Forensic Science Regulator
Overseeing Quality

Forensic Science Regulator Guidance

DNA Mixture Interpretation

FSR-G-222

Issue 3

Contents

1. Introduction

1.1 Background

1.1.1 The interpretation of mixed DNA results has been the subject of much interest and there are several rulings on admissibility and subjective opinions by the Court of Appeal in England and Wales. Most recently, this was the subject of an appeal in the cases of Dlugosz, Pickering and MDS [1], all of which involved results that were too complex or of insufficient quality for the calculation methods available at the time. The Appeal Court ruling allowed the use of subjective expert opinions in such cases.

1.1.2 The introduction of DNA multiplexes that are more sensitive, such as the DNA - 17 systems, has led to an increase in the number of complex mixed results being obtained from questioned samples. There have also been major advances in the development and implementation of software packages for the statistical evaluation of DNA mixtures. However, interpretation is not simply a matter of feeding data into a black box and recording the number that emerges: the role of the DNA specialist scientist is as important as it always was and the final assessment depends critically on scientific judgement.

1.1.3 The interpretation of DNA profiles of questioned origin depends on the propositions that are addressed from both the prosecution and defence perspectives. The purpose of this document is to provide guidance to forensic units (FUs) for this critical stage of the process with the objective of fostering consistent standards between practitioners and organisations. The guidelines are listed in Annex 1.

1.2 Approaches for Mixture Interpretation

1.2.1 Once a DNA sample has been analysed, the output takes the form of a computer record of the position and height of the fragments of DNA that have been detected. The record is in the form of an electropherogram (EPG) together with a data table that provides information about the allelic designation, size and abundance of fragments together with designations for some types of artefact.

1.2.2 For the purposes of this document and these guidelines, 'interpretation' is taken to cover the part of the process from the EPG to the calculation of the likelihood ratio (LR).

1.3 Publications on DNA Mixture Interpretation

1.3.1 The general principles for the interpretation of mixtures have developed from results obtained using single locus probes to the current short tandem repeat (STR) tests [2,3,4]. Initially these considered only the presence of alleles, but as more information was recorded about the heights and areas, the relative amounts were also considered.

1.3.2 Guidelines for the evaluation of autosomal DNA results have been produced by the Scientific Working Group on DNA Analysis Methods (SWGDAM) and the International Society of Forensic Genetics (ISFG) [5,6,7]. The current versions of both SWGDAM and ISFG provide guidance on complex mixtures.

1.3.3 The guidance in this document is based on published work. There has also been widespread consultation with all of the major providers of forensic science services in the UK and Ireland via the DNA Analysis Specialist Group of the Forensic Science Regulator (FSR). Broader public consultation has also been undertaken by the FSR.

1.4 Guidelines Within the Forensic Science Regulator's Codes

1.4.1 Existing FSR Codes of Practice and Conduct (the Codes) relevant to this topic are:

a. FSR-C-108 DNA Analysis;
b. FSR-G-202 The interpretation of DNA evidence (including low-template DNA);
c. FSR-G-213 Allele frequency databases and reporting guidance for the DNA (Short Tandem Repeat) profiling;
d. FSR-G-217 Cognitive Bias Effects Relevant to Forensic Science Examinations.
e. FSR-G-223 Software Validation for DNA Mixture Interpretation

1.4.2 FSR-C-108 provides an explanation of how the Codes are applied to the detection, recovery, analysis and use of DNA evidence. It provides a brief list of

factors that should be considered when using either qualitative or probabilistic methods including:

a. allelic drop-in and drop-out;
b. gross contamination;
c. the use of threshold values such as for heterozygote balance;
d. stutter;
e. other artefacts;
f. mixtures of two or more individuals;
g. methodology for reporting single test results or replicate analyses as a single assessment of evidential weight.

1.4.3 FSR-G-202 considers the principles applied to the interpretation of complex DNA profiles including those associated with low level target DNA. This expands on the outline given in FSR-C-108 and includes an assessment of allelic drop-out and drop-in, use of replicates and consensus interpretation methodology. However, the thrust of this document is intended to highlight the basic principles to adopt rather than stipulating explicit principles.

1.4.4 FSR-G-213 considers the suitable allele frequency population databases that should be used for interpreting DNA profiles for the UK, along with advice and guidance as to the approach for reporting match probabilities and likelihood ratios for profiles derived for the DNA-17 system.

1.4.5 FSR-G-217 is intended to assist readers in identifying cognitive bias and therefore help to prevent bias effects from occurring. DNA mixture interpretation is one of a number of processes covered in the document, which describes:

a. the various means by which bias can potentially be introduced in mixture interpretation; and
b. the means by which it can be managed, for example, by undertaking checking via repeat interpretation by an experienced and competent colleague prior to the reference result being known.

1.4.6 It also discusses whether the interpretation can be reliably conducted where there is no suitable option for quantitative evaluation.

1.4.7 FSR-G-223 provides guidance to assist organisations in the validation of autosomal DNA mixture interpretation software and Y- STR profiling software applications.

1.5 Standards for Mixture Interpretation

1.5.1 National and international standards [8,9] for testing and calibration in laboratories provide guidance on analytical methods. However, there is much less detail for the type of interpretation of analytical results required for DNA analysis.

2. Purpose And Scope

2.1.1 The purpose of this document is to provide guidance for forensic scientists (instructed by prosecution or defence) in the interpretation of autosomal DNA profiles in cases where a comparison is to be made between the DNA profiles of one or more known individuals and a mixed DNA profile from a sample of questioned origin.

2.1.2 The scope of this document is to provide guidance on the following.

a. Guidance on the structure and interpretation sections in statements about DNA mixtures.
b. Guidance on how the propositions for mixed results should be clearly specified in the statement. This relates to DNA level (sub-source) propositions only.
c. Agreed nomenclature to describe the features of DNA profile results, and explanation(s) regarding the interpretation of a mixture, for example, clear terminology regarding the interpretation of a mixture where it has been considered reasonable to condition on the presence of DNA from one or more persons. This is distinct from standard abbreviations and a glossary of terms; these are phrases included in reports intended to describe results but where scientists may intend to impart a range of meanings to the reader.
d. Guidance on suitable checks for the transfer of results to ensure that the DNA data provided have no transposition errors and that the FU's

guidelines for interpreting the presence of peaks, designation as allele, stutter, over-stutter or artefact are robust and transparent.

e. Guidance on the acceptable boundaries of interpretation in the context of DNA mixtures.

f. Guidance for DNA expert witnesses on the use and limitations of a qualitative opinion where no quantitative likelihood ratio (LR) has been calculated. This can also be used to inform lawyers and judges.

g. Provide advice on the requirements for:

   i. producing specially constructed DNA mixtures under controlled conditions that would be needed to support FUs wishing to provide qualitative evaluation (QE); and

   ii. by defining the standards against which scientists' evaluations are made.

3. Implementation

3.1.1 This guidance is available for incorporation into a forensic unit's quality management system from the date of publication. The Regulator requires that the Codes [10] are included in the forensic units schedule of accreditation by October 2017 and the requirements in this guidance are implemented by October 2018.

3.1.2 The guidance does not apply to cases where there was a Criminal Justice Service (CJS) disposal of any kind prior to October 2018, for example, no further action, caution, guilty plea or concluded trial.

3.1.3 With respect to any case where a qualitative evaluation of the strength of evidence was performed prior to October 2018 but where there was no CJS disposal prior to that date the recommendations are as follows:

a. If the source of the DNA is accepted by all parties as not at issue, there is no need for any further action;

b. If the source of the DNA (as opposed to the manner of its deposition) is a contested issue, then where possible, a statistical evaluation of the strength of evidence should be performed.

3.1.4 Should the CJS process be restarted in any particular case (e.g. by an appeal) then the advice of Crown Prosecution Service (CPS) should be sought about the nature of the appeal and any further work required.

4. Modification

4.1.1 This is the third issue of this document.

4.1.2 Significant changes to the text have been highlighted in grey.

4.1.3 The modifications made to create Issue 3 of this document were, in part, to ensure compliance with The Public Sector Bodies (Websites and Mobile Applications) (No. 2) Accessibility Regulations 2018. There is an updated copyright statement, some reformatting, and provision of text alternatives where information has been presented in a non-text format. Any references that have necessarily changed with the passage of time have been refreshed. The content of the document is otherwise unchanged save for in Section 4.

4.1.4 The Regulator uses an identification system for all documents. In the normal sequence of documents this identifier is of the form 'FSR-#-###' where (a) the '#' indicates a letter to describe the type or document and (b) '###' indicates a numerical, or alphanumerical, code to identify the document. For example, the Codes are FSR-C-100. Combined with the issue number this ensures each document is uniquely identified.

4.1.5 In some cases, it may be necessary to publish a modified version of a document (e.g. a version in a different language). In such cases the modified version will have an additional letter at the end of the unique identifier. The identifier thus becoming FSR-#-####.

4.1.6 In all cases the normal document, bearing the identifier FSR-#-###, is to be taken as the definitive version of the document. In the event of any discrepancy between the normal version and a modified version the text of the normal version shall prevail.

5. Terms And Definitions

5.1.1 Although this document has been written principally with practitioners in mind, it is possible that it may be referred to in court. The main technical terms and phrases used are listed in section 19, the Glossary.

