given that one proposition is true and given the conditioning information; and (ii) the probability of the findings given that the other proposition is true and given the conditioning information. We cannot measure a LR in the same way we can measure the length of a piece of string or the quantity of a drug from an item. This is because neither the LR nor probabilities exist in the real world, hence they are said to represent the 'belief' of the scientist. This belief is underpinned by modelling assumptions, which are a representation of the (unknowable) real world. Validation exercises are carried out to characterise software. Such exercises are performed with samples of known origin and these are used to study model behaviour relative to expectations of performance in terms of sensitivity and specificity. In order for the computed LRs to be calibrated, non-contributor tests should conform to Turing expectations [40,41][1]. As stated in Buckleton et al: *"With an LR when applied in the purest form, an estimate is not produced. Rather the resultant LR is termed an assigned LR to embrace the subjective probabilities that may have been used in its formation"* [12].

It follows that a probability or a likelihood ratio cannot be associated with 'uncertainty of measurement'. However, their values are dependent upon variables that are input into models. Variables/factors considered by probabilistic genotyping software usually include:

- number of contributors
- allelic proportions or frequencies from a given population
- Fst
- drop in rate
- drop out rate
- allele peak height

[1] According to Turing's rule, the expected LR for a false proposition is one, if the model is correct (p. 72, [41]). Turing's rule informs us that the fraction of non-donors producing an LR ≥ x is expected to be at most 1/x. Examples of such validations can be found in Buckleton et al. [69] They discuss that if the LR given the propositions that the DNA is from Smith or an unknown person is 1000, then we expect that after comparing this profile with a sample of 10000 non-contributors we would observe – on average – 10 or fewer individuals with LRs that are 1000 or higher. It is this expectation, that one would study when validating LR calculations.

- mixture proportion (Mx)
- stutter
- degradation

We discuss below how to estimate allelic proportions and how to account (i) for rare alleles, (ii) for the fact that there is sub-structure in human populations.

6.2.1 Allelic Proportions in Surveyed Populations

When, measuring the occurrence of an allele in the population, because this exists for a given population at a given time, there is a true value of the proportion of this characteristic. In such a case, statistical methods should be applied to account for the uncertainty associated with sampling.

- To determine allelic proportions, laboratories use samples from relevant populations (those that are typically represented in routine casework). Quality checks shall be done as described previously [24,31].
- The relative frequency of a given allele type (*a*) is calculated as $f_a=a_n/2N$ where a_n is the number of *a* alleles and 2*N* is the total number of alleles in the sample of size *N* for the population surveyed.
- As we do not study all the population but only part of it, there are statistical methods that account for the fact that only a sample (i.e., a selection) of the whole population has been studied. To estimate allelic proportions in the population of interest, some laboratories may use, for example, a Bayesian estimator and summarize the posterior distribution with the mean equal to $(x_i+[1/(k+1)])/(2N+1)$ where x is the number of observations of allele *i*, and *k* is the number of alleles typed at the locus under consideration (e.g., known alleles for the locus is {6,7,8,9} then k=4).
- During casework, rare alleles which were not observed during the survey of the population because of the inherent selection process will be encountered (this will often be the case with massively parallel sequencing). If the sampling process is not considered as described in the previous bullet-point, this would result in $f_a=0/2N = 0$. It is not meaningful to carry out calculations where the probability of an allele (*a*) is 0. If a rare allele is encountered, for example a simple adjustment is: Pr(*a*)=1/(2*N*+2); the minimum allele probability is 1/(2*N*+2). Note that this application of minimum allele frequency has nothing to do with compensating for sampling uncertainty. If using a Bayesian estimator, the size of sample will be accounted for in the estimation of non-observed alleles $(1/(k+1))/(2N+1)$.
- When a population is sampled, this does not take account of the underlying sub-structure. Sub-populations cannot be precisely defined or sampled, but if both defendant and perpetrator are assumed to originate from the same sub-population, they are more likely to share alleles from a common ancestor. Consequently, the probability of a given allele should take this into account. Fst (theta correction) is applied to consider sub-population effects. The Balding/Nichols formula [42] [43]is used, and is extended to accommodate mixtures [12]. The value of Fst is dependent upon the population of interest. For cosmopolitan populations Fst=0.01 is suitable, but higher values up to Fst=0.03 may be needed. Appropriate values can be found in Table 3 from Buckleton et al. [12].

When likelihood ratios are reported, an 'error rate' or 'confidence interval' is neither applied nor recommended. As with all probabilities, LRs depend on the data used and assumptions made. A *sensitivity analysis* may be applied to demonstrate the impact of the variation of these elements using simulation. This will show how sensitive our LRs are to the change of data and/or assumptions. But this is not a measurement of error. Sensitivity analysis is sometimes applied to evaluations given activity level propositions [44]. It is useful to understand the impact for example of the data used, particularly when there are few experiments. However, this method is used for investigation rather than evaluation. For reporting purposes, a point

estimate based upon the median or mean value is generally used. Some laboratories may prefer to report a quantile as a 'conservative' measure, but this is optional.

7. QUALITY ASSURANCE

Quality assurance is fundamental for confidence in the forensic service providers. Accreditation according to ISO/IEC 17025 provides formal external recognition and approval of quality assurance[1,10]. In many countries, this is a legal obligation in order to exchange DNA data [1].

7.1 Proficiency Testing/Collaborative Exercises

Proficiency tests (PT) shall be performed by a laboratory as a procedure for monitoring the validity of results produced (ISO/IEC 17025, clause 7.7.2) [10]. They should cover all technical procedures (e.g. searching, recovery, presumptive tests, DNA extraction, quantification, PCR, electrophoresis), data analysis and interpretation, statistical evaluation of results and reporting of conclusions. This monitoring shall be planned and reviewed.

PTs from bodies that are compliant with or accredited according to ISO/IEC 17043 are recommended. This International Standard specifies general requirements for the competence of providers of proficiency testing schemes.

Laboratories are advised to also participate in interlaboratory comparisons/exercises to complement existing PTs, as a further evaluation of the validity of results produced by the laboratory.

Any discrepancies observed in PTs shall be evaluated as part of the laboratory's quality management system according to ISO/IEC 17025, clause 8.7 [10].

7.2 Quality Controls

The laboratory shall make use of quality control samples for monitoring the validity of results (ISO/IEC 17025, clause 7.7.1) [10]. Critical steps to be monitored are the following:

- The use of presumptive tests: appropriate negative and positive control samples shall be used to verify the performance of presumptive tests. (i.e the controls should not merely test new lots but ensure an appropriate control for the duration of the use of the controls & tests).
- DNA extraction and purification: a negative control/reagent blank sample shall be included to monitor traceability and potential systematic contamination from various sources (e.g., reagents and consumables) for both trace and reference sample analysis. For trace analysis, positive control samples consisting of a known DNA-profile may be used to monitor the performance for batches of samples.
- DNA quantification: a negative control sample should be included to monitor traceability and potential systematic contamination from various sources (e.g., reagents and consumables). A positive control of known DNA quantity, e.g., positive control of STR kits, may be used in each batch to check the performance of the run and if used, a record should be kept to identify any deviations.
- DNA amplification: a negative control sample shall be included to monitor traceability and potential systematic contamination from various sources (e.g., reagents and consumables). A positive control sample consisting of a known DNA profile, e.g., positive control of STR kits, should be used to monitor the traceability and performance for each batch of samples.
- Logs of contamination and drop-in events should be maintained by the laboratory and made available for quality control and interpretation.

Further tools/procedures to identify contamination events, such as elimination databases, software to search casework results (including mixtures) for cross-contamination, environmental monitoring of DNA laboratory facilities and equipment should be in place in the quality assurance/control framework. Details on these controls/ tools are found in ENFSI Guidelines for DNA Contamination Prevention [28] as well as the relevant FSR guidance [29].

Controls and procedures to verify and check the performance of reagents and equipment should be in place. Where possible, an internal verification procedure for quality, such as batch testing to verify that materials/reagents are free from detectable DNA (if not certified as such) and perform as expected should be carried out and recorded. If available, the manufacturer elimination database can be used to confirm the origin of contaminants.

7.3 Data Collection for Control, Monitoring and Trend Analysis

The data from monitoring activities shall be analyzed, regularly, as predefined by the laboratory, and if applicable, used to improve the laboratory's activities. If the results of the analyses of the above-mentioned data in sub-chapter 7.2 are found to be outside pre-defined criteria, (stated in the method or in performance/acceptance criteria of the laboratory) appropriate action should be taken to prevent potentially incorrect results from being reported. Action should be taken in order to remedy the problem observed and to minimize the risk of re-occurrence.

