Forensic Science Regulator
Overseeing Quality

Forensic Science Regulator Guidance

Proficiency Testing Guidance for DNA Mixture Analysis and Interpretation

FSR-G-224

Issue 1

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1. Introduction

1.1.1 Within the UK forensic unit (FU) environment there is divergence both in terms of the multiplex short tandem repeat (STR) chemistries that are used to generate DNA profiles and the statistical methods used to evaluate mixed DNA profiles for casework purposes.

1.1.2 There is some variation in the way in which these results are communicated within a framework of report and statement writing. The level of divergence after the changes to the National DNA Database™ (NDNAD) in July 2014 to permit loading of profiles generated from different approved STR chemistries, including DNA-17, was assessed by a collaborative study commissioned by the Forensic Science Regulator (FSR).[1] Its purpose was to determine the 'lie of the land' by comparing outcomes from a set of known DNA mixture samples that were analysed and interpreted by different FUs using their latest methodologies.

1.1.3 Lessons learned from the study have been used as the basis for generating guidance on mixture interpretation [2] and herein on proficiency testing (PT). In addition, the guidelines build on an earlier report commissioned by the FSR, 'The interpretation of DNA evidence (including low-template DNA)'[3] which acknowledged that there was a need to develop standards for interpretation methodology.

1.1.4 Currently (as at March 2020) in the UK there is limited availability of PTs that are designed to test the detailed interpretation of DNA evidence, including evidence generated from mixtures that contain low-template [4] components. The development of a coherent PT approach will help to measure variation and will assist in the pragmatic development of standards.

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[1] Principal Forensic Services Ltd was awarded the contract by the Home Office on behalf of Andrew Rennison, the Forensic Science Regulator at the time, to run the study. The authors Barber, M., Evett, I., Pope, S., Sullivan, K., Tully, G., Whitaker, J. and Coble, M. produced a DNA Mixtures collaborative study report (unpublished) in 2014.

[2] Forensic Science Regulator: DNA mixture interpretation, FSR-G-222 and Software validation for DNA mixture interpretation, FSR-G-223. Available at: www.gov.uk/government/collections/dna-guidance

[3] Gill, P., Guiness, J. and Iveson, S. (July 2012) The interpretation of DNA evidence (including low-template DNA). Available at: www.gov.uk/government/publications/the-interpretation-of-dna-evidence

[4] Includes low DNA quantification levels and based on the mixture proportion of the minor contributions in a mixed profile would fall into the low-template range for the validated method used.

1.1.5 The purpose of this document is to provide:

a. guidance to providers of PTs (including collaborative exercises); and
b. a standard process for the design and operation of PTs intended to monitor DNA mixture interpretation methods used by UK FUs.

1.1.6 This will aid FUs in monitoring their own performance against others through participation in external PTs and as part of maintaining their ISO 17025 [5] accreditation. It will also help to monitor the performance and competency of those that only conduct statistical interpretation from the analytical profile data.

2. Guidelines Within the Forensic Science Regulator's Codes

2.1.1 Existing Forensic Science Regulator (FSR) Codes of Practice and Conduct (the Codes) relevant to this topic are:

a. DNA Analysis, FSR-C-108;
b. The interpretation of DNA evidence (including low-template DNA), FSR-G-202;
c. The control and avoidance of contamination in laboratory activities involving DNA evidence recovery and analysis, FSR-G-208;
d. Allele frequency databases and reporting guidance for the DNA (short tandem repeat) profiling, FSR-G-213;
e. Cognitive Bias Effects Relevant to Forensic Science Examinations, FSR-G-217;
f. DNA Mixture Interpretation, FSR-G-222;
g. Software Validation for DNA Mixture Interpretation, FSR-G-223; and
h. DNA Contamination Detection – the management and use of staff elimination databases, FSR-P-302.

2.1.2 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

[5] BS EN ISO/IEC 17025 *General requirements for the competence of testing and calibration laboratories*.

of factors that should be considered when using either qualitative [6] or probabilistic methods including:

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

2.1.3 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.

2.1.4 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 (LRs) [7] for profiles derived for the DNA-17 system.