6. Mixture Interpretation Guidelines

6.1 Introduction

6.1.1 This document considers the situation where DNA has been extracted and profiled from a biological sample of questioned origin. If the sample has been recovered from the scene of a crime then it will often be referred to as a crime sample, but there will be instances where this terminology may not be appropriate, for example, where the sample of questioned origin has been recovered from a victim or suspect. To avoid such issues, these will be termed questioned samples. From this sample, one or more DNA profiles will be generated: these are the questioned profile(s).

6.1.2 There will be profiles from one or more samples of undisputed origin. In general, these will be known as reference profiles and they fall into two classes.

a. In the simplest case, there will be a reference profile from a person considered to be a person of interest (POI) and the issue of whether or not the POI has contributed to the questioned profile is in dispute.
b. There may also be reference profiles from one or more persons who, given the circumstances of the crime, may reasonably be expected by both the prosecution and defence to have contributed to the questioned profile. These are referred to as conditioning profiles (genotypes).

6.1.3 Situations where there are two or more POIs are discussed in section 6.8.15.

6.2 Data and Observations

Structure of a profile

6.2.1 A DNA profile consists of a set of data that is generated by an appropriate biochemical process. It is viewed most simply as a set of tables, one for each locus. Each row of the table describes the properties of a peak above some pre-

set threshold and will include data for: peak height, molecular weight, and allele designation, where this has been possible and, potentially, other properties depending on the software. There will also be a graphical representation of the data and it is customary to refer to this as 'the profile'.

Observations

6.2.2 The questioned and reference profiles will be designated, usually by the profiling system (this guidance does not consider this process), and reviewed by the scientist. It is at this stage that the scientist will decide on which of the peaks should be considered for numerical calculations: certain peaks, for example, may be excluded from further analysis because the scientist considers them to be an artefact. The abstracted data that the scientist passes for calculation is termed the 'observations' to emphasise that the final assignment of evidential weight depends crucially on the scientist's judgement with regard to the data that are to be included in the calculation. The judgement is necessarily subjective but will be within a documented set of guidelines.

Genotypes

6.2.3 The pair of alleles at a given locus is known as the genotype for that locus and, combining across all loci yields the genotype for the particular profiling system that is in use. The profile from the reference sample will usually be of high quality so that it is possible to designate alleles for each locus unambiguously. With questioned samples it is often the case that it is not possible to infer a single genotype unambiguously and this is particularly the case with mixtures. If, for example, at a given locus alleles 10, 11, 12, 13 are designated then there are various combinations of genotypes from two persons that, in principle, could yield this collection of alleles, for example, {10, 11/12, 13}; {10, 12/11, 13}. Consideration of the heights of the peaks will enable different weights to be assigned to different genotype combinations and this process will, in general, be undertaken by means of software: a process known as deconvolution.

Informativeness

6.2.4 The first decision for the scientist is to decide on is whether the information content of the profile(s) from the questioned sample is appropriate for a

meaningful interpretation to be carried out. At one extreme, it might be that the profile contains too much information; for example, so many peaks that there is considerable uncertainty with regard to the number of contributors to be assigned. At the other extreme the profile has only a few peaks, all of which are around the level of a predetermined guidance threshold. The criteria for making a decision at this stage will be determined to some extent by the capabilities and limitations of the software.

6.2.5 Taylor [11] has considered the informativeness of DNA profiles in some detail and has shown how informativeness is a function of:

a. the number of contributors;
b. the amount of imbalance in the mixture; and
c. the overall quality and quantity of DNA.

6.2.6 At present informativeness can only be judged by subjective evaluation. However, some software packages enable deconvolution of a questioned profile in the absence of known contributors and this process can offer a guide to informativeness.

6.2.7 If informativeness is considered poor, practitioners will always consider the merits of re-testing the sample, given sufficient DNA, associated areas of the sample or other samples, as a potential means for improving the overall information content of the observations.

Replicates

6.2.8 If the questioned sample is of adequate quantity, two or more sub-samples may be prepared from it. Then, in a search for adequate informativeness, multiple analyses can be performed, and replicate profiles created.

6.2.9 There are various methods for combining the data from replicate profiles [12]. The principles for formulating propositions are the same whatever the number of replicates but the degree of variation between replicates can itself be informative. For this reason, it is preferable that as much of the data contained in the replicates as possible be employed in the subsequent calculations.

6.3 The Logical Approach

6.3.1 This section is concerned with evaluating the weight of evidence in a case where a DNA profile from a sample of questioned origin is a mixture of the profiles of two or more people. The increase in the sensitivity of DNA profiling techniques has led to an increase in the number of cases where the questioned sample proves to be a mixture. The logical approach to assigning weight of evidence in such cases is well established [13,14]. It is epitomised in three principles, which may be summarised as the framework of circumstances that occurs within the statement of the principles:

a. the need to address two propositions; and
b. the need to assign the probability of the observations, given each of the two propositions.
c. The ratio of the two probabilities is known as the likelihood ratio (LR) and is a measure of the extent to which the observations support one of the two propositions.

6.3.2 The approach is described in the following sections within the context of DNA mixtures.

6.4 Framework of Circumstances

6.4.1 For a meaningful evaluation, it is necessary that the scientist be provided with background information that explains all of the circumstances that are relevant to the interpretation of the profiles. This is called ‘the framework of circumstances’ and its provisional nature is recognised in that it might later be changed by new evidence.

6.5 Propositions

6.5.1 It is essential for any approach that it be balanced and to this end the scientist addresses two propositions. In complex cases there may be more than two propositions but under no circumstances should the scientist address only one proposition. These propositions should be as simple and concise as possible and must be clearly stated. In a criminal trial, one of them will represent what the scientist understands to represent the position that the prosecution will take at court and the other will represent that of the defence. Both propositions are provisional and are subject to change under direction of the respective

advocates and that of the court. The weight of evidence assigned by the scientist depends critically on the propositions and it is the scientist's responsibility to make all parties aware of this.

Hierarchy of propositions

6.5.2 In this document only propositions that relate to the origin of questioned material are considered. Propositions are always considered in pairs, representing the respective positions (or hypotheses) that the prosecution and defence may be expected to take in relation to this issue. It is conventional to denote these as H_p and H_d respectively. The following is a simple example.

H_p: The DNA in the questioned sample is that of Mr X.

H_d: The DNA in the questioned sample is from some unknown person, unrelated to Mr X.

6.5.3 In all fields of forensic science there is a basic criterion for any proposition. A proposition 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'?

6.5.4 The following example is a pair of propositions that would not satisfy this criterion.

H_p: DNA from the POI is present in the questioned sample.

H_d: DNA from the POI is not present in the questioned sample.

6.5.5 Because the propositions do not postulate a number of contributors to the sample and the nature of other unknown contributors (related or unrelated to the POI, for example) is not specified, there is no reasonable basis for assigning a probability to the observations.

6.5.6 The first pair of propositions above are at the lowest level of what is known as a hierarchy of propositions. If the nature of the material (such as blood, semen or hair) from which the profile has been generated is undisputed these are known as 'source level' propositions. For example, "the semen came from Mr X". In many cases the nature of the material cannot be established with certainty and

then the propositions are known as ‘sub source level’ or ‘DNA level’. The above pair are DNA level propositions.

6.5.7 The next level above source level is known as ‘activity level’, described in the following example.

H_p: Mr X fondled Miss Y.

H_d: Mr X had only social contact with Miss Y.

6.5.8 Such propositions may involve issues relating to the origin of DNA that might have been recovered from either Mr X or Miss Y (or from both) but also issues relating to how DNA might have been transferred and/or persisted during such activities. There is a higher level in the hierarchy, which is known as the ‘offence level’, consider the following example.

H_p: Mr X indecently assaulted Miss Y.

H_d: Mr X had only social contact with Miss Y.

6.5.9 Here again, the issues to be addressed involve considerably more than those of the source of the DNA. This guide relates only to DNA level (sub-source) propositions.

Mutually exclusive propositions

6.5.10 The logic of the approach requires that the two propositions must be mutually exclusive – that is, if one is true it necessarily follows that the other must be false. However, for forensic evaluation it is not necessary that they be exhaustive. That is, they do not need to cover all possibilities; it is sufficient that they represent the two competing positions of the prosecution and defence within an accepted framework of circumstances.

6.6 Likelihood Ratio

6.6.1 Once a pair of propositions is formulated, the scientist will consider the extent to which the information contained in the profiles supports one or other of them. This is done by calculating the LR, which is an indicator of evidential weight. The logic of forensic inference and the calculation of LRs are not covered in this document. There are a number of standard texts that include treatments of this subject [14,15,16,17,18]. An initiative by the Royal Statistical Society has

resulted in a series of freely available publications relating to evidence interpretation, including one specially devoted to DNA evidence [13].

6.7 Software

6.7.1 Recent advances in theory and software development have led to the availability of several software packages for the quantitative assessment of evidential weight in DNA mixtures cases. LRs for DNA results produced using some software have been presented in evidence in England and Wales, Scotland, NI and Ireland [19,20,21,22]. This guidance does not consider or comment on the relative merits of the various products; guidance for validation is discussed in FSR-G-223: Software validation for DNA mixture interpretation. This discussion assumes that the scientist has access to fully validated software and is competent to use it; it considers the issues leading to the calculation and the reporting of the outcome of the analysis.

6.8 Forming Propositions

Number of contributors

6.8.1 Statistical methods for assigning weight of evidence in mixtures cases have, prior to 2017, often required that the number of contributors be specified by the user. Increasingly, however, the theory and software are being extended to allow for uncertainty in the number of contributors [23,24,25].