The input for monitoring activities can be the data obtained from the use of quality controls the proficiency testing results, internal audits, peer review of data and expert reports.

Special attention should be given to monitoring contamination events observed in the laboratory which should be recorded.

7.4 Risk Assessment

The forensic unit shall carry out risk assessments at defined intervals with respect to the external and internal context of its work in order to identify potential risks (through brain storming, SWOT analysis, nonconformity records, complaints, customer satisfaction surveys and other means) which can affect the quality and validity of its services or ability to fulfil contractual obligations. In so doing, the laboratory can ascertain whether its quality control and quality assurance plans, operational and management policies and associated procedures are adequate to prevent or mitigate these potential risks. If it culminates in the conclusion that the existing control measures are inadequate or not able to safeguard the forensic unit from these risks, then additional control measures should be implemented. Techniques described in the standards for risk management may be applied to perform risk assessments in the laboratory [38,39].

Examples of potential risks that may be assessed can be divided in to the following general categories: external and internal risks.

7.4.1 External risks

- Customer activities: inappropriate handling of and compromised test items impacting quality of data, impartiality, confidentiality threats.
- External suppliers of services and products: failure to provide expected quality and timely service/product, impartiality and confidentiality threats.
- Natural disasters/pandemics/criminal and/or cyber-attacks.

7.4.2 Internal risks

- Consumables/reagents/reference materials: availability, suitability and quality.
- Equipment: availability, maintenance, calibration, data processing, IT systems and software requirements.
- Facilities and environmental conditions: safety, suitability for work performed.
- Methods and procedures: suitability of selected manual and automated methods, validation, monitoring.
- Personnel: impartiality and confidentiality, training and competence, coordination, well-being.
- Challenges of working with limited/degraded biological material.

8. HANDLING ITEMS

This section addresses specific considerations of handling items at the scene(s) and in the laboratory.

Chain of custody/traceability of all items shall be recorded and controlled.

8.1 At the Scene

Factors that could influence the result and should be considered include:

- examination of the scene, and/or persons
- avoidance of contamination
- search and recovery
- sampling
- preservation, packaging, storage and transport of items
- unique item identification (labelling) and chain of custody

8.2 In the Laboratory

The forensic unit shall have procedures for the receipt, identification, transportation, sampling, examination, protection, storage, retention and/or disposal of items, including all provisions necessary to protect the integrity of the item, and the interests of the laboratory and the mandating authorities/customers.

The laboratory shall ensure that items are appropriately handled from the time of submission to its facilities throughout item examination, sampling and analysis and finally in storage and return to the mandating authorities, or for the allocated time of retention for all other items.

The packaging and labelling of items shall be examined upon submission to the laboratory and recorded. Any deviations from specifications or observations are discussed with the customer to the suitability of the sample for examination. If there is a significant deviation that impacts the value of the findings, this shall be fully disclosed in the statement.

Items shall be uniquely identified as they arrive at the laboratory or as samples are taken by laboratory scientists from the primary items submitted. The identification system shall be designed and operated so as to ensure that items cannot be confused physically, or when referred to in records or other documents. The system shall also accommodate a sub-division of groups of items and the transfer of items within and from the laboratory when appropriate.

All items submitted for examination shall be securely stored so as to ensure their integrity by preventing against deterioration, contamination, and loss of identity so as to ensure the

generation of valid results if re-examination is warranted. Environmental conditions for the storage of items shall be specified, monitored and recorded according to SOPs.

Quality control procedures for item handling and examination methods shall be applied to safeguard the item (primary item, subsample from the item, extracted DNA, PCR product) and therefore validity of results generated for the respective sample.

Procedures should be in place to ensure that elapsed time between receipt, examination, sampling and DNA extraction is as minimal as possible so as to avoid deterioration/ degradation of DNA, e.g., if embedded in a matrix with chemicals. If delays are unavoidable then items should be kept in appropriate conditions to prevent deterioration until they can be processed.

9. INITIAL ASSESSMENT

9.1 Assessment at the Scene

The forensic unit should have procedures that provide direction and guidance for the initial assessment for routine examinations. For each case this should include consideration of the mandating authority's requirements, case specific information required to formulate propositions, incompatible activities, equipment and methods available to determine the examination strategy.

For non-routine examinations the same considerations apply and deviations should be recorded.

9.2 Assessment at the Laboratory

Police authority or other mandating authority requests or contractual agreements should be reviewed by the laboratory with respect to the nature of the service requested, the turnaround time that can be accomplished, and the spectrum of tests to be performed along with their limitations. Procedures for receiving, sampling and storing case items should take account of the following considerations:

- Urgency of the investigation.
- Direction of the investigation.
- Status of the crime scene, suspects and victims.
- Nature and severity of crime committed.
- Changes in the relative urgency of information.
- Developments from and/or changes in witness testimony.
- Developments in investigative leads from other forensic disciplines.
- Impact of results already reported.
- Correlation or conflict of other complementary findings.
- Possible contamination issues at the crime scene and availability of relevant elimination samples.
- Case information provided (e.g., what is the issue, alternate source of DNA, possibility of legitimate access to the scene or items, context of the case).
- Compromising the items due to extrinsic circumstances (e.g. heat, humidity, incorrect labelling, contamination, loss).
- Bulk of items delivered for examination and ability of the forensic unit to receive and adequately store these items until examination and sampling.
- Use of safety equipment for sampling and storage of items posing a biological, chemical or other hazard to staff such as drugs, petroleum infused materials, decomposing tissue

from disaster victims as well as explosives and armed weapons which will require examination and sampling by highly trained case examination staff.

10. PRIORITISATION AND SEQUENCE OF EXAMINATIONS

For setting the case examination strategy the following should be considered:

- client's requirements
- availability of items and amount of material
- number, nature and sequence of examination technique
- potential value of the information from each technique

10.1 Establish Priorities at the Scene

Preserving the quality of biological traces is essential to maximise the chance of obtaining optimal results. The quality of trace material found at the scene can be influenced by the way they are collected and stored as DNA is sensitive to humidity, temperature and direct sun light.

In general, guidelines should be provided for the following:

- Minimize the risk of contamination and deterioration of the trace material.
- Properly record the origin of the collected items.
- Determine the order of sampling by different forensic disciplines to prevent destruction/ alteration of the item (e.g. collection of fingerprints versus DNA sampling).
- Recovery methods, packaging and transport conditions required to preserve the integrity of the item. FSR-C-116 may be consulted for packaging clothing of sexual assault examinations.

10.2 Establish Priorities at the Laboratory

For both financial reasons as well as scientific reasons, the use of forensic DNA analysis may be evaluated within the general context of the case (if available). A pre-evaluation of the case can determine if the requested DNA analysis can potentially help answer the police authority's questions. The scientist shall evaluate the findings in the context of the hierarchy of propositions explained in chapter 12, and/or shall explain the limitations of reporting if case context is incomplete (see sub-chapter 13.1).

Choices should be made with regards to prioritization of items to be analysed as well as prioritization with regards to the use of other forensic disciplines in order to minimize the risk of loss or alteration of trace material and to make maximum use of all material available. Prioritization and sequence of examinations may be based on:

- police authority requirements
- issue with which forensic biology can help in the case
- urgency of case
- severity of case
- custody expiration of suspect(s)
- availability of items and amount of material
- sampling strategy (order of examination by different forensic disciplines to prevent destruction/alteration of the item)
- number, nature and sequence of examination technique
- potential value of information from each technique
- application of urgent case protocols
- implementation of all QC/QA steps

- dealing with backlog of non-urgent cases
- biological fluid identification in accordance with case history
- tests required to evaluate the case given activity level propositions (e.g. rape case analysis typically include semen, blood, saliva tests, sperm cell staining, Y-STRs)

Further guidance may be found in the SWGDAM Guidelines for the Collection and Serological Examination of Biological Evidence [45].

11. RECONSTRUCTION

Not applicable.

12. ASSESSMENT OF RESULTS AND INTERPRETATION

In this section, we summarise the principles for evaluative reporting. In addition, for further information, the Appendix describes the following in detail:

a) Details about the characterization of the nature of body fluids and limitations (investigative reporting).

b) A discussion on the importance of task relevant case information, the concept of propositions showing how it can be structured in the form of a hierarchy.

c) We discuss the different levels of the hierarchy, and explain when it is meaningful to consider the value of biological results considering propositions at a given level.

d) We conclude the Appendix chapter with a section on pre-assessment, which is particularly important when transfer, persistence and recovery of DNA need to be considered in the context of the case and further discuss how to assess biological results.

