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**Standard for Forensic DNA Interpretation and  
Comparison Protocols**

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# Standard for Forensic DNA Interpretation and Comparison Protocols

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## Foreword

Detailed and comprehensive DNA interpretation and comparison protocols are needed to ensure reliable and consistent interpretation and comparison of DNA data from single source and mixed DNA samples. This document describes specific requirements for a laboratory's DNA interpretation and comparison protocol(s) and provides direction for its development. These requirements include distinguishing single source data from mixed data, defining assumptions that may be used, defining limitations of the interpretation methods and determining when data are unsuitable for interpretation or comparison based on the laboratory's internal validation studies, published scientific literature and other appropriate scientific resources where available. A requirement for a documented policy to ensure that evidentiary data are interpreted prior to comparison to known reference data is provided. The goal is for the laboratory to consistently produce reliable, repeatable and reproducible interpretations and conclusions that are supported by internal validation data and laboratory protocols.

This document should be used in conjunction with ASB Standard 20 "Standards for Validation Studies of DNA Mixtures and Development and Verification of a Laboratory's Mixture Interpretation Protocol" and the standard approved by the OSAC and submitted to the ASB, ASB Standard 18 "Validation Standards for Probabilistic Genotyping Systems."

This standard was revised, prepared, and finalized by the DNA Consensus Body of the AAFS Standards Board. The draft of this standard was developed by the Biology/DNA Biological Data Interpretation and Reporting Subcommittee of the Organization of Scientific Area Committees. All hyperlinks and web addresses shown in this document are current as of the publication date of this standard.

**Keywords:** *DNA mixture, mixture interpretation, comparison, protocols, policy, procedure, internal validation, best practices*

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# Standard for Forensic DNA Interpretation and Comparison Protocols

## 1 Scope

This document provides requirements for a laboratory's DNA interpretation and comparison protocol. A protocol is needed for any DNA testing methodology that includes data interpretation and/or comparison. The protocol should encompass all variables permitted in the technical protocols that may have an impact on the data generated and the variety and range of test data anticipated in casework based on the types of samples routinely accepted and tested in the laboratory.

## 2 Normative References

There are no normative references for this standard. Annex C, Bibliography, contains informative references.

## 3 Terms and Definitions

### 3.1

#### **comparison**

The process of examining two or more DNA data sets to assess the degree of similarity or difference.

### 3.2

#### **evidentiary data**

Data derived from biological specimens of unknown source.

### 3.3

#### **internal validation**

1) In general, the accumulation 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. 2) In the context of probabilistic genotyping, the accumulation of test data within the laboratory to demonstrate that established parameters, software settings, formulae, algorithms and mathematical functions perform as expected; and that the information/results/data obtained is correct and consistent with expected values.

### 3.4

#### **interpretation**

The process of evaluating DNA data for purposes including, but not limited to, defining assumptions related to mixtures and single source profiles, distinguishing between alleles and artifacts, assessing the possibility of degradation, inhibition, and stochastic effects, and determining whether the data are suitable for comparison.

### 3.5

#### **reference data**

Data derived from biological specimens of a known individual.

### **3.6**

#### **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 (a non-allelic peak in the stutter position exceeding the stutter expectation of the laboratory).

### **3.7**

#### **unsuitable for comparison**

Data that cannot be used for comparisons for reasons including, but not limited to, poor or limited data quality, mixture complexity, or a failure to meet quality assurance requirements.

## **4 Requirements**

**4.1** The laboratory interpretation protocols and comparison protocols, including criteria for drawing conclusions from comparisons between evidentiary data and reference (or other evidentiary) data, shall be based on, developed from, and supported by internal validation studies.

NOTE Published scientific literature or other appropriate scientific resources, where available, may supplement internal validation studies.

NOTE Refer to Annex B, Requirements - Supporting Information, for additional information on the requirements in this section.

**4.2** The laboratory shall maintain and follow documented DNA interpretation protocols that address the following.

**4.2.1** Criteria for assessing the DNA data as originating from a single source or multiple sources.

**4.2.2** Criteria upon which assumptions may be made and the types of assumptions that may be used in data interpretation including, but not limited to, the number of contributors and the presence of assumed contributors.