6.8.2 For the present it is assumed that the number of contributors can be assigned unambiguously. In 0 possible approaches are discussed for cases where there is uncertainty with regard to the number of contributors.

6.8.3 As a general rule it would seem preferable to assign a minimum value to the number of contributors to a questioned sample without considering the reference profiles. However, this might be unnecessary and unrealistic in some cases (if the questioned profile has come from a vaginal swab in a rape case, for example, it would seem unreasonable to ignore the complainant's profile).

Guideline 1: The scientist should attempt to assign a value to the number of contributors to the questioned sample. The reasoning to support this should be recorded on the case file.

Conditioning profiles

6.8.4 It is often the case that the presence of the DNA of a given individual in a mixture may be expected to be undisputed between the prosecution and defence. The genotype of such an individual is known as a conditioning genotype. Conditioning on one or more undisputed genotypes can greatly reduce the complexity of subsequent calculations and consequently improve the informativeness of the outcome.

Guideline 2: The scientist should consider whether it is reasonable to use any of the known genotypes from given individuals for conditioning one or more of the propositions. The reasoning to support this should be recorded on the case file.

6.8.5 There are a number of interacting considerations. There should be a strong evidential basis for inferring that the given person's DNA would be observed and such presence would appear not to be disputed by the prosecution and defence. The framework of circumstances may specify a reason why the DNA of that person might be observed. In the examination of an extract from a vaginal swab taken from the complainant in a rape case, for example, the circumstances provide strong prior justification for conditioning on the complainant's genotype. The most important consideration is that the observations themselves should support the presence of the given genotype.

6.8.6 There will be cases where the conditions are not satisfied but conditioning can be adopted via a stepwise approach. Examples are considered in sections 6.8.15 and 6.8.11.

Straightforward exclusions

6.8.7 Having decided on the number of contributors and on any conditioning profiles the next step to consider is straightforward exclusion.

Guideline 3: The scientist will consider the genotype of the person(s) of interest to provide a preliminary assessment of whether or not there is a straightforward exclusion, given the assigned number of contributors and the conditioning genotypes(s), and taking account of the quality of the questioned profile(s), particularly in relation to the potential for drop out and other artefacts.

6.8.8 In the case where a straightforward exclusion is indicated, the scientist should not increase the number of contributors solely to achieve a higher LR. A trivial example will illustrate this. Imagine a single locus profile where the genotype of the POI is AA. The crime profile shows alleles A and B with equal intensity. If single person propositions are considered, this would imply an exclusion. However, if two-person propositions are considered, without any supporting features in the profile, then a higher LR might be achieved. This could be inappropriately interpreted. The reality is, of course, much more complicated than this but the principle is the same.

6.8.9 If the outcome for a given POI is a straightforward exclusion then no further analysis will be required for that individual and reporting is straightforward. For any situation where an exclusion is not straightforward or cannot be made, consider proceeding with full mixture evaluation.

Prosecution proposition

6.8.10 A single POI is considered here. Multiple POIs are considered in section 6.8.16.

Guideline 4: On the basis of the framework of circumstances and the outcome of the previous steps, the scientist will formulate one or more propositions that could be anticipated as representing the prosecution position in proceedings against the person of interest. For each proposition the number of contributors and the postulated contributors should be made clear.

6.8.11 For example, in a case where it is alleged that Ms C has been raped by the POI and the result from the questioned sample is a mixture of at least three people, the prosecution proposition might take the following form.

H_p: The questioned sample is a mixture of the DNA of Ms C, the person of interest and an unknown person, all unrelated to each other.

6.8.12 The notation H_p, H_d is in widespread use in scientific papers to represent the prosecution and defence propositions respectively. This notation is used here but would not be used in statements written for court purposes.

6.8.13 ‘One or more propositions’ is used in the guideline to reflect the consideration that the number of contributors might be uncertain and also that there may be uncertainty about the inclusion of conditioning profiles.

Defence proposition

Guidelines 5: On the basis of the framework of circumstances and the outcome of the previous steps, the scientist will formulate, to correspond with each prosecution proposition, a defence proposition. As with the prosecution proposition, the number of contributors and the postulated contributors should be stated. The genetic relationship between any unknown contributor and the person of interest or other known persons in the case should also be made clear, either in the proposition or the accompanying text.

6.8.14 Continuing the example in the previous section, the defence proposition might take the following form.

H_d: The questioned sample is a mixture of the DNA of Ms C and two unknown persons, unrelated to the person of interest and Ms C and to each other.

6.8.15 At the time of the scientist's examination it is often the case that the defendant's position is not known, particularly when there has been a "no comment" interview. In such cases, this will be a 'proxy' proposition and its provisional nature should be made clear.

6.8.16 Note that there is no logical requirement for the number of contributors specified in the defence proposition to be the same as that in the prosecution proposition [7,26]. However, it should be noted that it is not normally in the interests of the defendant to increase the number of unknown individuals specified in the defence proposition above the minimum required to explain the questioned profile [27]. This issue is discussed in more detail in 6.10.1.

Multiple Persons of Interest

6.8.17 If a crime has been committed by two or more people, and if there are profiles from two or more suspects to be compared with the questioned sample then the basic 'two proposition' approach described in the previous section may be inadequate [27]. The following example will illustrate the issues that should govern the interpretation of such cases.

6.8.18 Assume that the questioned profile may be reasonably taken to be a mixture of two genotypes. There are two POIs and the questioned profile consists of peaks

that correspond to the alleles in the suspects' genotypes and no others. Then it is tempting to address propositions of the following kind.

H_p: The DNA is a mixture of persons of interest 1 and 2 (POI 1 and POI 2).

H_d: The DNA is a mixture of two unknown people, unrelated to POI 1 and POI 2

6.8.19 However, if the questioned profile is partial and unbalanced then it would seem wrong to assign the same evidential weight to both POIs, particularly if the genotype of one has alleles corresponding to large peaks, whereas the other has alleles that appear as peaks close to the analytical threshold. Furthermore, it should not be expected that both defendants would take the same positions: one POI, for example, may claim innocence and implicate the other. The root problem here is that of attempting to use a two proposition framework in a case where there are several potential defence propositions. There is no simple solution, other than to be aware of the range of alternatives that are possible. At the very least, the scientist could be expected to consider a calculation for each of the following two prosecution propositions.

H_p: The DNA is a mixture of person of interest 1 (POI 1) and an unknown person who is unrelated to POI 1.

H_p: The DNA is a mixture of person of interest 2 (POI 2) and an unknown person who is unrelated to POI 2.

6.8.20 Each would be considered with the same defence proposition as before.

H_d: The DNA is a mixture of two unknown people, unrelated to POI 1 and POI 2.

6.8.21 However, as is pointed out by Buckleton et al.[28], if both LRs support the prosecution propositions it is still conceivable that the first pair of propositions lead to a LR of less than one, so the calculation for that pair should be checked and reported.

6.8.22 In the event that one of the POIs later pleads guilty, the scientist may be invited to repeat the interpretation conditioning on the presence of that POI's genotype.

H_p: The DNA is a mixture of persons of interest 1 and 2 (POI 1 and POI 2).

H_d: The DNA is a mixture of persons of interest 2 and 2 (POI 2) and an unknown person unrelated to POI 1 and POI 2.

6.8.23 See FSR-G-217 for a more extended discussion of this kind of case.

6.8.24 For greater numbers of POIs, Buckleton et al [28] advise as follows;

> "If there are M persons of interest, to an N contributor profile, then there are many pairs of hypotheses that could be considered. For complex profiles with many persons of interest the number of proposition pairs could number in the hundreds or thousands. This is clearly too many for an exhaustive exploration of likelihood ratios. A strategy in current use and described above, tries each of the M persons of interest in H_1 with the remaining N-1 contributors as unknown. H_2 is set as N unknown contributors. Since there are M persons of interest, M LRs will be produced and plausibly reported. This is a search strategy that forms part of the investigative phase."

6.8.25 A suitably competent interpretation specialist (or however this role may be named) should be consulted for advice on the propositions chosen.

6.8.26 An interpretation for intelligence use can be provided via a streamlined forensic report (SFR) or initial report. It should be made clear when this may not be suitable for later evaluative interpretation. An SFR produced for intelligence use should not be on a form that is admissible in court; it should also be marked as 'not for evidence'.

6.8.27 Another complication arises if the POIs are closely related to each other. For example, if the prosecution position is that the questioned material is from two brothers.

Guideline 6: Where the circumstances include multiple persons of interest, a simple pair of propositions is not adequate. Several pairs of propositions may be necessary according to the framework of circumstances. If necessary consider providing an investigative rather than an evaluative report.

Association of the questioned sample with the crime is not certain

6.8.28 Thus far, the guide has assumed that the questioned sample is clearly associated either with the crime (such as a stain at the scene of the crime) or with a POI (such as a stain on the POI's clothing). There will be cases where this assumption does not apply. Such cases require careful consideration and it is not possible to give comprehensive guidance. The following example, however, illustrates a potential approach.

6.8.29 A complainant, C, alleges that she was held in premises against her will for several days by the POI, who denies all knowledge of the crime. A flannel is recovered from the bathroom of the premises and a DNA profile from an extract is considered to be a two-person mixture, which is entirely explained by a mixture of the DNA of C and the POI. The prosecution proposition is straightforward.