At the time of writing this best practice manual, for various practical reasons, we note that it may not be common practice to undertake evaluation of forensic results given activity level propositions. However, this is an issue where the courts of law are regularly requesting assistance from the forensic DNA expert and surveys [46] have shown that 70% of DNA scientists thought that evaluative reporting given activity level propositions was useful. This study also showed that half were uncomfortable with reporting findings given activities, however 53% reported on the issues by transposing the conditional. This tendency can also be observed in court transcripts. Over the past twenty years, there have been numerous studies related to the methodology (also outlined in the Appendix). It is important therefore to outline the principles of interpretation, to avoid errors that are commonly made in the communication of results to the courts.

The purpose of these recommendations is to minimise risks of miscarriages of justice such as that illustrated by the much-publicised case of Amanda Knox and Raffaele Sollecito [47]. This case amply shows the dangers of not working within a coherent evaluative framework. Therefore, laboratories are recommended to follow the principles outlined in this section.

The ultimate decision on how to contextualise reports remains as applicable in each organization /jurisdiction. The state-of-the art methods referred to in this manual (Appendix) are to assist practitioners who report biological findings given activity level propositions; for those who do not necessarily report given propositions at this level, a summary of the principles that should be followed as a minimum requirement is provided below. Scientists have a responsibility to be transparent and to ensure that the limitations of their findings are properly explained.

1) The value of the findings is assessed given propositions where the forensic scientist can add value by using expert knowledge that is necessary to understand the value of the findings that is otherwise unavailable. To do so, the concept of the hierarchy of propositions described by Cook and others provides a useful framework [48]. One can refer to the ENFSI guideline for Evaluative Reporting and the ISFG recommendations for further details [13,15,49]. The hierarchy of propositions is widely used to help scientists and the court understand the meaning and limitations of the findings within the context of a case. Each level of the hierarchy is associated with a particular issue that allows to define the mutually exclusive propositions that are to be considered to evaluate the results. The higher the level in the hierarchy, the more value can be added, and the more knowledge and information is needed. (see Appendix section A1.5). It is important that if assessing the value of the DNA comparison only, then propositions should not be higher than source level. If the DNA of the POI may be present for legitimate reasons (e.g., the item was recovered on the POI's car or the POI knows the complainant), or if there are questions about transfer, then just considering the source of the DNA is generally not meaningful. The scientist (provided they have the expertise) can be more helpful by assessing the value of their findings given activity level propositions.
2) At least two mutually exclusive propositions shall be considered. Propositions cannot be exhaustive in general, but practice can proceed with an acceptable coverage, that is with the omission of propositions which are not pertinent to the case.
3) The scientist shall give an opinion on the probability of the findings, not on the probability of the propositions.
4) The value of the findings is determined by the ratio of the two probabilities (i.e., LR): (1) the probability of the findings given the case information and the first proposition and (2) the probability of the findings given the case information and the alternative proposition. Generally, one value (i.e., LR) will be assigned for each person of interest.
5) The scientist should not claim a level of precision that is neither needed, nor justified. For this reason, LRs are usually rounded down to one significant figure. When the LRs are smaller than one, one can first give the value and then indicate that the propositions have been inverted to give a LR larger than one, as smaller numbers are difficult to grasp. In that case, the scientist must take precautions so that the reader does not misunderstand the LR. A possible solution is to write: "This likelihood ratio indicates that the analytical results support the alternative proposition that it was only unknown persons, and not person XXX, who contributed to the DNA mixture. Because numbers smaller than one are difficult to understand, we have reversed the propositions in the wording below. This likelihood ratio means that it is of the order of a X times more likely to observe our analysis results if two unknowns (not the POI) are the origin of the DNA mixture observed for the YYY trace. rather than if it is the POI and an unknown."
6) The assumptions and the propositions, where applicable, shall be clearly stated and a caveat applied that informs the court that should new information become available that could affect the validity of the propositions, then a new evaluation may be required.
7) As far as possible, the case information (on which the propositions are based) shall be disclosed. If there is no information available from the defence prior to the court-proceedings, then when appropriate, the expert needs to formulate the alternative proposition based upon reasonable assumptions. In that case, the scientist should indicate that should these assumptions not be relevant to the case, a new interpretation and perhaps further analysis will be necessary based on the new case information and new alternative.
8) A caveat should also outline / explain what a LR is and is not. Some laboratories will add a caveat indicating that the laboratory does not provide any assessment on how likely it is that the first proposition or the alternative proposition is true. Indeed, this probability

(e.g. The blood came from Mr A), is the domain of the court, as one needs to combine all the information of the case in order to make such a statement. And a caveat on activities: e.g., “This report does not provide any information on the mechanisms or actions that led to the deposition of the biological material concerned. It only provides help regarding its source. Should an issue arise at any time regarding the activities that led to the deposition of this DNA, an expert might be consulted to re-assess the findings in light of the new information.”

The report shall be structured as per the current ISO 17025 quality assurance standard requirements and include the following:

a) A preamble to describe the purpose of the examinations carried out within the framework of circumstances.
b) If there is uncertainty regarding the source of the DNA, alternative propositions are stated at sub-source level e.g.,
    i. The DNA came from Mr X and two unknown persons unrelated to him.
    ii. The DNA came from three unknown persons unrelated to Mr. X.
c) The value of the evidence is described e.g.,
    i. The DNA profiling results are of the order of one billion times more likely if the first proposition (i) is true than if the alternative (ii) is true.

A verbal equivalent can be used in addition (but not as a substitute): e.g., “I have assigned a LR of the order of one million. Thus, according to our internal verbal scale, this analysis provides extremely strong support for the proposition that Mr. X is a contributor to the DNA obtained from Item I rather than not”.

9) If a laboratory does not report given activity level propositions then the report should make clear that the opinion only provides information regarding the source of the DNA [49]. A statement of limitation is required (as described in the previous paragraph) to make it clear that the findings described in the report do not enable one to answer questions about how the DNA was deposited. An example could be: “*The case has been reported given source / sub-source level propositions, which means that this report does not provide any information on the mechanisms or actions that led to the deposition of the biological material concerned. It only provides help regarding the origin of the DNA. Consequently, the results are not informative in the context of the activities given the knowledge that we have”.*

10) If activity level propositions are considered, then the source of data should be explicit. Where there is uncertainty in the value of a parameter, a sensitivity analysis may be carried out to show the effect upon the LR (ISFG DNA Commission II, supplement) [13].

An example of propositions at activity level is as follows:

i. The appellant drove the car at the time of the incident.
ii. An unknown person drove the car and the appellant was a passenger in the back seat.

Here there is no mention of 'transfer' in the propositions, but data are needed to inform the relevant probabilities. To avoid bias, the expert should ideally set the propositions, based on the case information, not the results. For a simple example to show how calculations are made refer to ENFSI Evaluative reporting guideline and the supplement of ISFG DNA commission II [13,15].

13. PRESENTATION OF RESULTS

The following principles apply for providing testimony in court (also see Appendix)

1) The expert shall not give opinions on matters that were not addressed in their report(s). There may be cases where matters are raised in cross examination, which are developed as a result of issues that have occurred during the trial, and which may need to be considered by the expert. If an opinion is given or interpretations made fall outside the scope of the accreditation, or is outside the scope of his/her expertise, then this is stated as such by the scientist. Where there has been good case management, it is rare to give an opinion that is not available in the report [50].

   Results shall be presented in a way that is comprehensible to the persons involved in the criminal justice system and be scientifically valid, robust and presented in a transparent way.

2) The value of the findings shall be provided in the form of a likelihood ratio, where the findings are considered given two alternative propositions that represent the positions of the prosecution and the defense as known.

3) In court, the scientist does not evaluate propositions, rather he/she evaluates the results if the propositions are true.

4) The likelihood ratio may be accompanied with a verbal equivalent expression the value of the findings [15]. However, the verbal scale shall not be used without an accompanying order of magnitude of the LR value (ISFG DNA commission part II section 10) [13]. Verbal equivalents are necessarily subjective and different verbal scales have been proposed. It is above all a matter of convention.

5) The expert shall explain the limitation of the DNA evidence reported given sub-source level propositions. When the source of the DNA is not disputed, the value of the DNA comparison given sub-source level propositions has no impact upon the value of the evidence given activity level propositions. The expert shall be pro-active to explain the dangers of carry-over of the LR value to a higher level of the hierarchy of propositions, [(ISFG commission part II, recommendation 2) [13].