2.1.5 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, and describes:

a. the various means by which bias (defined in glossary) can potentially be introduced in mixture interpretation;
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; and

[6] The use of qualitative evaluation is no longer an approved method for evaluating DNA mixtures since the publication of DNA Mixture Interpretation, FSR-G-222, issue 2 (31 October 2018).

[7] A likelihood ratio is an estimate and varies depending on the statistical method used. It may be necessary to use a range of commonly used interpretation models to obtain the magnitude of variation to be expected.

c. whether the interpretation can be reliably conducted where there is no suitable option for quantitative evaluation .

2.1.6 FSR-G-222 sets out DNA mixture interpretation and reporting so that any PT provider will understand what participating UK forensic units are required to comply with, in order to design the test(s) and reporting formats to provide appropriate PTs for performance monitoring purposes.

3. Scope

3.1.1 Guidelines for the provision of PTs used for the assessment of DNA mixture analysis and interpretation methods, including statistical evaluation and report/statement writing.

3.1.2 The guidelines are intended to be used in conjunction with ISO/IEC 17043: 'Conformity assessment – General requirements for proficiency testing' and are supplementary to the 'Technical Requirements' section (section 4) of this guidance.

3.1.3 'The DNA Mixture Interpretation Guidance, FSR-G-222 and Allele frequency databases and reporting guidance for the DNA (short tandem repeat) profiling', FSR-G-213 are relevant to the interpretation and reporting of results.

3.1.4 These guidelines apply to England and Wales. Scotland, Northern Ireland and the Republic of Ireland may also institute parallel arrangements for their jurisdictions.

4. Modification

4.1.1 This is the first issue of this document.

5. Terms and Definitions

5.1.1 The terms and definitions set out in the Forensic Science Regulator's Codes of Practice and Conduct (the Codes), the DNA mixture Interpretation Guidance FSR-G-222 (for ease of reference definitions are reproduced) and the Glossary apply.

5.1.2 Abbreviations are spelled out in Abbreviations in this guidance.

6. Implementation

6.1.1 The requirements in this guidance are available from publication for immediate implementation for DNA mixtures PTs [8] provided by PT providers and used by forensic units as part of their quality performance monitoring and compliance with ISO 17025.

7. Overall Approach

7.1.1 The PT should be conducted as an external quality assessment (EQA) programme. This fits most closely to an interpretive-type model for PT schemes, as outlined in section A.4 of ISO 17043.

7.1.2 In summary it comprises the following steps.

a. Determine the design of the PT and the plan for delivery.
b. Determine the characteristics and suitability of the test items to be provided.
c. Produce test items and develop case scenarios.
d. Determine acceptable criteria for responses and interpretations – expectations of performance.
e. Distribute test material and case scenarios to participants.
f. Receive results and interpretations from participants.
g. Evaluate participants and interpretations against the expectations of performance.
h. Produce report and issue advisory comments.

8. Test Design

8.1.1 The proposed design should address the objectives and purpose of the PT as defined by the end user participant who understands the requirements to be met by their stakeholders, for example:

a. the Forensic Science Regulator (FSR);
b. the Forensic Information Databases Service (FINDS);
c. the Crown Prosecution Service (CPS); and
d. the courts.

[8] These would also apply to producing DNA mixture collaborative exercises.

8.1.2 These should be documented, along with the plan for delivery of the test.

8.1.3 The type of test items that may be considered are:

a. extracts of real DNA mixture samples;
b. mixed body fluid samples (for example, stains made from known mixtures of blood); and
c. electronic DNA profile data files (.fsa or other suitable format) to enable profile interpretation using either the organisation's own analysis and threshold parameters or provision of the analysis parameters for the method used to generate the data.

8.1.4 The full end-to-end process requires the mixed body fluid type sample(s) rather than the extracted DNA, which assesses the variability in the end-to-end process. The latter point focuses on variability in the interpretation process(es) alone.