H_p: The questioned sample is a mixture of the DNA of C and the POI.

6.8.30 The defence could take several positions.

a. In particular:

i. the questioned sample is a mixture of two unknown persons;
ii. the questioned sample is a mixture of C and an unknown person;
iii. the questioned sample is a mixture of the POI and an unknown person.

b. In absence of guidance from the defence, there is scope for a two-stage approach.

6.8.31 First, consider propositions of the following kind.

H_p: The questioned sample is a mixture of C and an unknown person.

H_d: The questioned sample is a mixture of two unknown persons, unrelated to each other.

6.8.32 Subject to the outcome of this calculation, C could be treated as a conditioning profile for the second stage, where the propositions might be of the following form.

H_p: The questioned sample is a mixture of C and the person of interest.

H_d: The questioned sample is a mixture of C and an unknown person, unrelated to each other.

6.8.33 But all of this is, of course, subject to the direction of the court, if proceedings follow. It may be that the SFR or initial report with an investigative opinion is accepted by defence and no further evaluation is provided. It is therefore important that the SFR or initial report includes information on the limitations of the findings.

Guideline 7: In a case where the association between the questioned sample and the crime is uncertain consider a staged approach to forming propositions. Also consider providing an investigative opinion in an initial report, ensuring that the limitations are clearly stated.

Relationship between the person of interest and unknowns

6.8.34 It is now long established practice to treat unknown persons in propositions as though they were genetically unrelated to the POI (and, for that matter, any of the conditioning individuals) unless there was clear information in the circumstances to suggest otherwise. However, it should be recognised that this is only a provisional device and the sophisticated software packages that are now available provide the scientist with the power that is needed to consider propositions that invoke individuals who are related in some way to the POI. No further guidance is given here but scientists are encouraged to keep this issue in mind.

Guideline 8: Always consider a relative of the person of interest as an alternative source under the defence proposition if the framework of circumstances suggests this to be a relevant issue.

6.9 Options for the Case Where a Calculation is Not Possible

Uncertainty in the number of contributors to complex mixtures

6.9.1 Classical methods of mixtures interpretation have always required that the number of individuals be specified in the propositions. In the early days, software could cope with only two contributors but programs are now available that can handle mixtures with four, five and even more contributors. It is still the case that the interpretation is more straightforward if the number of contributors is specified but recent developments (Taylor et al.[29]) have established the mathematics for dealing with uncertainty about that number. Bright et al.[30] say:

> "The accurate assignment of the number of contributors in conjunction with the adoption of continuous models has become one of the most contentious issues in forensic DNA profile interpretation. The most difficult profiles to specify the number of contributors are those with peaks that may be either allelic, or artefactual, or both, and which are termed ambiguous in this paper. In our caseworking experience, trace DNA contributions, profiles with high stutter

above an assigned threshold and stutter in a forward (a+1) position introduce uncertainty and often result in the inflation of the assumed number of contributors to a profile."

6.9.2 There are several strategies for dealing with uncertainty in the number of contributors. The approach that the scientist will take will depend on the capabilities of the available software, but they include the following features:

a. sensitivity;
b. probability distribution;
c. optimisation; and
d. the major/minor approach.

Sensitivity

6.9.3 If there is uncertainty with regard to N, the number of contributors, one approach would be to explore sensitivity by considering pairs of propositions with differing numbers of N. Consider the following examples.

H_p: The questioned sample is a mixture of C, the person of interest and an unknown person.

H_d: The questioned sample is a mixture of C and two unknown persons, unrelated to each other.

Followed by:

H_p: The questioned sample is a mixture of C, the person on interest and two unknown persons, unrelated to each other.

H_d: The questioned sample is a mixture of C and three unknown persons, unrelated to each other.

6.9.4 The scientist will need to report both calculations and explain, taking into account all relevant circumstances and observations, which may be of greater use to the court, following the Criminal Procedure Rules Code of Guidance for Experts section 19.4 (f).

6.9.5 It should be noted that the computational load will be considerably higher in relation to the latter two.

Probability distribution

6.9.6 In principle, it is more rational to ask the scientist to assign probabilities to the range of numbers of contributors than to demand certainty for a single value. This could be regarded as the purist approach but it is not without its difficulties, not the least of which being the complexity of consequent calculations. Taylor et al. [24] have considered the mathematics of this in detail and have successfully created a practical software implementation. In essence, the LR is computed by averaging both numerator and denominator (separately) over the appropriate probability distribution for the number of contributors.

6.9.7 Once the facility to undertake this kind of analysis becomes routinely available, it will probably become the method of choice. However, this is not yet universally the case and the scientist has to consider the sensitivity approach. The reporting of such results is challenging, especially where there are no other suitable results in the case. The provision of several LRs relating to differing calculations for the same DNA result requires careful explanation to clarify the reasons for the variation for the CPS and the courts.

Guideline 9: If there is uncertainty with regard to the number of contributors to a questioned profile, and the background information does not assist in assigning relevant propositions, then calculations should be carried out for all combinations of propositions that appear reasonable in the circumstances of the case. The range and outcome of the calculations should be reported.

Optimisation

6.9.8 Another possible approach is to seek the values of N that maximise the numerator and denominator of the LR separately. Again, this is an approach that requires appropriate software.

Major/minor approach

6.9.9 An approach has widely been followed in the past in the type of case where a mixed profile can be clearly factored into major and minor components. If, for example, there is a set of prominent peaks that can unambiguously be assigned to a single contributor and that set of peaks is then found to be indistinguishable

from a POI then, following this approach, propositions of the following kind are addressed.

H_p: The person of interest is the major contributor to the mixture.

H_d: The major contributor is an unknown person, unrelated to the POI.

6.9.10 The computation is then as it would have been for a single profile. The ISFG document Gill, et al. (2018) [7] and this document do not differ in their aim on the topic of the major: minor approach, but the guidance in FSR-G-222 paragraphs 6.9.12 – 6.9.14 considers a wider range of less clear-cut situations.

6.9.11 There are a few remarks to be made about this.

6.9.12 This approach may be supportable for a mixture where there is a clear unambiguous single strong profile at every locus, assigned without reference to the profile of the POI. However, it is not possible to think of a prescriptive approach for the very wide range of situations that occur in casework. So even though a mixture may have stronger peaks, this is often not sufficient to justify the use of these simplifying strategies.

6.9.13 An important issue is that such propositions relate only to part of the mixture and Taylor et al [24] have called them “sub-sub-source” propositions. Their paper also explains the need to consider what is known as the “N! effect” (multiple trace problem), which may lead to a substantial reduction in the LR. So, if the propositions consider only the major donor, that is part but not all of the mixture, it is to be expected that the LR for this pair of propositions is greater, possibly substantially so, than if propositions relating to the entire mixture were addressed.

6.9.14 The ‘major/minor’ approach becomes increasingly difficult if there are loci where the disparity between the components becomes small. A bespoke analysis (calculating the LR for affected loci separately for propositions considering issues affecting deconvolution) may be feasible if this affects only one or two loci, but the approach cannot be supported at multiple loci. This bespoke analysis should provide reassurance that by setting that locus aside tends towards the conservative. Overall, there can be severe problems with this as a simplifying strategy.

Guideline 10: The major/minor approach to interpreting a profile is permissible if pursued with due regard for logic, taking into account all loci, and only where it is not based on the results of the comparison of the trace with the person whose DNA presence is contested.

6.10 Changing the Number of Contributors

6.10.1 It is worth noting that if the defence counsel suggests an increase in the number of contributors to a mixture then it is helpful if this is notified in advance of the trial. This is because the computational load will be high and will require time to complete calculating the updated LR. Also, whereas it is right that the defence proposition is 'under the control' of the defence and there is some justification in attempting to find a proposition that maximises the denominator, it may be argued that the prosecution proposition, by the same token, is under the control of the prosecution. As mentioned in section 6.8.12 there is no logical reason why the number of individuals cited in the denominator should be the same as that in the numerator [7,26] and, provided the software allows, the scientist may consider a pair of propositions of the following kind.

H_p: The questioned sample is a mixture of C, the person of interest and an unknown person.

H_d: The questioned sample is a mixture of C and three unknown persons, unrelated to each other.

6.10.2 In this context, Taylor et al.[24] say:

> "Proposing an unreasonable number of contributors under the defence hypothesis, H_d, and holding the number under the prosecution hypothesis at a reasonable assignment will increase the likelihood ratio (LR), favouring the prosecution hypothesis, H_p (Budowle et al.[31])."

6.10.3 Evett and Pope [27] have made a similar point and an earlier paper by Mortera and Lauritzen gives a formal proof [32].

6.10.4 Note that careful scrutiny is required when, for a non-zero LR, it is necessary for the prosecution proposition to cite a greater number of contributors than is necessary for the defence proposition. Consider the following example.

H_p: The questioned sample is a mixture of C, the person of interest and an unknown person.

H_d: The questioned sample is a mixture of C and an unknown person, unrelated to each.

6.10.5 It is nevertheless reasonable for the defence to challenge this.

6.10.6 Carrying out an evaluation without specifying the number of contributors has recently been considered in some detail by Taylor [11]. The paper includes citations to earlier work by other groups who have studied this rather difficult subject.

6.10.7 Bright, et al.[30] say:

> "When using the continuous method, the assumption of an incorrect number of contributors to a mixed DNA profile does not affect the weight of evidence assigned to a clear major contributor.
>
> The assumption of an increased number of contributors may significantly decrease the LR assigned to known minor and trace contributors to the mixture."