6) From section 4.1 of the ISFG DNA commission [13], statements like:

   “Secondary transfer was an unlikely explanation for the presence of the appellant's DNA on the door handle”

   are not acceptable because this amounts to giving an opinion on the propositions and may lead the court to believe that based only on the DNA, one can infer that that it is very probable that the appellant touched the door handle (which is the prosecutor's fallacy, aka a transposed conditional).

   ISFG DNA commission II Recommendation 3 [13], states:

   “Scientists must not give their opinion on what is the ‘most likely way of transfer’ (direct or indirect), as this would amount to giving an opinion on the activities and result in a prosecutor’s fallacy (i.e., give the probability that X is true). The scientists' role is to assess the value of the results if each proposition is true in accordance with the framework (the probability of the results if X is true and if Y is true).”

7) If answering questions on DNA transfer in the given case, it follows that the expert shall be transparent regarding how his/her opinion was made. In general, for equality of arms, this opinion will be also available in a written report based on data and where the scientist explains the value of the findings in the context of the alleged activities. The assumptions made and the limitations associated with the opinion (experiments used, opinions made in the absence of a report will be disclosed).

8) LRs given activity level propositions are typically many orders of magnitude lower than those calculated given sub-source level propositions. It is useful to demonstrate this even if there are limited data available.

9) During court cross examination, questions may arise that were not considered in the original statement, and new information is required to help the court. The scientist must state the limitations of the current report; then he/she can suggest that in order to answer the court query, further work is necessary which may be outlined. The court may then issue an adjournment to enable the work to be carried out. An example modified from a draft OSAC (2022-S-0024 Best Practice Recommendations Draft) is provided below, showing how the scientist can handle such a situation: [51].

   "What is relevant is whether the observed DNA profile is more likely if an object was handled by the person of interest or if he did not handle it but had contact with an unknown person who did. However, to help with this question, I would need to run or to refer to experiments to determine how often and under what circumstances DNA is detected when a person does not handle the object themselves."

10) The scientist shall avoid the prosecutor's fallacy: e.g. "The probability *that* the DNA came from Mr. X is one in a billion." (ISFG DNA comm part I, section 7) [49]. One shall indicate instead that the probability of the findings if the DNA came from a person unrelated to Mr. X is one in a billion. Beginning one's sentence by "The DNA results are…" is helpful to avoid this fallacy [52].

11) During court proceedings when the expert is questioned, he/she will need to be vigilant to ensure that the prosecutor's fallacy is not inadvertently committed by lawyers and judges, correcting mistakes if they arise.

12) Caution is required when using the word "match" in statements because it might imply "identity". The expert avoids any verbal statement that might imply that he/she is making an opinion on the identity of the questioned DNA (otherwise the prosecutor's fallacy may be committed).

14. HEALTH AND SAFETY

14.1 Overview of Requirements

The Occupational Health and Safety Policy and related procedures of the Organization/Institute (where the forensic facility is part of a bigger organisation) based on national legislation should be followed and should include plans/instructions/guidelines/equipment and training for health and safety in relation to the following potential work-related hazards: chemical, biological, electrical, radiation, physical and hazards (rarely occurring but likely in certain geographical areas or regional/local conditions) that may occur during working hours which are not related to the working environment (e.g., natural disasters [earthquake, adverse weather conditions affecting the work environment and or infrastructure] terrorist attack, explosion etc.). In the latter situations, evacuation plans including search and location of all team members in time to evacuate the danger zones shall be in place as applicable. Formulation of a business continuity plan is also recommended to allow the continuation of service provision under the spectrum of potential hazards which can impact the laboratory.

In the context of a pandemic such as COVID-19, strict adherence to precautions provided by the Ministry of Health/WHO, is required by the forensic unit to minimize infection of its staff.

All accidents/incidents shall be reported in order to identify the root cause and avoid reoccurrence where possible through further training or improvement of safety procedures.

Field specific safety precautions outlined below shall also be taken where applicable.

14.1.1 Personal Protective Equipment (PPE)

Required PPE (*i.e equipment designed and manufactured to be worn or held by a person for protection against one or more risks to that person's health or safety*) should be available, used, controlled and disposed of in the appropriate manner in accordance with the nature and level of exposure to hazardous substances during examination.

14.1.2 General Work Place Hygiene

The working environment shall be maintained clean, well ventilated and proper waste management shall be followed in accordance with the nature of the waste.

14.1.3 Chemical Hazards

14.1.3.1 Safety Requirements for Chemicals used in the Forensic Facility

Required knowledge of the dangers of chemicals used in analytical procedures and appropriate training in their safe handling, including appropriate ventilation and disposal of residual chemicals shall be acquired and implemented at all times.

14.1.3.2 Safety Requirements for Handling Items Containing Potentially Hazardous Chemicals

Appropriate safety precautions including adequate ventilation shall also be used when examining and sampling narcotics, petroleum and other items harbouring dangerous chemicals delivered to the forensic unit for examination in order to avoid exposure.

14.1.4 Biological Hazards

Required knowledge and training of the potential biohazards of crime scene samples as well as reference samples and avoidance of infection shall be acquired and implemented at all times during sampling and handling procedures. Appropriate ventilated hoods shall be used for item examinations where necessary.

14.1.5 Physical Hazards

Required knowledge of the potential physical hazards associated with the examination and sampling of dangerous items (fire arms, explosive devices, knives, tools, needles, syringes, etc.) shall be acquired and implemented in order to avoid injury.

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16. AMENDMENTS TO PREVIOUS VERSION

Not Applicable (First Version).

APPENDICES

A1. APPENDIX TO CHAPTER 12: ASSESSMENT OF RESULTS AND INTERPRETATION

A1.1 Overview

In this chapter, we present the principles for evaluative reporting, the characterization of the nature of body fluids and limitations (investigative reporting). We then discuss the importance of task relevant case information, the concept of propositions and show how it can be structured in the form of a hierarchy. We discuss the different levels and explain when it is meaningful to consider the value of biological results considering propositions at a given level. We conclude the chapter with a section of pre-assessment, which is particularly important when transfer, persistence and recovery of DNA need to be considered in the context of the case and further discuss how to assess biological results.

At the time of writing this best practice manual, for various practical reasons, we note that it may not be common practice to undertake evaluation of forensic results given activity level propositions. However, this is an issue where the courts of law are regularly requesting assistance from the forensic DNA expert. There have been numerous studies related to the methodology and guidelines, hence it is both imperative and prudent to outline best practice in this context. The absence of evaluation of forensic results given activity level propositions does not remove the responsibility of the scientist to ensure that the limitations of reporting findings given sub-source propositions are made explicit.

The ultimate decision on how to contextualise reports remains as applicable in each organization/jurisdiction. The state-of-the art methods referred to in this manual are to assist practitioners who report the findings given activity level propositions; for those who do not report at this level, guidance is provided on the limitations of reporting given sub-source propositions so that the dangers of carry-over of LRs given activity level propositions are made explicit.

A1.2 Principles of Interpretation for Evaluative Reporting

Results shall be assessed and presented with balance, integrity, transparency, logic and impartiality. The scientist shall only assess, report and give opinions in areas where she/he has been proven to be competent by the laboratory approved training and competency tests. For the purpose of evaluative reporting, one can apply the following principles.
The evaluation of the findings is made in the light of case information (just as the examination strategy is).

- At least two mutually exclusive propositions shall be considered. Propositions cannot be exhaustive in general, but practice can proceed with an acceptable coverage, that is with the omission of propositions which are not pertinent to the case.
- The scientist shall give an opinion on the probability of the findings, not on the probability of the propositions[2].

[2] For practical reasons, for investigative purposes, it is generally accepted that forensic scientists report 'exclusions', when they observe from the EPG that there is an incompatibility between the DNA profile of a stain and a POI. Exclusions are rarely presented in court in evaluative reports, unless it is pertinent to the defence case. On the other hand, when there is a compatibility between profiles, an assessment of the value of the DNA comparison is necessary to convey the meaning of the results. In the case where exclusions are reported, it is good policy to caution the reader about not over-interpreting this result and specify the assumptions made (e.g., In our opinion, provided there was no error in the process, the POI is excluded as a possible source of the DNA. Note that the inference that the DNA of this person is absent from the stain may need to be assessed in the context of the case by considering factors such

- The value of the findings is determined by the ratio of the two probabilities (i.e., LR): (1) the probability of the findings given the case information and the first proposition and (2) the probability of the findings given the case information and the alternative proposition. Generally, one value (i.e., LR) will be assigned for each person of interest.