**4.2.3** Criteria for evaluating other considerations used in the interpretation of the data, such as the presence of major and minor contributors, the possibility of allele sharing, the relative mixture ratio for contributors, the possibility of inhibition or degradation for one or more contributors, the possibility of stochastic effects, and the presence of stutter.

**4.2.4** The limitations of the interpretation methods used such as characterizing and defining the maximum number of contributors, and issues associated with low-level data, low-level contributors and potential contamination events.

**4.2.5** Criteria for defining what are interpretable data versus data that cannot be interpreted.

**4.2.6** Criteria for defining data that are suitable for comparison versus data that are unsuitable for comparison.

**4.3** The laboratory shall have a documented policy requiring the interpretation of evidentiary data and documentation of any interpretation, including all assumptions used, prior to the comparison to any reference data.

**4.3.1** Interpretation of evidentiary data shall include documentation of the suitability of the single source or DNA mixture data for comparison.

**4.3.1.1** If the data or a subset of the data [e.g., major contributor(s)] are deemed suitable for comparison, the loci eligible for use in the comparison and in a subsequent statistical calculation(s) shall be documented in the case record.

**4.3.1.2** If the data or a subset of the data [e.g., minor contributor(s)] are deemed unsuitable for comparison, the qualitative reason(s) shall be documented in the case record.

**4.3.2** The subsequent interpretation of new evidentiary data shall be done by completing the interpretation and its documentation prior to comparison to any previously generated reference data.

**4.3.3** When an assumption of an expected contributor is used for interpretation, the use of that assumption shall be documented in the case record along with the DNA data of the assumed contributor.

**4.4** The laboratory shall maintain and follow documented protocols for drawing conclusions from the comparison of suitable evidentiary data derived from single source, mixed, and limited quality/quantity samples to reference (or other evidentiary) data.

**4.4.1** Laboratory protocols shall describe the criteria used for concluding that the source of the reference data is included, excluded, or inconclusive when compared to evidentiary data when those terms are used by the laboratory. If a comparison is deemed inconclusive, the reason(s) shall be documented in the case record.

**4.4.2** All re-evaluations of, and changes to, the original evidentiary data interpretation shall be thoroughly documented within the case record. The laboratory shall have protocols that address re-evaluation of evidentiary data after the comparison to reference (or other evidentiary) data has been performed.

## **Annex A**

(informative)

### **Foundational Principles**

The evaluation and interpretation of any DNA data, from a single individual as well as from DNA mixtures, and the comparison of that data to other DNA data are critical components of all forensic DNA testing. Detailed protocols for the interpretation and comparison of DNA data based on sound validation studies provide test results and conclusions that are reliable and consistent to customers of forensic science service providers.

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.

Additional standards regarding biological relationship testing are available through the American Association of Blood Banks (AABB).



## **Annex B**

### **(informative)**

#### **Requirements—Supporting Information**

It is the intent of this document that any DNA data: 1) that fall outside the acceptable range of the interpretation and/or comparison method employed; 2) for which no suitable/appropriate documented protocol exists; or 3) for which no suitable internal validation studies exist to support the method, will not be interpreted or compared by the laboratory until the standards are sufficiently met and approved by the appropriate authority(ies) within the laboratory. Having an adequately detailed protocol tightly connected to internal validation studies that addresses the expected variables of DNA data ensures more consistent and reliable interpretation, comparison, and reporting by all members of the laboratory.

The following information is provided to aid both the personnel responsible for developing the DNA interpretation protocols for the laboratory and anyone responsible for assessing if the requirements are sufficiently met by the laboratory. It is recognized that each laboratory performing DNA testing is required to conduct its own internal validation studies to assist in defining the limitations of the testing. In addition, each laboratory has individual case and sample acceptance policies and uses different technologies, methods, and protocols to generate DNA data. While each of the requirements listed shall be addressed in the development and use of the laboratory interpretation protocol(s), the approaches used, the type of data evaluated, and the details of the protocols will vary between laboratories.