6.11 Acceptable Boundaries of Interpretation

6.11.1 The objective of any evidential evaluation of a comparison between reference samples and a mixed questioned profile should be a quantitative expression of weight of evidence in relation to a clearly stated pair of propositions. It follows that the boundaries of interpretation are set by the capabilities of the validated software that is available to the scientist.

6.11.2 The laboratory should have available or access to fully validated processes for interpreting DNA mixtures up to and including:

a. at least three donors;
b. alternatives of close relatives; and
c. stochastic effects from low template DNA (LTDNA) samples.

6.11.3 The software should not be used for calculations outside these validated processes. Assumptions, such as the inclusion of a conditioning donor or the exclusion of minor contributors, must be well founded and defensible within the circumstances of the case and the provenance of the sample. These assumptions should not be made with the main aim of simplifying a mixture to enable a calculation to be performed within the limitations of the software, but should consider what is relevant and provide justification for this opinion.

Guideline 11: A quantitative evaluation is possible provided that:

a. A clearly stated pair of propositions can be formulated;
b. All aspects of the observations (particularly artefacts) that are excluded from the evaluation are done so without uncertainty;
c. The calculation is within the validated capabilities of the software; and
d. The population databases [33] that are used can be shown to be relevant within the context of the case circumstances.

7. Guidance On The Use And Limitations Of A Qualitative Opinion When A Quantitative Likelihood Ratio Has Not Been Calculated

7.1 Unresolved Interpretive Issues: R. v. Dlugosz

7.1.1 From time to time the case will arise where, because of one or more unresolved interpretive issues, a quantitative analysis of the observations is not within the capabilities of the systems available to the scientist. In relation to such a case, the judgment in R. v. Dlugosz [1] (at paragraph 24) says:

> "(Nonetheless,) it does seem to us that provided it is made clear to the jury the very limited basis upon which an evaluation can be made without a statistical database, a jury can be assisted in its consideration of the evidence by an expression of an evaluative opinion by the experts. We consider that on the materials with which we have been provided, there may be a sufficiently reliable scientific basis on which an evaluative opinion can be expressed in cases, provided the expert has sufficient experience (which must be set out in full detail in the report) and the profile has sufficient features for such an opinion to be given. If the admissibility is challenged, the judge must, in the present state of this science, scrutinise the experience of the expert and the features of the profile so as to be satisfied as to the reliability of the basis on which the evaluative opinion is being given. If the judge is satisfied and the evidence is admissible, it must then be made very clear to the jury that the evaluation has no statistical basis. It must be emphasised that the opinion expressed is quite different to the usual DNA evidence based on statistical match probability. It must be spelt out that the evaluative opinion is no more than an opinion based upon [the expert's] experience which should then be explained. It must be stressed that, in contrast to the usual type of DNA evidence, it is only of more limited assistance."

7.1.2 The judgment clearly relates to cases where no formal calculation of evidential weight is feasible and it states (emphasis added):

> "We consider that … there may be a sufficiently reliable scientific basis on which an evaluative opinion can be expressed."

7.2 Counting Matching Alleles

7.2.1 There have been several cases (including R. v. Dlugosz, R. v. Thomas [34] and R. v. Walsh [35]) where an interpretation of a complex DNA mixture has been provided by referring to the number of alleles found in the questioned sample that match alleles in the profile of the defendant. Part of the justification for this appears to have rested on an internal report that was written within the Forensic Science Service (FSS) before its closure. This internal report was written to advise reporting officers against the practice of reporting the number of matching alleles for the purpose of evaluation. Some scientists also refer to unpublished work by Buckleton, Triggs and Gill presented at a conference. However according to these scientists, it appears that this was in fact a reference to the same FSS internal report, which was discussed in response to a question during a workshop.

7.2.2 The weight of evidence is assessed through the likelihood ratio (LR), which is the ratio of the probability of the observations given the prosecution proposition to the probability of the observations given the defence proposition. These two probabilities are, respectively, the numerator and the denominator of the LR. The number of alleles in a mixture that match alleles in the profile of a person of interest (POI) is, in a broad way, related to the magnitude of the denominator of the LR. The allele counting approach is presented to convey to the jury an impression of the smallness of the denominator. However, it is only one side of the picture and the reality is that the numerator of the LR in a mixtures case will also be a small number. The LR depends on both the numerator and denominator and giving the jury the impression that one is small without explaining that the other is also small is unbalanced and biased in favour of the prosecution. Evett and Pope 2014 [27] have explained why it is unsatisfactory to use the number of matching alleles as an indicator of evidential weight.

Guideline 12: The practice of using the number of matching alleles as an indicator of evidential weight in relation to DNA mixtures should be discontinued because it is potentially prejudicial.

7.3 Calibration of Expert Opinion Against Software

7.3.1 The notion that a forensic scientist may gain the necessary experience for reliable evidence evaluation from carrying out lots of casework is as old as forensic science. However, modern thinking has moved a long way from regarding casework as a source of reliable knowledge and embraces the notion of the testing of experts under controlled conditions [14,36,37,38,39,40]. This is known as calibration.

7.3.2 Following R. v. Dlugosz, one FU has offered a service whereby a scientist may offer an evaluation of a mixture without carrying out a software calculation. This was intended as an interim approach prior to the introduction of specialist statistical software accompanied by the training of appropriate reporting scientists in the use of the software. Such an evaluation has come to be known as a 'qualitative evaluation (QE)'. Such opinions may be framed in terms of an expression that indicates verbally the strength of support for the prosecution proposition (such as 'at least strong support'), following a verbal scale that is aligned with that for qualitative opinions in other areas of forensic science. The FU has carried out an extensive programme of calibrating scientists' opinions against comparisons that have been carried out using validated software. From this process a panel has been drawn up of scientists whose QEs have been shown to be reliable in relation to the calculated assessments. All such evaluations are internally peer-reviewed. Where possible statistical calculations are recommended, but unless authorised will not be undertaken.

7.4 Rapid Investigative Opinions

7.4.1 During an investigation there may be a substantial time delay before a statistical analysis can be carried out and it can be helpful for an investigator to be given a preliminary assessment of the scientist's expectation of the outcome. This may be by means of an email, an initial report or a streamlined forensic report, with suitable caveats to make the limitations of use apparent. Such an assessment may be given in informal language on the understanding that it does not satisfy the requirements of an opinion to be presented at court.

Guideline 13: In a case where a statistical analysis appears feasible, it is reasonable to present a qualitative evaluation as an interim measure, perhaps

in the context of an initial report. It is necessary to emphasise the provisional nature of the opinion.

Guideline 14: The reliability of qualitative evaluations from any forensic unit wishing to provide this service must be established by a continuous programme of calibrating the qualitative evaluations against statistical analysis using the resources currently available within the organisation.

Guideline 15: Qualitative evaluations should only be presented as investigative opinions for intelligence purposes, rather than as evaluative opinions.

7.5 Expert Opinions Outside the Capabilities of the Available Software

7.5.1 Although a scientist's opinions may have been shown to be reliable in cases where the calculation of evidential weight has been possible, it does not follow that such opinions are necessarily reliable in cases in which calculation has *not* been possible. There is a wide range of such cases, from those that are just outside the capabilities of the software to those where there are issues that are outside the bounds of reliable knowledge. For the former type of case, the reasonable course would be to consult a specialist who has particular knowledge of the available software with a view to carrying out a bespoke statistical analysis. With regard to the latter type, the Regulator's guidelines on cognitive bias (FSR-G-217) include a detailed discussion of the interpretive difficulties that may arise with complex mixtures and gives examples of how prosecution bias is a real danger. If it were required to establish the reliability and freedom from bias of the scientists' evaluations in such cases, it would be necessary to carry out a programme of testing scientists under blinded conditions on complex mixtures from two sets of ground truth cases:

a. one set where the prosecution proposition is known to be true; and
b. another set where the defence proposition is known to be true.

7.5.2 Given the range of such cases, the problems and scale of such an exercise would not be justifiable. Resources would be much better directed to implementing the best available software.

Guideline 16: The practice of offering a qualitative evaluation in a case where, because of unresolved interpretative issues, it has not been possible to carry out a quantitative evaluation by means of validated software, should not be continued.

7.6 Expressions of Possibility

7.6.1 The Dlugosz judgment [1] contains the following at paragraph 7:

> "There was no dispute in the first and third appeals that DNA evidence from a mixed profile could be used simply to establish that the defendant might have been a contributor or could not have been a contributor. It was accepted that it is often useful for a jury simply to know that fact without any further elaboration."

7.6.2 For the scientist to tell the court that the defendant "might have been a contributor" to the mixed profile is unbalanced in favour of the prosecution unless the scientist adds that the defendant "might not have been a contributor". Taken together the two statements are equivalent to saying "it is possible that Mr X contributed to the mixture and it is also possible that Mr X did not contribute to the mixture". This statement is, of course, uninformative and implies no evidential weight in support of either proposition [41,37,42]. The court has no more information than if the DNA profiling had not been carried out. The same remarks are applicable to other phrases that express possibility, such as 'could have' and 'consistent with'.

Guideline 17: Expressions of possibility should not be presented in a manner that favours the prosecution. If an assessment of evidential weight is not possible, the scientist should make it clear that he/she can give no guidance to the court with regard to probative value.