A1.3 Tests used for Investigating the Nature of Body Fluids

The results of tests used for investigating the nature of body fluids shall be assessed by considering the possibility of false positives and false negatives. They shall not be presented as factual results, nor be presented as a 'confirmatory test'. For example, where spermatozoa are observed by microscopy, a report that sperm are present would be an opinion, but not a fact. Criteria for reporting opinions should be detailed in laboratory SOPs. If there is a given person of interest and the issue is the nature of the trace (which body material?), one should be aware that the material might be present for legitimate reasons (as background or prevalent DNA). In such cases, activity level propositions may be helpful.

One should note that it is extremely difficult to associate a DNA profile to a given body fluid. An example of an exception would be when multiple spermatozoa have been observed by microscopy and where a single DNA male profile has been obtained in the male so-called spermatic fraction. In other situations, one must be extremely cautious for example, if there is a mixture of two individuals and a positive blood test is obtained, it does not mean that the DNA has come from blood of both individuals [55].

A1.4 Importance of Task Relevant Case Information

When available (for example through discussion with investigators or mandating authorities), task-relevant information should be taken into account. Information is useful for two tasks: first the case circumstances will allow to identify the issue (and thus the level of hierarchy) with which forensic biology/DNA profiling can contribute. Secondly, the circumstances of the case will condition the scientist's judgement of the probability of the findings. If for example, the question lies on how the DNA was transferred or how long it can persist, then information about times and actions will inform the scientist's judgements. Another example would be case information pertaining to the physical description of the offender if the issue relates to the donor of the DNA. Indeed, this information will inform a decision about which population survey(s) to use. There are other aspects of the framework that are not task relevant and could potentially bias the scientist. An example would be to be told that the suspect was recognised by a witness. Such information is not needed and should therefore not be requested by the scientists. Laboratories are encouraged to introduce a formal system for case information management, for instance through separation of tasks and sequential unmasking of task-relevant information.

In some cases, especially in the investigation phase when the issue is the source of DNA, there might be little information. In order to proceed, the scientist can make assumptions based on what is known (e.g., where the incident took place) being clear that little information was provided to formulate propositions. A caveat will in this case indicate that if the information changes, a new evaluation will be needed. Also see sub-chapter A2.2 (5).

A1.5 The Hierarchy of Propositions

The value of the findings is assessed given propositions where the DNA scientist can add value by using expert knowledge that is necessary to understand the value of the findings that is otherwise unavailable. To do so, the concept of the hierarchy of propositions described by

as persistence of DNA. This separate evaluation can be done on demand by the laboratory when considering the alleged activities.). If using a probabilistic genotyping software and/or if this result is to be presented in court, it is recommended to report the LR, even if it is smaller than 1.

Cook and others provides a useful framework [48]. One can refer to the “ENFSI guideline for Evaluative Reporting” and the ISFG recommendations for further details [13,15,49]. The hierarchy of propositions is widely used to help scientists and the court understand the meaning and limitations of the findings within the context of a case. Each level of the hierarchy is associated with a particular issue that allows to define the mutually exclusive propositions that are to be considered to evaluate the results. The higher the level in the hierarchy, the more value can be added, and the more knowledge and information is needed.

The propositions in this hierarchy will reflect the positions (as understood by the scientist) of the two parties, for example prosecution and defence respectively. One basic criterion for proposition formulation is: “*that they should be formulated in such a way that it is reasonable for the scientist to address a question of the form – ‘what is the probability of the observations given this proposition and the framework of circumstances’?”* [13]. Another criterion is that propositions should be formulated at the appropriate level of the hierarchy, according to guidelines. Propositions should also be distinguished from explanations that do not have the aforementioned properties. To prevent bias, propositions should ideally be formulated before the comparisons. This ensures propositions (including the number(s) of contributors) are not based upon the results of the comparison (i.e., the number of contributors/NOC is based upon the stain-profile, before comparison with persons of interest). For more information on formulation of propositions we refer to relevant publications [13,48,49,54–57].

There are 3 main levels in the hierarchy of propositions: source, activity and offence. The issues associated with these three levels are as follows: (1) whether or not a given person is the source of the material, (2) whether a given person has done one activity or another and (3) whether a person has committed an offence or not. It must be remembered that DNA scientists do not give an opinion on these propositions. They assess their results given these propositions. It is important to emphasize that each of the levels of hierarchy is different so that the LR calculation given propositions at one level shall not be carried over to the next level; this would be misleading. Therefore, strictly adhering to the hierarchy of propositions is an important foundation to prevent miscarriages of justice occurring.

Examples of the hierarchy of propositions are given in table 2 below. As mentioned, to rise in the hierarchy of propositions the scientists need to add value, considering different results and factors in their evaluation. Here, we give no example of offence level propositions. This is because it is rare to add value when considering results given offence level propositions (e.g., where findings from different forensic disciplines are combined in the case where multiple activities took place), rather than activity level propositions. The ultimate issue of guilt/innocence is not the province of the scientist, but nor are the activities, and nor is the source of the DNA. Indeed, it is not the province of the scientist to express an opinion on any proposition (whatever the level) but they shall only assign the probability of their findings given the propositions within the framework of the case circumstances.

Table 2: Examples of mutually exclusive propositions at a different level in the hierarchy of propositions

Level	Question	Results/*Factors*	Example of pairs of propositions
Sub-sub-Source	Is Mr S the source of part of the DNA mixture?	DNA profiling comparison	Mr S is the major contributor of the DNA mixture. An unknown unrelated person is the major contributor of the DNA mixture.
Sub-source	Is Mr S the source of the DNA?		Mr S is the source of the DNA. An unknown unrelated person is the source of the DNA.
Source	Is Mr S the source of the body-fluid?		Mr S is the source of the semen. An unknown unrelated person is the source of the semen.
Activity	Did Mr S perform the activity?	Presence/absence of DNA Quantity/quality of the DNA (DNA profiling comparison) * Presumptive tests Multiple traces from same activity Transfer, persistence, prevalence background, contamination. * If the source of the DNA is contested.	Mr S and Ms C had penile-vaginal intercourse. Mr S and Ms C only had social activities as described in the case information.
			Mr S forced the door with his screwdriver. An unknown person forced the door with Mr S's stolen screwdriver.
			Case relevant information to consider: e.g., time frame, POI has washed/not washed, alleged activities with the object.

In the statement the case information that is relevant would be described as well, as propositions and case information are entwined. See task-relevant information (paragraph A1.4).

A1.6 Evaluation of DNA Profile Comparisons when the Issue is who is the Donor of a Biological Fluid/tissue (Source Level Propositions)

Source level propositions are considered when the issue regards who (which individual) is the source of a given biological material, for example blood, semen or saliva, teeth, bone etc. If source level propositions are considered, the DNA scientist assumes the nature of the body fluid as the propositions are: e.g., Mr S or an unknown is the source of the semen. This assumption can be justified if the presumptive test is positive and there is no issue about the nature of the body fluid as determined by its extrinsic characteristics and generates a single source profile.

With mixtures it cannot be assumed that the presence of a given cell type (e.g., blood or saliva) is associated with all contributors to the crime-stain (maybe one or more contributors have deposited skin-cells). In addition, it cannot be assumed that all individuals contributed DNA at the same time – i.e., some or all of the contributors may have had nothing to do with the alleged activities. These types of issues are dealt with under the context of activity level propositions.

A1.7 Evaluation of DNA Profiling Results: Comparisons when the Issue is Who is the DNA Donor? (Sub-Source Level Propositions)

One can routinely produce a DNA profile from very small quantities of biological material. If the nature of the material is unknown, one will speak of sub-source propositions. These address the question of who is the donor of the DNA? (The nature of the material e.g., skin cells, saliva, blood, is unknown).

A1.8 Evaluation of DNA Profiling Results: Comparisons when the Issue is who is the Major or Minor Contributor to the DNA Profile? (Sub-Sub-Source Level Propositions)

If one is concerned with the question of who is the donor of a portion of the DNA mixture? (i.e., a major or minor contribution), then one will refer to sub-sub-source propositions [52,58–60]. If it is important that the person of interest is compatible with the major component, then this might be an indication that the issue lies in the activities. If the relative quantity is not an important factor, then sub-source propositions are generally more meaningful. Indeed, DNA scientists are in general able to provide more value when considering propositions that are at a level higher than sub-sub-source as it allows to assess all the results and not part of the results.