8.1.5 This builds on the dual approach utilised in a large PT based in the USA, which was conducted by the National Institute of Science and Technology (NIST) in 2013. [9,10]

8.1.6 Samples should be selected (in terms of donor profiles, mixture ratios, number of contributors and input levels of DNA) so as to test challenging but frequently encountered aspects of mixture interpretation as well as more straightforward interpretation. Tests should include the following.

a. At least one sample that reflects the poorer quality of samples and resultant profiles observed in typical casework, for example, low-template characteristics, degradation.
b. Samples with at least two contributors and also some examples of more complex mixtures (up to four contributors) with contributors present in varying proportions.
c. A 'challenging' sample with more than four contributors present. Although this may be beyond some evaluation software, how such profiles are reported will be informative.

[9] National Institute of Science and Technology 2013.
[10] Buckleton *et al*.(2018).

d. Samples should be provided within an appropriate casework scenario; providing a context for the results and interpretation.

e. Appropriate reference samples or profiles should also be provided.

8.1.7 Points that need to be considered in the design of the PT include the following.

a. The donor pool – from which samples are to be selected. Relatedness among donors should be no greater than that expected in a similar size sample from the general population. For a continuing programme of tests, the pool should be large enough that participants do not perceive obvious repetition of donors.

b. Essentially each donor within the pool should be independently profiled using at least one of the currently accredited polymerase chain reaction (PCR) chemistries.

c. Sample composition – When choosing sample donors the amount of profile overlap and the distribution of 'non-matching' heterozygote (defined in glossary) and homozygote loci within each profile will need to be considered with respect to the achievement of the expected result. When defining template levels and mixture proportions, system detection limits may need to be accounted for; for example:

   i. the participant's stochastic thresholds (minimum template level that would give a satisfactory result); and

   ii. the detection limits (the lower limit for obtaining a full profile as determined by the laboratory through internal validation).

d. Analytical and interpretation processes – In the UK there is no requirement for FUs to use fully 'prescribed' DNA profiling methods; all appear to employ different combinations of both manual and automated processes. Differences between systems may affect test results. Therefore in advance of a PT, participants should be requested to provide details of the systems they intend to use so that appropriate test material can be produced. This would include details of:

   i. the quantification system;

ii. amplification, short tandem repeat (STR) multiplex, type of thermal cycler, cycle number, template level;
iii. electrophoresis platform, sequencer type, injection time and injection voltage;
iv. analysis software and analysis guidelines; and
v. expert interpretation systems and manual interpretation methods.

9. Sample Characterisation

9.1.1 Sample material should be characterised through repeated analysis in order to ensure the following requirements.

a. As far as possible stability and homogeneity is established and comparable, and stable samples can be provided to each participant.
b. Expectations of performance can be defined including:
   i. quantification result [11] propositions that address the casework scenario with which participants are presented;
   ii. mixture proportions;
   iii. LRs; and
   iv. identification and designation of alleles.
c. Results generated can meet with the desired expectation of performance.

10. Production Of Test Samples And Case Scenarios

10.1.1 Production of test samples and case scenarios should be carried out in accordance with the organiser's documented/accredited procedures and the process of sample preparation documented in full.

10.1.2 The aims and objective of the PT should be documented within both the sample preparation paper work and instructions provided to participants.

10.1.3 The preparation of the samples and the testing to characterise the samples prior to distribution shall be conducted, taking due regard of the DNA anti-contamination requirements set out in The control and avoidance of

[11] The expected quantification results are dependent on the quantification method employed. It may be necessary to use the commonly used tests to determine an expected range of estimates.

contamination in laboratory activities involving DNA evidence recovery and analysis, FSR-G-208.

11. Performance Criteria

11.1.1 The PT organiser should determine and document performance criteria for the PT with respect to:

a. profile type and completeness, including expected allele designation, mixture proportion, drop-in and any other allelic or non-allelic process artefacts; and
b. interpretation approach, where a LR approach would be expected with an unequivocal statement of the propositions addressed.

11.1.2 When setting the criteria the PT organiser should take into account differences between participants' processing systems and consider if the introduction of an 'acceptable range' would be appropriate. For example, for estimated DNA concentration mixture proportion and LR.

11.1.3 The inclusion of the assessment of quantification, LRs and mixture ratios introduces 'quantitative' elements, which need to be characterised (see section 9) and documented within the expectation of performance along with any acceptable range; for example, due to process differences and interpretation models.