7.7 Bespoke Statistical Analyses

7.7.1 In the case where there are unresolved interpretive issues that preclude a routine statistical analysis, consideration should be given to consulting a specialist who has an advanced knowledge of the statistics of interpreting mixtures. The specialist should have a deep understanding of the mathematics underlying the statistical models in current use. There are various strategies, depending on the capabilities of the available software and, in every case, the objective should be a quantitative statistic accompanied with a detailed explanation of the reasoning and assumptions underlying the analysis.

Guideline 18: In the case where it has not been possible to carry out a calculation because there are interpretive issues that are outside of the resources available to the scientist, consideration should be given to consult a specialist who has extensive mathematical knowledge of the interpretation of mixtures. The objective will always be a quantitative assessment accompanied by a reasoned justification of the analysis.

8. Checks For Transfer Of Results

8.1.1 Suitable checks are required for the transfer of results to ensure that the DNA data provided have no transcription or transposition errors. Errors in profiles have occurred with both staff elimination database (SED) searches and loading of profiles to the National DNA Database® (NDNAD) because of mis-designations due to transcription errors. This type of error has been observed where the interpretation of a DNA result, usually a mixture, has involved either manually designating profiles or copying designations onto paper with the associated potential for:

a. transcribing the allele incorrectly; or
b. misreading the handwritten designation.

8.1.2 Procedures are in place to detect such errors, for example, the near match reports produced by the NDNAD to investigate close matches where only one allele is different between the profiles being compared. As the use of software to designate and transfer profile data automatically has grown, such errors have decreased. But the transcription errors apparent in a collaborative exercise show that where electronic transfer is not possible, steps need to be in place for effective quality checks.

8.1.3 One type of check is the simple witnessing of the data entry. This requires great concentration on the part of the witness and may not pick up all errors. Another type is for the checker to enter the data independently, and for suitable software to compare the two sets and identify any differences. This is more successful at identifying errors at the cost of greater input on the part of staff.

9. Guidelines For Transfer Of Results

Guideline 19: Forensic processes should, where possible, transfer information electronically to reduce the risk of transcription errors. If data are to be transferred manually then appropriate checks to safeguard the integrity and quality of the data should be clearly undertaken and recorded. The process of transfer and checking should be clearly described within the appropriate standard operating procedures, and regularly included in audit processes.

Guideline 20: Any data and interpretation compiled for any proficiency test should follow the same process, and meet the same requirements and standards, as the casework procedure.

10. Requirements For Forensic Units' Guidelines For Interpreting The Presence And Designation Of Peaks

10.1.1 The interpretation and designation of peaks within a DNA profile result is conducted within a framework of laboratory specific data. These determine values such as:

a. expected size of forward and over stutters;
b. locus specific incidence of stutters; and
c. range of heterozygous imbalance.

10.1.2 It is desirable that interpretation guidelines are written, based on the data, in such a way as to reduce any variation and subjectivity between practitioners, thereby ensuring consistency and reproducibility in outcomes. The compliance of practitioners in applying the guidelines should also be monitored within a system of audit and proficiency testing.

Guideline 21: Forensic units should ensure, through a system of audit and compliance checking, that laboratory specific interpretation guidelines are fit for purpose, drive consistency and reduce variation in interpretation outcomes.

Guideline 22: The data to support the interpretation guidelines should be referred to in statements as per the requirement of the Criminal Procedure Rules part 19.

10.1.3 In cases where the analysis and interpretation of DNA samples and results are split between different FUs or other scientists, agreements must be made on which set of guidelines to use for the interpretation of the results. This includes DNA results that:

a. are produced by one FU and transferred to another for interpretation and evaluation using software not available at the first;
b. were produced by the Forensic Science Service (FSS) and are now being re-examined as a result of reopening or reviewing a historic case;
c. are produced by a forensic laboratory from outside the UK and submitted to an FU for comparison with a reference sample;
d. are produced by an FU and supplied to an expert for review on behalf of the defence.

Guideline 23: When a DNA result is transferred to another organisation, the thresholds and interpretation guidelines used to interpret the DNA result should be those of the forensic unit that produced the DNA result at the time of the original analysis.

11. Statement Writing – Interpretation And Conclusion Wording

11.1.1 In many instances the initial output from a forensic DNA examination is an initial report or a streamlined forensic report. This may be one of:

a. a DNA match report, produced without any input from a forensic scientist;
b. a results report that lists the outcome of DNA tests;
c. a forensic unit's DNA table;
d. an initial report; or
e. an abbreviated statement, with brief details of the type of result obtained but without interpretation or specialist likelihood ratio (LR) calculations.

11.1.2 The Criminal Procedure Rules (CrimPR) give courts explicit powers to proactively manage the preparation of criminal cases waiting to be heard in order to get rid of unfair and avoidable delays. This enables investigators,

scientists, prosecutors and the defence to comply with the CrimPR in the interests of justice by providing key forensic evidence in a simple form, allowing the early identification of issues.

11.1.3 Part 19 of the CrimPR provides assistance with the required structure and contents of an evaluative statement.

a. An expert must give details of any literature or other information that the expert has relied on in making the report. Obviously for DNA interpretation this may be an extensive list. However, examples of papers covering the range of opinion relating to the issues in the specific case must be included.
b. Where there is a range of opinion on the matters dealt with in the report the expert must summarise the range of opinion, and give reasons for their own opinion. If the expert is not able to give an opinion without qualification, the qualification must be stated.

11.1.4 In the sections of a full evaluative statement dealing with the description of DNA mixtures, their interpretation, LR calculations or subjective opinions and conclusions, these should all meet the requirements under part 19 of the CrimPR.

11.1.5 However, although the CrimPR provide guidance on the contents, there is no general agreement between individual forensic scientists or FUs on the suitable wording for these descriptions. This is considered further in the section below.

12. Agreed Nomenclature To Describe The Features Of The DNA Profile Results

12.1.1 Forensic units do not have universally agreed nomenclature to describe the features of DNA profile results and explanation(s) regarding the interpretation of a mixture. For example, clear terminology regarding the interpretation of a mixture where it has been considered reasonable to condition on the presence of DNA from one or more persons.

12.1.2 It is appropriate that DNA-reporting scientists should be able to express themselves individually when writing reports or providing oral testimony. However, for the benefit of the end users such as the court, the Crown

Prosecution Service, juries and other lay people, it is also desirable that the language adopted is consistent and has common usage and meaning amongst all FUs. The goal of reducing the risk of misunderstanding of results and concepts, and improving the quality of the forensic process, should act as a driver for improving consistency of terminology amongst experts.

12.1.3 It is clear that the phrases used by forensic scientists have very specific gradations of meaning to the writer. These may, however, be less clear to a non-scientific reader. Forensic scientists are attempting, as individuals, to convey meaning through carefully nuanced phrasing that has evolved as a result of discussions with lawyers and the police to enable the reader to grasp very specific difficult concepts. One example provided shows a distinction being made between 'could have'; 'cannot be excluded'; and 'unable to determine whether or not'. Below are all examples that are in common usage, which appeared in a single report. These show the need for agreed wording so as not to confuse the reader with subtle differences in wording.

> "A mixed DNA result was obtained which indicated the presence of DNA from at least five contributors. The majority of DNA detected appears male. No major contributor could be determined. However, there are generally more prominent components within this result.
>
> The reference DNA profile of A is fully represented within the generally more prominent components of this mixed DNA result such that he could be a substantial contributor of DNA.
>
> The reference DNA profile of B is almost fully represented in this mixed DNA result such that he cannot be excluded as being a possible contributor of DNA.
>
> In my opinion, there is no clear indication that either C or D are substantial contributor. However, I am unable to determine whether or not they may have contributed DNA at a low level.
>
> This finding is not suitable for a standard statistical evaluation, but could be reviewed for suitability for a specialist statistical evaluation or a subjective evaluation in line with the Court of Appeal ruling in R. v. Dlugosz."

12.1.4 The wording used has very specific meanings for the scientist, who will distinguish between, for example:

a. a major contributor and a contributor with more prominent alleles;
b. a substantial contributor, a contributor at a low level and a possible contributor; and
c. a possible contributor and a contributor who cannot be excluded.

12.1.5 It is uncertain whether the lay reader will necessarily appreciate the subtleties that the wording has for the scientist.

12.1.6 Research into the meaning that forensic scientists intend to convey and that understood by non-scientific readers and listeners has shown the gap in understanding [17,43,44,45] and has shown that words and phrases such as ‘consistent with’ (see 7.6) and ‘evidence to link’ are often understood by prosecutors and the police to convey a greater strength than the scientist intends to imply.

12.1.7 The documents provided by FUs show divergence in the use of terms when describing commonly encountered DNA profile features. For example, the terms ‘DNA allele’, ‘DNA band’ and ‘DNA marker’ are used interchangeably. While a major component is well defined, this is not the case for a prominent component. Terms describing different types of profiles such as ‘a complex mixture’, ‘a mixture with a clear major component’, ‘a mixture with prominent alleles’ or ‘no reportable result’ have no generally agreed description of the intended meaning.

12.1.8 Forensic scientists at each FU should agree on understandable terms and phrases to describe:

a. the features of a DNA profile;
b. comparison with reference profiles;
c. interpretation; and
d. an agreed nomenclature to describe different DNA profile types, categories and descriptions.