Sub-sub-source level propositions are not meaningful if any of the following circumstances apply:

a) If both minor and major components have been compared to the POI.

b) The components cannot be clearly classified into major/minor.

c) The probabilistic genotyping method takes into account peak height, or assigns different rates of drop-out to different contributors.

Sub-source propositions are more meaningful when the issue is whether or not a POI is the source of the DNA, irrespective of the proportion of DNA contributors. For probabilistic genotyping systems take account of peak height and automatically return LRs given sub-sub-source propositions. However, it is easy to convert this LR given sub-sub-source propositions to a LR considering sub-source propositions. This is done by applying a conversion factor, dependent upon the number of contributors, as described by Taylor and others [60]. For simplicity one can “divide the *LR* for an *N* person profile by *N*!”, e.g. divide the LR given sub-sub-source propositions by 6 for a three-person mixture (3x2x1 equals 3 factorial). The difference is small especially if the LR is large – although consideration is certainly needed when LRs are in the thousands.

A1.9 Evaluation of presence/absence of DNA (or of relative quantities) when the Issue is the activities that led to the DNA deposition? (Activity Level Propositions)

If the DNA profile is produced from a very small quantity of biological material, there may be questions as to whether the DNA resulted from direct or indirect transfer. The use of these terms is problematic because it involves two aspects: the DNA transfer (which is an aspect where the DNA scientist may have knowledge on) and the activities (which is a matter for the court to decide and where the scientist does not have knowledge). Therefore, it is advised that activity level propositions are formulated without the terms transfer and relate to a true activity (transfer is not an activity, driving a car or pulling down knickers is). Activity level propositions are meaningful when there are possible legitimate reasons for the presence of DNA from the POI (e.g., if the persons know each other or if the person has been on the premises for legitimate reasons) or when one can help assessing the absence of DNA or by considering the possibility of contamination.

A1.10 Pre-assessment

Pre-assessment can be used (as a means) to specify the main potential findings from examinations of the items submitted and then to assign their probabilities considering each proposition.

Based on this analysis and the available case information, a strategy shall be decided/agreed by the scientist in consultation with the mandating authorities. If the issue solely relates to whether the person is the source of the DNA, usually the examination (DNA analysis) is carried out without pre-assessment. If transfer, persistence or the nature of the body fluid has an impact in the case, then it is advised to carry out pre-assessment. (e.g., consideration should be given to the sampling strategy including substrate controls where body fluid attribution is relevant).

The pre-assessment stage [63] is particularly important to avoid post hoc rationalisation (i.e., bias) [64] when the scientist determines the expectations of the results if a particular proposition is true. At this stage, the scientist will also decide what type of results to assess (e.g., presence/absence of DNA; major DNA profile), and whether there are sufficient data and case information. An example of pre-assessment where the suspect is accused of sexual assault by digital penetration is given below:

Table 3: Case example of pre-assessment where DNA is recovered from the fingers of the accused

Outcomes (E)	**Pr(E|Hp,I) if digital penetration**	**Pr(E|Hd,I) if social activities**	**Likelihood ratio Pr(E|Hp,I)/ Pr(E|Hd,I)**
Large quantity & full female profile	0.82	0.16	≈ 5
Small quantity & partial female profile	0.09	0.16	≈1
No female profile	0.09	0.68	≈1/7
Total	1	1	

Once the probability of the possible results has been assigned (e.g., recovering a large quantity & full female profile, a small quantity & partial female profile, no female profile) and that pre-assessment has shown that it is useful to proceed, the scientist can carry out the examination. If there are insufficient data, the results will be reported as uninformative.

A1.11 Main Steps for Interpretation of DNA Profiles

To evaluate DNA profiles, the framework outlined in the DNA commission documentation [49,63] shall be used. The main steps are summarised below.

1) To assess the value of a DNA profile, the first aspect to consider is whether the profile has sufficient information (from the laboratory's guidelines) for comparative purposes. It may be possible to condition the DNA profile on known individuals – for example the person from whom the swab was taken. In that case one will consider the presence of the DNA of this person in both alternative propositions (e.g., The DNA mixture is from Mr C and Mr S or from Mr C and an unknown). Using all the information available enhances selectivity and sensitivity. Mixed DNA profiles are often encountered; validated probabilistic genotyping software tools should be used to evaluate such results. (see section A1.11).
2) In cases where there is no suspect available, a national or international DNA database search may result in the nomination of one or more potential candidates for the DNA profile. This information is regarded as investigative, i.e., it provides leads to direct the investigation. The DNA results that support a proposition that the POI is a donor need to be investigated by the relevant authority with respect to other DNA and non-DNA information in the case.
3) Providers of DNA database reports shall be aware of the possibility of adventitious matches. When reporting a database match between a scene-related DNA profile with a person, apart from indicating the value of the DNA comparison, a caveat should be included, indicating the possibility of an adventitious match and that the information obtained should be considered together with other case related information. Further guidance is available in ISFG and ENFSI documents [13,15].
4) If following the POI's interview, the issue changes from source to activity, then additional task relevant case information is required. If the person has legitimate access or has carried out activities that could explain the presence of his/her DNA, activity level propositions will need to be considered.

Reports shall mention that if case information changes, this impacts propositions and the value of the results (LR) will change. The scientist should be informed, preferably before court appearance as it takes time, effort and access to expert software to carry out evaluations.

A1.12 Use of Software to Evaluate DNA Results Given (Sub) Sub-source Level Propositions

The sensitivity and discrimination power of STR typing systems facilitate the detection and analysis of complex and low-level DNA mixtures. Interpretation of mixtures or low template DNA shall be carried out using developmentally validated and in-house verified probabilistic genotyping software to assess the value of the comparisons. A likelihood ratio (LR) that takes into consideration the probability of dropout and/or peak heights given the number(s) of known (e.g., person of interest) and unknown contributors, and allelic relative frequencies or proportions from the relevant population (e.g., populations from the STRiDER database) will be produced.

The limitations of the data and/or methodologies used to assign LRs should be known and taken into consideration when using the data, and also need to be communicated to the relevant authorities in the written report or as an accompanying technical note.

A1.13 Evaluation of Biological Traces Considering Activity Level Propositions

When the issue is when or how the DNA was deposited, activity level propositions are meaningful. They shall be used to assess the significance of the combined laboratory results (extrinsic characteristics of the trace, results of tests for biological body fluids and cell types, DNA profiling, quantification results). They also allow to account for factors such as transfer, persistence, recovery, consideration of background and prevalent DNA.

Once the DNA comparison has been evaluated given sub-source level propositions, under the assumption that it is agreed that the POI is a donor, it may not be disputed that the DNA is from the POI. If case pre-assessment has shown that it is useful to proceed, then the scientist can carry out the evaluation considering activity level propositions. Typically, the outcome of a DNA case can be subdivided into three possibilities listed in Table 3. The transfer, recovery and persistence probabilities are assigned by results of experimentation and a Bayesian Network or formulae can be used to carry out the calculations (an example is given in the ISFG document) [13]. The advantage of the Bayesian network is that all possible outcomes can be assigned without prior knowledge of the results – i.e., all that is needed is an understanding of the case circumstances and probabilities to inform the model. Note that where there is an absence of DNA that is “compatible” with the POI it will in general support the defence proposition (the LR<1) – therefore the absence of evidence is not necessarily neutral.

A1.13.1 Formulation of Propositions

Propositions need to be formulated in a meaningful way; for example, it is important to avoid use of the word ‘transfer’ in propositions [13]. This is because propositions are assessed by the court, but DNA transfer is a factor that scientists need to consider for the interpretation of their results. It is meaningful to assign the probability of DNA being transferred if an activity took place (e.g., the POI drove the car). However, it is not meaningful to assign the probability of DNA being transferred if transfer took place. This example shows why it is important to distinguish propositions from results or from factors that are accounted for by the scientist’s interpretation. This also applies to contamination: if the word ‘contamination’ is in the propositions, then the scientist cannot take this into account, as they do not assess propositions.

A1.13.2 Considerations when Helping to Address Activity Level Propositions

Important considerations when helping to address activity level propositions are listed below: (see also section A2.2 below).

1) A LR assigned for a DNA profile comparison considering sub-source propositions cannot be carried over to higher levels in the hierarchy of propositions, (i.e., the calculations given sub-source, source and activity level propositions are all separate and indeed, differ with respect to scale by orders of magnitude). Carry-over of the LR would be misleading and may culminate in a miscarriage of justice. In situations where a likelihood ratio cannot be determined because of technical reasons, limitations should be clearly stated. At the time of writing, evaluation given activity level propositions are not often mandated to the laboratory and DNA results are usually reported given sub-source level propositions; it shall be outlined that the probability of the DNA results given sub-source propositions do not help to address the question relating to how the DNA was deposited. However, the scientist can help address the question of activity using experimental data, either generated for this purpose or taken from published literature.
2) To assess factors such as transfer, persistence, prevalence, background and contamination that may have to be accounted for in an evaluation, both case information and specialized knowledge are needed.