12. Distribution Of Test Material

12.1.1 It is envisaged that test material will be sent to participants using a trackable service (for example, recorded delivery, special delivery or courier for wet samples). The PT organiser should ensure that the packaging and packaging process used for test samples meets with current UK postal regulations for biological specimens.

12.1.2 Each participant should provide details of a single point of contact within their respective organisation to ensure:

a. receipt and internal distribution of materials; and
b. that the instructions provided are understood and complied with.

12.1.3 Any contact email address should be checked to ensure that data sent by the organisers are received by participants and to confirm that there are no problems with system protection firewalls.

12.1.4 DNA extracts in solution should:

a. be provided in appropriate tubes to minimise loss due to DNA adhering to the sides of the tube;
b. be of sufficient amount to enable multiple analyses to be conducted; and
c. be of a concentration that does not require large dilutions to be conducted prior to analysis.

12.1.5 Similar considerations should be given to body fluid samples (wet or dry) and dried DNA extracts.

12.1.6 All instructions, data, PT results forms and other written material pertinent to the PT should be made electronically available by establishing a secure file sharing site. All participants should check and confirm that they are able to access the shared facility prior to commencement of the PT. Where file sharing is not possible, encrypted memory sticks or secure email could be used for data transfer, provided confirmation of receipt is given.

12.1.7 Instructions should be clear, comprehensive and unambiguous using common agreed terminology. For example, where information on the amount of DNA added to a polymerase chain reaction (PCR) is requested, the units (for example, nanograms) must be unambiguously stipulated.

12.1.8 The PT results form should be designed to capture data in a format that allows for straightforward compilation and comparison of results both between participants and against the expected outcomes of the PT.

12.1.9 To minimise transcription errors the results form should have the capability to be electronically populated – as a minimum to copy/paste information and results, or to have the facility to add result tables.

12.1.10 There should be separate space provided to capture any potential allele designations that although present, fall outside/below the participant's thresholds for designation, for example, samples with low DNA template.

12.1.11 Ideally a hard copy of both the PT instructions (including the name of the participant contact) and the results form should be included with the test samples.

13. Requirements For The Completion Of The Proficiency Test

13.1.1 To allow for the evaluation of potential intra-laboratory variation within and between FU sites, participants should submit assessments from more than one individual, and ideally from at least three staff members representing a wide spectrum of experience.

13.1.2 Participants should use their standard casework analysis procedures, including the usual independent checking of critical results. Where participants would normally utilise the services of a third party for the assessment of challenging analytical results, it should be requested that they also do so in the PT.

13.1.3 Results should be written up in the usual reporting format that the FU employs for real casework, and the conclusions drawn clearly stated.

13.1.4 Assessments by both the analyst and the checker should be provided along with any electropherograms used in the interpretation process.

13.1.5 The PT results form should be completed as required.

14. Receipt Of Results

14.1.1 All results and associated data and reports from participants should be returned to the PT organiser within the specified timescales.

14.1.2 To avoid any commercial sensitivity regarding the PT organiser's knowledge of the results generated by particular participants, the results should be submitted using an anonymisation code.

14.1.3 On receipt of the results and within specified timescales the PT organiser should acknowledge receipt and provide an individual 'initial summary of performance' for each participant.

14.1.4 The PT organiser should also initiate the investigation of any anomalous results; by both the PT organiser (into sample preparation) and the participant (sample processing).

15. Evaluation Of Performance

15.1.1 The PT organiser should assess responses from participants against the expectations defined in section 11:

a. undertaking a review of any differences between expectations and results in each case; and
b. taking participants' detection limits, thresholds and interpretation guidelines into account.

15.1.2 Once individual performance has been evaluated and reported an overall performance report should be generated that collates the results, giving:

a. a description of the extent of variability in outcomes from the prepared mixtures;
b. an analysis of the extent of variability in outcomes from the analysis of data files;
c. a comparison between methods and wording used in statements;
d. graphical /visual summaries of results to demonstrate profile type and completeness, for example, a spreadsheet showing profile completeness using colour coded (for example, Red/Amber/Green) boxes; and
e. LR ranges.