12.1.9 This should allow FUs to record their observations clearly and accurately in ways that assist end users to understand the meaning consistently and without ambiguity. The understandable terms and phrases should also include the following.

a. Descriptions of the range of mixtures.
b. If a phrase such as those listed below is used to describe the types of contributor, then the intended meaning should be defined:

i. a major contributor;
ii. a strong contributor;
iii. a prominent contributor;
iv. a substantial contributor;

v. a minor contributor;
vi. a low level contributor;
vii. a possible contributor;
viii. included as a contributor;
ix. excluded as a contributor;
x. cannot be excluded as a contributor;
xi. fully represented; and
xii. almost fully represented.

12.1.10 Phrases intended to convey a weight or description should not be used unless they can be clearly defined, qualified and agreed by other scientists.

13. Requirements For Samples For Proficiency Tests

13.1.1 The main aim of proficiency testing in forensic DNA analysis is to assess how well the analysis and interpretation of results obtained from evidential material reflect the true nature of samples constructed from measured amounts of DNA from known donors.

13.1.2 The following are requirements for proficiency test samples.

a. DNA or body fluids from known donors is mixed in measured proportions so that the ground truth of the mixture is available. The results can be measured against an expected/known outcome. Casework samples are not a complete substitute as relative proportions of the donor samples are unknown.
b. Limitations: There are no artificial mechanisms to mimic naturally occurring degradation or inhibition effects.
c. The test samples provided to each participant must be the same construction so that inter-laboratory comparisons can be made.
d. The amount of the sample provided should be sufficient for a participant, using appropriate analytical methods, to detect all expected DNA components.
e. The expected evaluation and interpretation outcomes should be independent of the DNA amplification polymerase chain reaction (PCR) chemistry used for analysis.

14. Acknowledgements

14.1.1 This guidance was produced following the award of a competitive tender to Principal Forensic Services. The authors would like to thank Cellmark Forensic Services, Professor Mike Coble (University of North Texas Health Science Center), Eurofins Forensic Services, Professor Peter Gill (University of Oslo Hospital), Forensic Science Ireland, Key Forensic Services Ltd, Scottish Police Authority, members of the Forensic Science Regulator's DNA Analysis Specialist Group and the forensic science regulation unit (FSRU).

15. Review

15.1.1 This published guidance will form part of the review cycle as determined by the Forensic Science Regulator.

15.1.2 The Forensic Science Regulator welcomes comments. Please send them to the address as set out at the following web page: www.gov.uk/government/organisations/forensic-science-regulator, or send them to the following email address: FSREnquiries@homeoffice.gov.uk

16. Legal Judgments

16.1.1 The legal judgments considered for these guidelines were:

a. R. v. Dlugosz and Ors, Neutral Citation Number: [2013] EWCA Crim 2. Available at: www.bailii.org/ew/cases/EWCA/Crim/2013/2.html [Accessed 6/08/2020]

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18. Abbreviations And Acronyms

Abbreviation or acronym	Meaning
CrimPR	Criminal Procedure Rules
CPS	Crown Prosecution Service
DNA	Deoxyribonucleic acid
EN	European norm
EPG	Electropherogram
FU	Forensic unit
FSR	Forensic Science Regulator
FSS	Forensic Science Service
ILAC	International Laboratory Accreditation Cooperation
ISFG	International Society of Forensic Genetics
ISO	International Organization for Standardization
LTDNA	Low Template Deoxyribonucleic acid

LR	Likelihood ratio
NDNAD	National DNA Database®
NIST	National Institute of Standards and Technology
PCR	Polymerase chain reaction
POI	Person of interest
QE	Qualitative evaluation
RSS	Royal Statistical Society
SED	Staff Elimination Database
SFR	Streamlined forensic report
STR	Short tandem repeat
SWGDAM	Scientific Working Group on DNA Analysis Methods
UK	United Kingdom
UKAS	United Kingdom Accreditation Service

19. Glossary

Activity Level:

A proposition that relates to a particular activity that is relevant to the deliberations of the court. This may represent a position taken by the prosecution (H_p) or defence (H_d). See 'Hierarchy of propositions'.

Allele:

A genetic variant at a particular location within an individual's DNA. DNA profiling tests examine a range of alleles that are known to vary widely between individuals. Alleles are represented by peaks in a DNA profile.

Allelic Drop-In:

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

Allelic Drop-Out:

Alleles missing from a DNA profile, so that it is partially represented.

Artefact:

Artefacts are 'nuisance' peaks in a profile; often associated with the amplification and detection processes, such as spikes, dye blobs, spectral pull-up. They do not represent genuine alleles; they are screened out by the scientist or the software.

Assignment Of Evidential Weight:

Evidential weight is a function of the likelihood ratio (LR). Evaluation consists of assigning a value to the LR. Values greater than one mean that the observations support the prosecution proposition or hypothesis (H_p) and values less than one mean that the observations support the defence proposition (H_d).

Autosomal DNA:

Any chromosome that is not a sex-determining chromosome.

Calibration:

In its broad forensic sense, calibration is the process of assessing the performance of a system and/or a person in assigning the weight of evidence under controlled conditions. This requires two sets of cases (real or simulated):

a. in one set the prosecution proposition is known to be true;
b. in the other set, the defence proposition is known to be true.

Broadly, the requirement is that a large likelihood ratio (LR) (greater than one) should be generated from the former set and a small LR (less than one) should be generated from the latter.

In the context of this report, there are two situations where the notion arises:

a. in assessing the performance under controlled conditions of the scientist who provided the qualitative evaluations (QEs); and
b. in validating the performance of the software for carrying out quantitative evaluations.

Complex DNA Profile:

A crime-sample profile that may exhibit allele drop-out/drop-in phenomena, and may be a mixture. The complexity may only become apparent when the DNA profile does not exactly match the reference profile from a known individual under the prosecution hypothesis (H_p).

Condition(ing):
When assigning the evidential weight (see Assignment Of Evidential Weight) a genotype/profile is expected to be present in the mixture (see Conditioning Genotype/Profile). It therefore forms part of the prosecution and defence propositions (hypotheses) and is effectively cancelled out, that is, it is treated as neutral.

Conditioning Genotype/Profile:
The genotype/profile of an individual whose presence in a mixture is not in contention. For example, in a sexual assault the conditioning genotype may be that of the victim, as their DNA can reasonably be expected to be present. In some circumstances this may also apply to the consensual partner of the victim. Conditioning is performed within the case specific circumstances.

Contamination:
A spurious DNA profile(s) in a crime stain comprising three or more alleles from one or more individual(s). The contributors are considered to be of no relevance to the case (for example, may be introduced into plastic ware during the manufacturing process, or may have originated from a scientist processing the samples in the laboratory). It is distinct from allele drop-in [46].

Designation:
The process of determining whether a peak observed in an electropherogram of short tandem repeat (STR) amplification products can count as being a particular allele or a stutter, over-stutter or artefact.

Deconvolution:
Preparation of a list of putative combinations of genotypes of contributors to a mixed DNA profile, based on quantitative peak height information and any underlying assumptions.

DNA-17 System:
Short tandem repeat (STR) multiplex system (kit) with 17 STR loci (including the gender marker amelogenin).

DNA Profile:
A set of data that is generated by an appropriate biochemical process. It is viewed most simply as a set of tables, one for each locus. Each row of the table

describes the properties of a peak above some pre-set threshold and will include data for:

a. peak height;
b. molecular weight;
c. an allele designation, where this has been possible; and
d. potentially, other properties depending on the software.

There will also be a graphical representation of the data and this is known as an electropherogram.

Electropherogram (EPG):
Plot showing output of analysis of DNA sample.

Evaluative Opinion:
An opinion expressed by a scientist that meets the standards of balance, robustness and transparency required for presentation to a court of law.

Framework of Circumstances:
Background information that summarises all of the circumstances that are relevant to the interpretation of the profiles.

Genotype:
A characterisation from the alleles present at a genetic locus. If, for example, in a mixture there are two different alleles at a locus such as a short tandem repeat (STR) containing six or nine repeat units, then possible contributor genotypes are ‘six, six’, or ‘six, nine’ or ‘nine, nine’.

Ground Truth:
A data set made from known source material, such as DNA extracted and analysed from stains produced using body fluids from known donors, used for validation, proficiency and competency testing purposes.

Hierarchy of Propositions:
Propositions or hypotheses address issues of interest to the court. At one level, the issue is where a particular item of evidence came from (source level or sub-source level); at another the issue is of an activity (activity level) that a person may have carried out; and at another the issue relates to an offence (offence level) that a person may have carried out. Whatever the level, there will be two

propositions that respectively represent the prosecution (H_p) and the defence (H_d) positions relating to the issue.

Informativeness:
A DNA profile from a reference sample from a known individual will usually be of sufficient quality for the genotype of that individual to be inferred unambiguously. A questioned sample, on the other hand, may be of insufficient quality and/or quantity for unambiguous designation of all of the constituent alleles even after replicate analysis. 'Informativeness' is used in this context as a fairly rough qualitative indication of the extent to which the level of information enables the designation of alleles in a crime sample. If the informativeness is low then an evidential interpretation of the profile may not be possible. This is usually a subjective judgement made by the scientist, bearing in mind the capabilities of the available statistical software.

Initial Report:
A short report provided by the relevant Forensic Unit giving the initial key findings.

Investigative Opinion:
An opinion expressed by a scientist for the purpose of assisting a police officer in the investigation of an offence. This opinion will not, in general, meet the standards of robustness and reliability required for presentation in a court of law.

