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Standard for Validation Studies of DNA Mixtures, and Development and Verification of a Laboratory's Mixture Interpretation Protocol

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Foreword

It is imperative that laboratories only interpret mixed DNA data for which there are supporting internal validation studies and data, and relevant and appropriate laboratory-approved interpretation protocols. Internal validation allows for the determination of the capabilities and limitations of a system and provides the rationale for the interpretation of data developed within that system.

DNA samples containing mixtures often include a range of DNA input, from low template (i.e., where stochastic effects occur) to high template, and a range of contributor numbers and ratios. Validation studies performed using known samples created under specified conditions enable the laboratory to assess observed versus expected data (e.g., for STR testing, genotypes detected, peak heights with associated ratios of input DNA and allele sharing, and estimated number of contributors) and determine the accuracy and limitations of the testing. These data allow the development of interpretation parameters that are supported by the data. Following development, it is critical for a laboratory to verify that the interpretation protocols work as designed.

The draft of this standard was developed by the Biology/DNA Interpretation and Reporting Subcommittee of the Organization of Scientific Area Committees. It was prepared and finalized as a standard by the DNA Consensus Body of the ASB. All hyperlinks and web addresses shown in this document are current as of the publication date of this standard.

This standard should be used in conjunction with ASB Standard 040, entitled "Standards for Forensic DNA Interpretation and Comparison Protocols" which has not yet been published.

All hyperlinks and links are valid and operational at the time of publication of this document.

Keywords: *DNA mixture validation, mixture interpretation guidelines and protocols, verification of mixture interpretation protocols*

Abstract: This standard is designed to provide direction and guidance to laboratories for the development of DNA mixture interpretation protocols that consistently produce reliable and reproducible interpretations and conclusions, and which are supported by internal validation data.

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Standard for Validation Studies of DNA Mixtures, and Development and Verification of a Laboratory's Mixture Interpretation Protocol

1 Scope

1.1 This standard sets forth the requirements for the design and evaluation of internal validation studies for mixed DNA samples and the development of appropriate interpretation protocols for mixtures based on the validation studies performed. This standard includes a requirement that the laboratory verify and document that the mixture interpretation protocols developed from the completed validation studies generate reliable and consistent interpretations and conclusions for the types of mixed DNA samples typically encountered by the laboratory.

1.2 This standard applies to any type of DNA testing technology and methodology used, including but not limited to, STR testing, DNA sequencing, SNP testing, haplotype testing, traditional and rapid protocols, etc., where mixtures of DNA may be encountered, analyzed and interpreted.

Laboratories are advised to review their previous validation for compliance with this standard, supplement validation where necessary, and modify existing protocols accordingly.

2 Normative References

There are no normative reference documents. Annex C, Bibliography, contains informative references.

3 Terms and Definitions

For purposes of this document, the following definitions apply.

3.1
consistent/consistency
Obtaining a similar output, within an acceptable limited range of variation (as defined by the laboratory protocol and validation data), when using the same methods and procedures over time.

3.2
internal validation
The accumulation and evaluation of test data within the laboratory for developing the laboratory standard operating procedures and demonstrating that the established protocols for the technical steps of the test and for data interpretation perform as expected in the laboratory. The parameters (e.g., any variable that impacts interpretation) included in a test protocol used by the laboratory should be supported by validation studies conducted with samples of known origin similar to the types of samples routinely accepted and tested by the laboratory.

3.3
mixed DNA sample
Any biological sample containing DNA from more than one individual.

3.4
stochastic effects
Changes in a DNA profile that generally occur when suboptimal or limiting quantities of DNA are tested. This may be due to sampling variation (e.g., pipetting) of the target DNA that goes into the

polymerase chain reaction (PCR) and/or random events between primers and target DNA during PCR amplification. The effects may be observed at one or more loci, and include: 1) peak height imbalance of sister alleles in a heterozygous pair; 2) loss of data (referred to as "allele drop out" when one or more alleles are missing at a locus and "locus drop out" when all alleles are missing from a locus); 3) allele drop-in (allelic peak(s) in an electropherogram that are not reproducible); and 4) elevated stutter peaks.

3.5
validation
The process of performing a set of experiments that establish the efficacy, reliability, and limitations of a method, procedure or modification thereof; establishing recorded documentation that provides a high degree of assurance that a specific process will consistently produce an outcome meeting its predetermined specifications and quality attributes.

4 Requirements

4.1 Refer to Annex B, Requirements – Supporting Information, for additional normative information on the following requirements.

4.2 The laboratory shall perform DNA mixture studies as part of the internal validation to support interpretation protocols prior to their use for casework samples in the laboratory. The mixture studies shall include, at a minimum, mixed DNA samples that:

4.2.1 Are representative of those typically encountered and interpreted by the testing laboratory.

4.2.2 Span the dynamic range of the detection platform.

4.2.3 Include each number of contributors to be interpreted by the laboratory.

4.2.4 Are constructed from extracted DNA samples of known origin (having known genotypes or sequences, etc.) combined: a) in varied input ratios based on the estimated DNA template amounts of the individual contributors; and b) with varied degrees of allele sharing.