15.1.3 Feedback from the PT organiser to all participants in order to clarify any points at issue and share experiences of the test should be provided on the conclusion of the PT. This can be done by various means, for example, as a workshop, summary report or with the PT organiser's list of collaborators.

15.1.4 The PT organiser should use lessons learnt from the performance of the PT for developing and preparing future PTs.

16. Monitoring Performance

16.1.1 All PTs completed should be monitored and reviewed through the FU's own quality management processes and disclosed as part of the FU's accreditation requirements to the national accreditation body. PTs provided through a national scheme, such as the FINDS, will be monitored through the data integrity processes as agreed with each FU.

17. Acknowledgements

17.1.1 This guidance was produced following the award of a competitive tender to Principal Forensic Services. The authors would like to thank Cellmark Forensic Services, Eurofins Forensic Services, Forensic Science Ireland, Key Forensic Services Ltd, the Scottish Police Authority, Forensic Science Northern Ireland, members of the Forensic Science Regulator's DNA Analysis Specialist Group and the Forensic Science Regulation Unit (FSRU).

18. Review

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

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

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20. Abbreviations

Abbreviation	Meaning
BS	British Standard
CD	Compact disk
CPS	Crown Prosecution Service

DNA	Deoxyribonucleic Acid
EQA	External quality assessment
FINDS	Forensic Information Databases Service
FSR	Forensic Science Regulator
FSRU	Forensic Science Regulation Unit
FU	Forensic unit
IEC	International Electrotechnical Commission
ISO	International Organisation for Standardisation
LR	Likelihood ratio
NDNAD	National DNA Database™
NIST	National Institute of Science and Technology
PCR	Polymerase chain reaction
PT	Proficiency test
QA	Quality assurance
STR	Short tandem repeat

21. Glossary

Allele:

A genetic building block that makes up the genotypes of a DNA profile. 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. [Back]

Allelic Drop-In:

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

Allelic Drop-Out:

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

Amplification:

The process of generating multiple copies of targeted DNA areas using polymerase chain reaction (PCR). [Back]

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. [Back]

Bias:

A feeling for or against something or someone that is not based on fair judgement. [Back]

Complex DNA Profiles:

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). [Back]

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. [Back]

DNA-17:

DNA short tandem repeat (STR) multiplex systems with 17 STR loci (including amelogenin). [Back]

DNA Mixture:

A sample containing more than one DNA profile. [Back]

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. [Back]

Donor Profile:

A confirmed profile from a sample of undisputed origin. [Back]

Electrophoresis:

A method for separating DNA fragments according to their size. [Back]

Elimination Database:

A collection of DNA profiles used solely for the purposes of detecting potential contamination events. [Back]

Forensic Unit:

A term used in ILAC-G19 to mean:

“a legal entity or a defined part of a legal entity that performs any part of the forensic science process”.

It is interchangeable with provider. However, it is used in this document as these are small teams or sole practitioners that for accreditation purposes may be considered separate legal entities in larger organisations, forensic science providers and police forces. [Back]

Heterozygote:

An individual having different alleles at a particular locus; usually seen as two distinct peaks in an electropherogram. [Back]

Homozygote:

An individual having the same (or indistinguishable) alleles at a particular locus; seen as a single peak in an electropherogram. [Back]

Likelihood Ratio:

This is a statistic that is a measure of the extent to which a set of observations supports one of two propositions. [Back]

Low-Template DNA (low-level target DNA):

A term describing very low amounts of DNA of interest for amplification (PCR). [Back]

Mixture Ratios (MX):

The relative proportion of DNA within a DNA mixture for each contributor. [Back]

Number of Contributors:

Known or determined count of the individuals contained within a DNA mixture. [Back]

Qualitative Evaluation:

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. [Back]

Quantitative Evaluation:

The calculation of a numerical LR 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. [Back]

Short Tandem Repeat (STR):

A microsatellite consisting of one to six or more nucleotides that is repeated adjacent to each other along the DNA strand. [Back]

Standard:

A standard is an agreed way of doing something that is a level of quality or attainment. [Back]

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). [Back]

22. Further Reading

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