This document is organized in a manner intended to mirror the chronology of DNA data interpretation. First, DNA data interpretation and comparison protocols are derived from developmental and internal validation data (Section 4.1), after which the interpretation protocols are applied to evidentiary DNA data. In casework analyses, the DNA data from evidentiary samples will be assessed in accordance with the limitations defined in the protocol to determine whether the data (either in part or as a whole) are suitable or unsuitable for interpretation and comparison (Section 4.2). This assessment shall be performed prior to any comparisons to reference data (Section 4.3). Once DNA data (or a portion thereof) have been deemed suitable for comparison, comparisons may be performed to reference or other evidentiary data (Section 4.4). When comparisons are made between sets of data, one of three conclusions may be drawn: (1) The DNA may have originated from the same source; (2) The DNA did not originate from the same source; or (3) no conclusion can be drawn (i.e., the comparison is inconclusive due to insufficient criteria to either include or exclude). Additional details pertaining to specific requirements are described below.

Section 4.1 - This section is intended for use in conjunction with the ASB Standard 20 “Standards for Validation Studies of DNA Mixtures and Development and Verification of a Laboratory’s Mixture Interpretation Protocol”, the standard approved by the OSAC and submitted to the ASB, ASB Standard 18 “Validation Standards for Probabilistic Genotyping Systems” and the “Quality Assurance Standard for Forensic DNA Testing Laboratories.” Additional guidance for developing protocols responsive to this standard may be available in publications on the SWGDAM website (see Bibliography).

Section 4.2.5 and 4.2.6 - Samples in their entirety or an unresolvable subset of the data (e.g., multiple minor contributors to a mixture with a single major contributor) may not meet the laboratory's criteria for interpretation or for comparison.

Section 4.3.1.2 – When making this determination, the qualitative reasons for reaching this conclusion shall be documented in the case record. These qualitative reasons may include, but are not limited to, data that are *too limited* due to the possibility of allelic drop-out, degradation, preferential amplification, and/or masking of minor alleles by the major donor or in stutter positions; data that are *too complex* due to the total number of possible contributors present, the possibility of allele sharing between multiple contributors, and/or the possibility of allelic dropout of lower level contributors; and/or data associated with contamination or control failure.

Section 4.3.2 - It is recognized that the analysis of supplemental evidentiary data may occur after the reference data have been interpreted. The analysis and interpretation of the new evidentiary data shall occur prior to comparison to the previously generated reference data.

Section 4.4.1 – The ambiguity of whether reference data are represented in the evidentiary data may result in the inability to draw a conclusion from the comparison. There are multiple possible causes for an inconclusive comparison, which may include qualitative factors (e.g., alleles being in stutter positions, masking or sharing of alleles, allelic dropout, inhibition, or degradation) or uninformative statistical values, as defined by the laboratory. When making this determination, the underlying reasons for reaching this conclusion shall be documented in the case record.

Section 4.4.2 – After completion of the initial interpretation of evidentiary data, additional DNA data may be used as a basis for re-interpretation (e.g., the use of a non-sperm fraction to inform a sperm fraction interpretation, learning that one of the known or possible contributors is tri-allelic or has a null allele at a locus, some of the possible contributors are related and/or the extent of degradation of the DNA for one contributor). Any re-interpretation of evidentiary data that occurs at any time shall be documented in the case record to include the reasons for the re-interpretation.

**Annex C**  
(informative)

**Bibliography**

- 1] Federal Bureau of Investigation, *Quality Assurance Standards for Forensic DNA Testing Laboratories*, available at <https://www.swgdam.org/publications>
- 2] Publications available at <https://www.swgdam.org/publications>
- 3] ASB Standard 018, *Validation Standards for Probabilistic Genotyping Systems*
- 4] ASB Standard 020, *Standards for Validation Studies of DNA Mixtures for the Development and Verification of a Laboratory Mixture Interpretation Protocol*
- 5] AABB Standards available at [www.AABB.org](http://www.AABB.org)

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