3) In relation to evaluation given activity level propositions, the expert shall convey the limits and relevance of experimental data, if available, derived from simulated transfer, persistence and recovery experiments, either from peer-reviewed publications or from unpublished experiments used to simulate the circumstances of a particular case. The data shall be disclosed for purposes of transparency. The scientist should only give an opinion if there is relevant information and data. If there are no data then the scientist shall state the limitations of the findings and indicate that the DNA results do not help to discriminate the activities.
4) Sufficient background information related to the case may not be available to allow an evaluation of the results given activity level propositions. The problem with not considering the activity level is that the court is only provided with information regarding the source of the DNA, but this does not assist the deliberations regarding the value of the results in the context of the alleged activities. In this situation, the scientist must state the limitations of the findings and indicate that the DNA results do not help to discriminate the activities.

A1.14 Examples for Statements

A1.14.1 Tests Used for Investigating the Nature of Body Fluids/Tissue

If there is a given person of interest and the issue is the nature of the trace (which body fluid/cell type), one should be aware that the material might be present as background. In such cases, activity level propositions may help the court [13].

An investigative report could read:

“In my opinion (based on obtained test results [list test results], sperm is present on the item analysed. This sperm could arise from two different ways:

a) From the disputed activity
b) It may be present due to reasons unrelated to the activity (e.g., as background)”.

It is more complicated for other body fluids, since we have false positives to deal with.

The report could read:

“I have carried out a test that indicates the possible presence of body fluid X. This test is not confirmatory.” Reference should be given regarding the probability of false positives if available. “*In addition, we cannot conclude that the body fluid has come from a given individual (even if DNA compatible with this person is detected). Body fluids may be found in the environment as background from unknown sources or may be directly/indirectly transferred from the POI. In this case, activity level propositions allow the court to be helped in a more meaningful way”* [64].

A1.14.2 Multiple Persons of Interest

Multiple POIs:

An example of propositions that are exhaustive where the DNA mixture would be compared to POI 1 and POI 2 is given below.

Evaluation of the DNA comparison for POI 1:

- POI 1 and POI 2 are the source of the DNA mixture or POI 1 and an unknown person are the source of the DNA mixture
- POI 2 and an unknown person are the source of the DNA mixture or two unknown persons are the source of the DNA mixture

Evaluation of the DNA comparison for POI 2:

- POI 2 and POI 1 are the source of the DNA mixture or POI 2 and an unknown person are the source of the DNA mixture
- POI 1 and an unknown person are the source of the DNA mixture or two unknown persons are the source of the DNA mixture

In practice, one can assess the value of the DNA comparison with exhaustive formulae as previously described [60,61,67].

The approach is particularly valuable when the two persons do not explain the mixture, but each has a LR larger than one such that the results support the proposition that they are contributors to the mixture. Close relatives will also be more easily discriminated when using this approach [59,66].

A2. APPENDIX TO CHAPTER 13: PRESENTATION OF RESULTS

A2.1 Overview

Findings can be presented to the court in writing and/or verbally as per the national legal system. Presentation of opinions shall clearly state the results of the evaluation and interpretation of the examination. The expert should only give an opinion if there is relevant case information and data for assessing the findings. The same quality standards should apply for findings presented orally or in writing. The expert shall be trained and know how to apply the principles of interpretation [13,49,61].

Written reports should include the available task-relevant information and shall fulfil the requirements according to ISO 17025, (clause: 7.8) [10] in a concise and unambiguous manner as required by the existing legal system. For personal security of the reporting scientists, special measures acknowledged by the courts may be taken by the forensic unit such as the use of a pseudonym in the reports if allowed by the national legal system.

Written reports shall be peer reviewed and confirmed according to laboratory SOPs (sub-chapter 5.2).

When requested, the expert provides an explanation of the laboratory methods, data analysis and interpretation methods to the court in a comprehensive manner. Within this context, the quality control and quality assurance steps applied can be addressed to provide the required confidence pertinent to the validity of the results (and their evaluation) generated by the laboratory represented by the expert.

The difference between (sub)-source and activity level propositions shall be explained. Two alternative propositions representing the two parties' (e.g., prosecution and defence) views of events, based upon the case-circumstances shall be clearly stated. If it is not possible, and the source of the DNA is not disputed, then no value can be attributed to the results and they shall be considered as uninformative [67] (see Appendix 1).

In relation to reporting, the expert shall convey the limits of their interpretation. For example, for assessment given activity level propositions, one will comment on the relevance of experimental data derived from simulated transfer experiments, either from peer-reviewed publications or from unpublished experiments used to simulate the circumstances of a particular case[3] [15]. The implications of not being able to carry out the experiments due to lack of resources should be conveyed to the investigator who may be able to assist the laboratory by providing financial support/resources/time allowance.

For evaluation given sub-source level propositions, one will indicate that if the case information changes, a new evaluation will be needed and that this evaluation provides no information on how or when the DNA was deposited. One should also outline that a likelihood ratio indicates the extent to which DNA analysis results support one proposition over another. It is not possible, on this basis alone, to determine which is the most likely proposition. To assign this probability, the DNA analysis results should be combined with other information in the case. This is not considered to be the domain of the forensic DNA expert.

Conclusions made will require supporting valid, peer reviewed literature relevant to methodologies, principles and/or concepts and in accordance with the ENFSI guideline [15].

Experts remain within the limits of their assignment and shall resist responding to questions that take them outside their field of expertise. In particular, they shall not comment on whether

[3] There are often unique circumstances that the peer review literature does not cover – under these circumstances the lab can carry out experiments to address such situations. The experimental design and data shall be disclosed to the court, See A2.2 (8) below.

such or such scenario is likely or not, as this would amount to transposing the conditional (i.e., give an opinion on what happened). Recommendations are given below on what DNA scientists cannot say in court (i.e., that direct transfer is more likely) and what gives little assistance to the court (i.e. providing a list of explanations as opposed to giving the value of the results).

A2.2 Principles

The following principles apply for providing testimony in court:

1) The expert shall not give opinions on matters that were not addressed in their report(s). There may be cases where matters are raised in cross examination, which are developed as a result of issues that have occurred during the trial, and which may need to be considered by the expert. If an opinion is given or interpretations made fall outside the scope of the accreditation, or is outside the scope of his/her expertise, then this is stated as such by the scientist. Where there has been good case management, it is rare to give an opinion that is not available in the report [50].
2) Results shall be presented in a way that is comprehensible to the persons involved in the criminal justice system and be scientifically valid, robust and presented in a transparent way.
3) The value of the evidence shall be provided in the form of a likelihood ratio where the findings are considered given two alternative propositions that represent the positions of the prosecution and the defense as known.
4) As far as possible, the case information (on which the propositions are based) shall be disclosed. If there is no information available from the defence prior to the court-proceedings, then when appropriate the expert needs to formulate the alternative proposition based upon reasonable assumptions.
5) The assumptions and the propositions, where applicable, shall be clearly stated and a caveat applied that informs the court that should new information become available that could affect the validity of the propositions, then a new evaluation may be required.
6) In court, the scientist does not evaluate propositions, rather he/she evaluates the results if the propositions are true.
7) The likelihood ratio may be accompanied with a verbal equivalent expression for the value of the findings [15]. However, the verbal scale shall not be used without an accompanying order of magnitude of the LR value (ISFG DNA commission part II section 10) [13]. Verbal equivalents are necessarily subjective and different verbal scales have been proposed. It is above all a matter of convention.
8) The expert shall explain the limitation of the DNA evidence reported given sub-source level propositions. When the source of the DNA is not disputed, the value of the DNA comparison given sub-source level propositions has no impact upon the value of the evidence given activity level propositions. The expert shall be pro-active to explain the dangers of carry-over of the LR value to a higher level of the hierarchy of propositions, (ISFG commission part II, recommendation 2) [13].
9) If activity level propositions are not considered, then the expert should define the limitations of source (if applicable) and sub-source propositions in the form of a caveat in the statement e.g., "*the case has been reported given source/sub-source level propositions, which means that this report does not provide any information on the mechanisms or actions that led to the deposition of the biological material concerned. It only provides help regarding the origin of the DNA. Consequently, the results are not informative in the context of the activities given the knowledge that we have"*.