Likelihood Ratio (LR):
This is a statistic [47] that is a measure of the extent to which a set of observations supports one of two propositions.

Locus (Plural Loci):
A specific location or position of an allele on a chromosome. Short tandem repeats (STRs) are examples of loci that are of interest in forensic science because they are polymorphic and are therefore highly discriminatory when several are analysed in combination to generate a DNA profile.

Mixture:
A DNA profile that contains more designated alleles than would be expected if there were only one contributor to the sample.

Molecular Weight:
The weight or size of a DNA fragment typically expressed as the number of nucleotides it contains.

Offence Level:
A proposition that relates to a criminal offence, e.g. sexual assault, that is relevant to the deliberations of the court. This is the domain of the jury/court.

Peak:
A DNA profile consists of a series of peaks. Most of these will represent alleles. However, there will also be a number that are artefacts.

Peak Height:
The height of a peak typically measured in relative fluorescence units and generated during electrophoresis and fluorescence detection of DNA amplification (PCR) products generated during the analysis of a DNA profile.

Person of Interest (POI):
Also referred to as a significant individual, a person whose profile is the subject of the evaluation.

Propositions:
In a criminal or civil trial it is usually necessary for a forensic scientist to help address two propositions or hypotheses: one (often called H_p) that represents the prosecution position with regard to a particular issue and the other (H_d) that represents the defence position with regard to the same issue.

Qualitative Evaluation (QE):
The judgment in R. v. Dlugosz is interpreted as supporting the practice whereby a scientist presents a qualitative evaluation of weight of evidence in a case where, because of one or more unresolved interpretative issues, it is not possible for the scientist to provide the court with a quantitative evaluation.

Quantitative Evaluation:
The calculation of a numerical likelihood ratio in relation to a pair of propositions or hypotheses. This will almost always be achieved by means of validated software and will incorporate reference to one or more databases of allele proportions.

Questioned Profile:

A DNA profile generated from a questioned sample.

Questioned Sample:

A sample associated with a crime or from an article associated with a person of interest, whose source is not known.

Reference Profile:

A profile from a sample of undisputed origin.

Replicate Profiles:

Profiles generated by repeated amplification and analysis of the same DNA sample.

Short Tandem Repeat (STR):

Is a microsatellite consisting of one to six or more nucleotides that is repeated adjacent to each other along the DNA strand.

Source Level:

A proposition relating to the origin of a DNA sample that has been attributed to a body fluid or tissue. Also see SUB-SOURCE LEVEL below.

Statement:

A statement is one form of a report. It is formatted to comply with the provisions of s9 Criminal Justice Act 1967.

Streamlined Forensic Reports:

Case management reports; there are two types.

a. Level 1 Streamlined Forensic Reports (SFR1s) are supposed to be a summary of the expert's evidence served on the defence to obtain agreement of the evidence under the provisions of Rule 19.3(1) of the Criminal Procedure Rules (CrimPR). There are a number of consequences of this.

   i. They are not intended to be used in evidence so the requirements that apply to statements (see below) do not apply.

   ii. They are not served under Rule 19.3(3) of the CrimPR and, as a result, the provisions of Rule 19.4 of the CrimPR do not apply.

iii. As the provisions of Rule 19.4 CrimPR do not apply many of the declarations required by part 19 of the Criminal Practice Directions do not apply.

b. Level 2 Streamlined Forensic Reports (SFR2s) are intended to be used as evidence and must comply with the provisions of Rule 19.4 CrimPR and the relevant sections of part 19 of the Criminal Practice Directions. SFR2s may also have to comply with the provisions applying to statements.

Stutter:
A stutter is an artefact of the amplification process that leads to smaller peaks close to the main allelic peak. The most common stutter peak is one that represents one repeat unit smaller than the allelic peak (-4). Stutters with other numbers of repeats are also possible, but less common. Over-stutters are one repeat unit larger than the allelic peak (+4).

Sub-Source Level:
A proposition that relates to the origin of DNA without specifying what kind of body fluid or tissue carried the DNA. Also known as a 'DNA level' proposition.

20. Further Reading

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Annex 1

21. Guidelines

21.1 Propositions

21.1.1 Guideline 1: The scientist should attempt to assign a value to the number of contributors to the questioned sample. The reasoning to support this should be recorded on the case file.

21.1.2 Guideline 2: The scientist should consider whether it is reasonable to use any of the known genotypes from given individuals for conditioning one or more of the propositions. The reasoning to support this should be recorded on the case file.

21.1.3 Guideline 3: The scientist will consider the genotype of the person(s) of interest to provide a preliminary assessment of whether or not there is a straightforward exclusion, given the assigned number of contributors and the conditioning genotypes(s), and taking account of the quality of the questioned profile(s), particularly in relation to the potential for drop out and other artefacts.

21.1.4 Guideline 4: On the basis of the framework of circumstances and the outcome of the previous steps, the scientist will formulate one or more propositions that could be anticipated as representing the prosecution position in proceedings against the person of interest. For each proposition the number of contributors and the postulated contributors should be made clear.

21.1.5 Guideline 5: On the basis of the framework of circumstances and the outcome of the previous steps, the scientist will formulate, to correspond with each prosecution proposition, a defence proposition. As with the prosecution proposition, the number of contributors and the postulated contributors should be stated. The genetic relationship between any unknown contributor and the person of interest or other known persons in the case should also be made clear, either in the proposition or the accompanying text.

21.1.6 Guideline 6: Where the circumstances include multiple persons of interest, a simple pair of propositions is not adequate. Several pairs of propositions may be necessary according to the framework of circumstances. If necessary, consider providing an investigative, rather than an evaluative report.

21.1.7 Guideline 7: In a case where the association between the questioned sample and the crime is uncertain consider a staged approach to forming propositions. Also consider providing an investigative opinion in an initial report, ensuring that the limitations are clearly stated.

21.1.8 Guideline 8: Always consider a relative of the person of interest as an alternative source under the defence proposition if the framework of circumstances suggests this to be a relevant issue.

21.1.9 Guideline 9: If there is uncertainty with regard to the number of contributors to a questioned profile, and the background information does not assist in assigning relevant propositions, then calculations should be carried out for all combinations of propositions that appear reasonable in the circumstances of the case. The range and outcome of the calculations should be reported.

21.1.10 Guideline 10: The major/minor approach to interpreting a profile is permissible if pursued with due regard for logic, taking into account all loci, and only where it is not based on the results of the comparison of the trace with the person whose DNA presence is contested.

21.2 Practice of Qualitative Evaluation

21.2.1 Guideline 11: A quantitative evaluation is possible provided that:

a. a clearly stated pair of propositions can be formulated;
b. all aspects of the observations (particularly artefacts) that are excluded from the evaluation are done so without uncertainty;
c. the calculation is within the validated capabilities of the software; and
d. the population databases [33] that are used can be shown to be relevant within the context of the case circumstances.

21.2.2 Guideline 12: The practice of using the number of matching alleles as an indicator of evidential weight in relation to DNA mixtures should be discontinued because it is potentially prejudicial.

21.2.3 Guideline 13: In a case where a statistical analysis appears feasible, it is reasonable to present a qualitative evaluation as an interim measure, perhaps in the context of an initial report. It is necessary to emphasise the provisional nature of the opinion.

21.2.4 Guideline 14: The reliability of qualitative evaluations from any forensic unit wishing to provide this service must be established by a continuous programme of calibrating the qualitative evaluations against statistical analysis, using the resources currently available within the organisation.

21.2.5 Guideline 15: Qualitative evaluations should be presented as investigative opinions for intelligence purposes, rather than as evaluative opinions.

21.2.6 Guideline 16: The practice of offering a qualitative evaluation in a case where, because of unresolved interpretative issues, it has not been possible to carry out a quantitative evaluation by means of validated software, should not be continued.

21.2.7 Guideline 17: Expressions of possibility should not be presented in a manner that favours the prosecution. If an assessment of evidential weight is not possible, the scientist should make it clear that he/she can give no guidance to the court with regard to probative value.

21.2.8 Guideline 18: In the case where it has not been possible to carry out a calculation because there are interpretive issues that are outside of the resources available to the scientist, consideration should be given to consult a specialist who has extensive mathematical knowledge of the interpretation of mixtures. The objective will always be a quantitative assessment accompanied by a reasoned justification of the analysis.

21.3 Interpretation of DNA Results

21.3.1 Guideline 19: Forensic processes should, where possible, transfer information electronically to reduce the risk of transcription errors. If data are to be transferred manually then appropriate checks to safeguard the integrity and quality of the data should be clearly undertaken and recorded. The process of transfer and checking should be clearly described within the appropriate standard operating procedures, and regularly included in audit processes.

21.3.2 Guideline 20: Any data and interpretation compiled for any proficiency test should follow the same process, and meet the same requirements and standards, as the casework procedure.

21.3.3 Guideline 21: Forensic units should ensure, through a system of audit and compliance checking, that laboratory specific interpretation guidelines are fit for purpose, drive consistency and reduce variation in interpretation outcomes.

21.3.4 Guideline 22: The data to support the interpretation guidelines should be referred to in statements as per the requirement of the Criminal Procedure Rules part 19.

21.3.5 Guideline 23: When a DNA result is transferred to another organisation, the thresholds and interpretation guidelines used to interpret the DNA result should be those of the forensic unit at the time of the original analysis that produced the DNA result.

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