NOTE The use of known samples allows for the assessment of observed versus expected data.

4.3 The data from the validation studies performed by the laboratory shall be the basis for the interpretation parameters and protocols developed by the laboratory and shall provide guidance for the types of mixed DNA profiles that will be interpreted by the laboratory. The studies shall:

4.3.1 Support all of the interpretation methods and protocols used for DNA mixture analysis.

The validation summary shall describe how the data from the validation studies performed led to the parameters used in the interpretation protocol.

4.3.2 Aid in assessing and defining the limitations of the methodologies used for the range of samples to be tested and the interpretation of the data generated.

NOTE Limitations may result from a number of factors including sample degradation and inhibition, the number of contributors that may be interpreted, and stochastic effects.

4.3.3 Establish testing methodology and interpretation parameters for samples containing mixtures of DNA, including criteria for establishing the minimum and assumed number of contributors to a DNA mixture.

4.4 The laboratory shall verify and document that the mixture interpretation protocols developed from the validation studies generate reliable and consistent interpretations and conclusions for the types of mixed DNA samples typically encountered by the laboratory.

4.4.1 Verification of the mixture protocols shall be performed on mixed DNA samples of known origin that are different from those in the initial validation studies used to establish the protocol.

Verification of the mixture interpretation protocol shall demonstrate that its use results in the correct inclusion of true contributors, exclusion of non-contributors, and the parameters considered in the interpretation protocols.

NOTE Parameters may include, but are not limited to, assessment of the number of contributors, and evaluation of contributor ratios.

4.4.2 Verification shall include a demonstration of consistency in the analysis and interpretation of mixed DNA data among analysts in the laboratory or laboratory system.

4.4.3 Verification shall be performed on new, existing, and modified mixture interpretation protocols.

4.4.4 Verification of the protocol shall be performed by individuals in a blinded manner without knowledge of the expected results.

5 Conformance

Documented conformance to these requirements need to be: (1) approved by the laboratory's DNA Technical Leader or other appropriate personnel (2) communicated to all analysts during training, and (3) made readily available for review (e.g., by auditors or inspectors, stakeholders who use reports generated by the DNA mixture test protocols and parameters, etc.).

Annex A
(informative)

Foundational Principles

Quality internal validation studies provide the critical foundational data needed for the development and implementation of protocols for DNA mixture interpretation. The standards stated here are for the: 1) design and evaluation of internal validation studies that shall be used by a DNA testing laboratory for the development of the interpretation protocol(s), to include establishing limitations for the types of samples to be tested and data to be interpreted (Sections 4.2 and 4.3); and 2) verification and documentation that the protocol developed from the completed validation studies generates reliable and consistent interpretations and conclusions for the types of mixed DNA samples typically encountered by the laboratory (Section 4.4). Standards with specific requirements for the final interpretation protocol are not provided in this document; those standards are included in ASB Standard 040, entitled "Standards for Forensic DNA Interpretation and Comparison Protocols". While this standard may also be used for the partial validation of probabilistic genotyping software, additional standards for the validation of probabilistic genotyping software and protocols for statistical analyses are included in ASB Standard 018, "Validation Standards for Probabilistic Genotyping Systems." See ASB standards relating to DNA for additional information regarding assessing consistency in the laboratory.

This document is intended for use in conjunction with the Federal Bureau of Investigation's *Quality Assurance Standards for Forensic DNA Testing Laboratories* [8] and with any future standards approved for the development and implementation of protocols for DNA data interpretation and comparison. It is the intent that this standard be applied to any existing interpretation and comparison protocol and that the protocol be revised as needed. Laboratories are advised to review their previous validation for compliance with these requirements, supplement validation data where necessary, and modify existing protocols accordingly. Any subsequent modifications to any DNA testing protocol shall include an evaluation for its impact on the DNA interpretation protocol and these requirements.

It is the intent of this document that if no suitable internal validation studies exist to support an interpretation of mixed DNA data, then: 1) the appropriate studies shall be conducted; 2) a protocol shall be developed based on the validation studies; 3) the protocol shall be verified for reliability and consistency within the laboratory prior to the interpretation of the DNA data; and 4) the appropriate authority(ies) within the laboratory shall approve the validation studies and protocol validation to ensure that this standard is sufficiently met. The proper use of an adequately detailed protocol that is tightly connected to internal validation studies and addresses the expected variables of DNA data ensures more consistent and reliable interpretation, comparison, and reporting by all members of the laboratory.

This document applies to any type of DNA testing technology and methodology used, including but not limited to, STR testing, DNA sequencing, SNP testing, haplotype testing, traditional and rapid protocols, etc., where mixtures of DNA may be encountered, analyzed interpreted and compared. Any terminology used in these requirements that suggests one type of testing or data should be understood to apply to all other DNA testing or data (e.g., STR profile vs. sequence), where appropriate.