10) From section 4.1 of the ISFG DNA commission [13], statements like:

“Secondary transfer was an unlikely explanation for the presence of the appellant's DNA on the door handle”

are not acceptable because this amounts to giving an opinion on the propositions and may lead the court to believe that based only on the DNA, one can infer that that it is very probable that the appellant touched the door handle (which is the prosecutor's fallacy, aka a transposed conditional).

ISFG DNA commission II Recommendation 3 [13], states:

“Scientists must not give their opinion on what is the ‘most likely way of transfer’ (direct or indirect), as this would amount to giving an opinion on the activities and result in a prosecutor’s fallacy (i.e. give the probability that X is true). The scientists' role is to assess the value of the results if each proposition is true in accordance with the framework (the probability of the results if X is true and if Y is true).”

Avoid using the term 'transfer' in propositions [13].

11) It follows that the expert shall be transparent regarding how his/her opinion was made. This opinion will be based on data and the value of the evidence assigned considering the activities. The assumptions made and the limitations associated with such experiments will be disclosed. Where there is uncertainty in the value of a parameter, a sensitivity analysis may be carried out to show the effect upon the LR (ISFG DNA Commission II, supplement) [13]. LRs given activity level propositions are typically many orders of magnitude lower than those calculated given sub-source level propositions. It is useful to demonstrate this even if there are limited data available.

12) An expert report shall be structured as per the current ISO 17025 quality assurance standard requirements and include the following:
    a) A preamble to describe the purpose of the examinations carried out within the framework of circumstances.
    b) If there is uncertainty regarding the source of the DNA, alternative propositions are stated at sub-source level e.g.,
        i. The DNA came from Mr X and two unknown persons unrelated to him
        ii. The DNA came from three unknown persons unrelated to Mr. X.
    c) The value of the evidence is described e.g.,
        i. The DNA profiling results are of the order of one billion times more likely if the first proposition (i) is true than if the alternative (ii) is true.

    A verbal equivalent can be used in addition (but not as a substitute): e.g., “I have assigned a LR of the order of one million. Thus, according to our internal verbal scale, this analysis provides extremely strong support for the proposition that Mr. X is a contributor to the DNA obtained from Item I rather than not”. Some laboratories will add a caveat indicating that the laboratory does not provide any assessment on how likely it is that the first proposition or the alternative proposition is true. Indeed, this probability (e.g. The blood came from Mr A), is the domain of the court, as one needs to combine all the information of the case in order to make such a statement. And a caveat on activities: This report does not provide any information on the mechanisms or actions that led to the deposition of the biological material concerned. It only provides help regarding its source. Should an issue arise at any time regarding the activities that led to the deposition of this DNA, an expert might be consulted to re-assess the findings in light of the new information.

    If a laboratory does not report given activity level propositions then the report should make clear that the opinion only provides information regarding the source of the DNA

[49]. A statement of limitation is required (as described in the previous paragraph) to make it clear that the scientist is unable to help the court further.

An example of propositions at activity level (e.g., where data of secondary DNA transfer is important as described in 11 above) is as follows:

i. The appellant drove the car at the time of the incident.
ii. An unknown person drove the car and the appellant was a passenger in the back seat.

Here there is no mention of 'transfer' in the propositions, but data are needed to inform the relevant probabilities. To avoid bias, the expert should ideally set the propositions, based on the case information, not the results. For a simple example to show how calculations are made refer to ENFSI Evaluative reporting guideline and the supplement of ISFG DNA commission II [13,15].

During court cross examination, questions may arise that were not considered in the original statement, and new information is required to help the court. The scientist must state the limitations of the current report; then he/she can suggest that in order to answer the court query, further work is necessary which may be outlined. The court may then issue an adjournment to enable the work to be carried out. An example modified from a draft OSAC (2022-S-0024 Best Practice Recommendations Draft) [51] is provided below, showing how the scientist can handle such a situation:

"What is relevant is whether the observed DNA profile is more likely if an object was handled by the person of interest or if he did not handle it but had contact with an unknown person who did. However, to help with this question, I'm certain that if we were able to run some trials, we could determine how often and under what circumstances DNA is detected from a secondary transfer event."

13) Avoid the prosecutor's fallacy: e.g. "The probability *that* the DNA came from Mr. X is one in a billion." (ISFG DNA comm part I, section 7) [49]. One shall indicate instead that the probability of the findings if the DNA came from a person unrelated to Mr. X is one in a billion.

14) LRs are usually rounded down to one significant figure, when the LRs are smaller than one, the propositions are inverted to give a LR larger than one, as smaller numbers are difficult to grasp. One should ensure that there is no transposed conditional. Beginning one's sentence by "The DNA results are…" is helpful to avoid this fallacy [52].

15) During court proceedings when the expert is questioned, he/she will need to be vigilant to ensure that the prosecutor's fallacy is not inadvertently committed by lawyers and judges, correcting mistakes if they arise.

16) Avoid making propositions such as "The matching DNA came from Mr. X". The results (i.e., the "match") shall not be interwoven with propositions. Such propositions can be formulated only after the analysis of data. Propositions should be formulated before the data analysis has been carried out [49]. Caution is required when using the word "match" in statements because it might imply "identity". The expert avoids any verbal statement that might imply that he/she is making an opinion on the identity of the questioned DNA (otherwise the prosecutor's fallacy may be committed).

17) It may be necessary to carry out more than one LR calculation using different pairs of propositions if there is uncertainty in the case circumstances. for example glove 'wearing' / 'not-wearing' [13].

A2.3 Possible Way of Reporting the Value of a Test Used to Investigate the Nature of Biological Fluids

For investigative purposes the information regarding the nature of the biological material is obtained by considering the probability of observations (e.g., results of indicative tests, quantification, DNA analysis, mRNA results, and, if applicable, colour of the sample) given the proposition that the item contains the biological fluid of interest and the probability of these same observations given the alternative proposition that the item does not contain this fluid. **The ratio of these probabilities is called a likelihood ratio**. The LR can then be assigned using a Bayesian network (a probabilistic graphical model) taking into account all observations and the possibility of false negatives and false positives [68]. The probability that the item contains the given fluid before making our observations (i.e., the so-called prior probability which is based on extrinsic characteristics of the trace) can be combined with the likelihood ratio in order to determine the probability that the sample contains the biological fluid of interest after our observations (i.e., the so-called posterior probability).
Regarding the nature of the biological material on the analysed sample, if we assigned a likelihood ratio of the order of 10 (for example), this means that it is of the order of 10 times more probable to make our observations if the item contains blood than if it does not. If we assume a prior probability of 50% that there was blood, then there is an equivalent probability of 50% that there was no blood. This gives a posterior probability that the item contains blood of the order of 90% (and therefore of the order of 10% that it does not contain blood).

Caveat presumptive test

Our conclusions regarding the nature of the biological material analysed are meant for investigative purposes. A new interpretation will be necessary if case information indicates that the prior probability that we have considered is not appropriate and/or if the interest were to focus on the activities alleged by the parties (i.e., how/when the material got there). In this situation, phenomena such as transfer, persistence as well as the presence of background shall be considered. In addition, the presumptive test cannot be used to assign the presence of a body fluid to a given contributor of a mixture. Unless, of course state-of-the-art methodology, internally validated is applied to address this issue.

A2.4 Example of Reporting when There are Multiple Persons of Interest

The DNA mixture from the item is in our opinion from 3 persons. The DNA profiles of person A and person B are compatible with this DNA profile for all 16 loci available. To determine the value of these compatibilities, we have considered the probability of the results given the proposition that Person A contributed to the mixture, with or without Person B, and the probability of the results given the alternative proposition that unknown persons contributed to the mixture, with or without the person B. We proceeded in the same way for the person B.

The ratio of these probabilities is called the likelihood ratio. In order to determine the latter, we have used the software ZZZ and the genetic characteristics of the population XXX (Publication/s), as well as an Fst correction of 1% to take into account the population sub-structure.

For person A, we assigned a likelihood ratio of the order of one billion. This means that it is of the order of a billion times more probable to observe the results if person A contributed to the DNA mixture derived from item YYY than not.
For person B, we assigned a likelihood ratio of the order of one million. This means that it is of the order of a million times more probable to observe the analytical results if person B contributed to the DNA mixture highlighted derived from item YYY than not.

To assign the probability, for example, that a person is the source of all or part of the DNA derived from an item, the DNA results must be combined with the other information of the case. This is generally not considered to be the domain of the forensic DNA expert.