Annex B
(normative)

Requirements – Supporting Information

The following additional information is provided to aid laboratory personnel responsible for conducting and evaluating validation studies, the development of the DNA interpretation protocol for the laboratory, and the validation of the laboratory protocol, as well as anyone responsible for assessing if the requirements are sufficiently met by the laboratory. It is recognized that each laboratory performing DNA testing has individual case- and sample-acceptance policies and uses different technologies, methods, and protocols to generate DNA data. Specific studies conducted and approaches used, the type of data evaluated, and the details of the protocols will vary between laboratories. Repeated testing and data analysis are critical to the understanding of variability. While specific requirements for the minimum number of studies and sample sets used for the validation studies and the verification process are not detailed in this standard, the laboratory shall perform sufficient replicate studies to address the variability inherent to the various aspects of DNA testing, data generation and the analysis and interpretation of the data. Additional guidance may be available in the references provided in the Bibliography (See Annex C).

Section 4.2—The samples used for mixture validation studies shall include a range of DNA input, from low template (i.e., where stochastic effects occur) to high template, and a range of contributor numbers and ratios. Some mixtures shall be constructed to provide data with high vs. low allele sharing. (e.g., by combining DNA from close relatives such as a parent and a child). Using known samples created under specified conditions permits the laboratory to assess observed versus expected data (e.g., for STR testing, genotypes detected, peak heights with associated ratios of input DNA and allele sharing, and estimated number of contributors) and determine the accuracy and limitations of the testing and interpretation parameters (e.g., detection/analytical threshold, stochastic threshold, peak height ratios, number of contributors). Validation sample mixture ratios should be based on the DNA template amounts of the individual contributors, rather than biological fluid amounts, to provide more consistent sample preparation and evaluation. The phrase "dynamic range of the detection platform" is intended to cover all options permitted in the laboratory for the use of the specific detection platform with the range of samples tested in the laboratory. For example, a laboratory using capillary electrophoresis for detection of results must validate all permitted injection times, voltages, sample input volumes, etc. and have appropriate verified interpretation protocols for all available parameters. If the laboratory intends to interpret DNA mixture data resulting from testing degraded DNA, the laboratory shall conduct internal validation studies with mixtures of degraded DNA and/or use documented data generated from other source(s) using the same testing system and parameters as support for the interpretation and comparison protocol. Internal validation studies conducted with known inhibitors added are only required if the laboratory will typically be testing DNA samples containing the known inhibitor and that inhibitor has an effect on the data generated with the test system used.

If the laboratory protocol allows for the interpretation of mixed DNA data for up to four contributors, the supporting validation studies shall include mixed DNA samples from two, three, and four contributors. This does not preclude including a subset of mixtures in the validation that contain more than four contributors. If the laboratory has not performed any validation studies using five- or more-person mixtures, the mixture interpretation protocol shall state that only DNA samples assumed to contain four or fewer contributors and no mixed DNA data assumed to contain five or more contributors may be interpreted.

Section 4.3—The data from the validation studies shall be the basis for the interpretation parameters and protocol developed by the laboratory and shall provide guidance for interpreting all types of mixtures including, but not limited to, those DNA samples that contain limited data, potential stochastic effects, and high numbers of contributors. The results of the studies shall be used to establish an assumed number of contributors to a DNA mixture if necessary for interpretation and comparison. Limitations on data interpretation and criteria for declaring DNA data unsuitable for interpretation or comparison shall be defined in the protocol and shall be based on the validation studies performed in the laboratory along with information or data from published scientific literature from other appropriate scientific resources, where available. The validation summary shall include documentation that makes it clear how the validation studies performed led to the parameters described in the interpretation protocol.

Section 4.4—For verification of the mixture interpretation protocol, the laboratory shall use data (e.g., electropherograms, sequences) from DNA mixtures generated and processed under similar testing conditions to those routinely used by the laboratory. The data for all contributors to the DNA mixtures used in the verification shall be known and available for the assessment of the data and the proposed interpretation protocol. DNA mixture data from different sets of contributors than used in the initial validation studies shall be used to verify the protocol. These supplemental mixtures shall span the range of data anticipated to be interpreted by the laboratory. The demonstration that the use of the various testing and interpretation parameters (e.g., variable injection times, number of contributors, mixture ratios) available in the laboratory protocol result in correct conclusions (i.e., inclusion of true contributors and exclusion of non-contributors to the mixture, and consistency for making "inconclusive" determinations) is required for verification of the protocol. The validation of the protocol shall be completed prior to implementation of the protocol for casework. The laboratory shall define the acceptable range of variability in the interpretation of DNA mixtures for use in the evaluation of the consistency within the laboratory. Additional validation studies and/or protocol development shall be necessary if deficiencies in the protocol or inconsistencies within the laboratory are identified through this verification process.

Annex C
(informative)

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