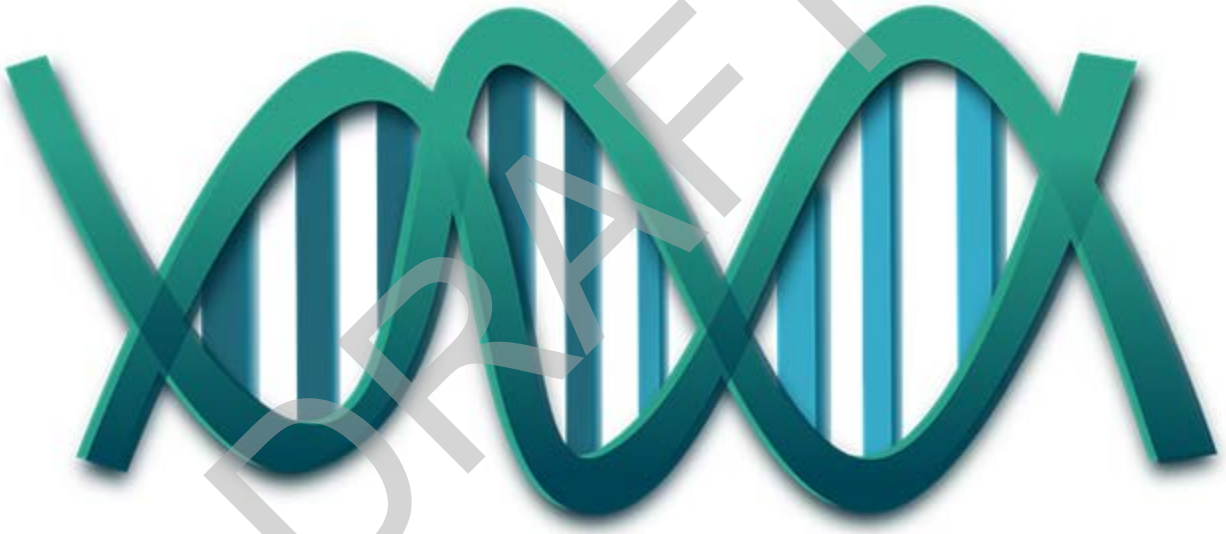
This document has been accepted by the Academy Standards Board (ASB) for development as an American National Standard (ANS). For information about ASB and their process please refer to asb.aafs.org. This document is being made available at this stage of the process so that the forensic science community and interested stakeholders can be more fully aware of the efforts and work products of the Organization of Scientific Area Committees for Forensic Science (OSAC). The documents were prepared with input from OSAC Legal Resource Committee, Quality Infrastructure Committee, and Human Factors Committees, as well as the relevant Scientific Area Committee. The content of the documents listed below is subject to change during the standards development process within ASB, and may not represent the contents of the final published standard. All stakeholder groups or individuals, are strongly encouraged to submit technical comments on this draft document during the ASB's open comment period. Technical comments will not be accepted if submitted to the OSAC Scientific Area Committee or Subcommittees.

Standards for Forensic DNA Interpretation and Comparison Protocols
DRAFT

DRAFT DOCUMENT

ASB Standard XXXX
2016

Standards for Forensic DNA Interpretation and Comparison Protocols



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STANDARDS FOR FORENSIC DNA INTERPRETATION AND COMPARISON PROTOCOLS

<ASB and ANSI approval dates>

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

ABSTRACT

This document describes requirements for a laboratory's DNA interpretation and comparison protocol and provides direction for its development. The goal is for the laboratory to consistently produce reliable, repeatable and reproducible interpretations and conclusions that are supported by internal validation data.

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 regardless of the possible variables affecting the DNA data. Specific requirements for a laboratory's protocol for the interpretation and comparison of DNA data are provided. These requirements include distinguishing single source data from mixed data, and defining assumptions that may be used, limitations of the interpretation methods and when data are unsuitable for interpretation 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. This standard was developed by the Biology/DNA Biological Data Interpretation and Reporting Subcommittee of the Organization of Scientific Area Committees. *[to be added at the ASB level: It was prepared and finalized as a standard by the DNA Consensus Body of the ASB.]*

These standards should be used in conjunction with the standards previously approved by the OSAC and submitted to the ASB, entitled "Standards for Validation Studies of DNA Mixtures and Development and Verification of a Laboratory's Mixture Interpretation Protocol." [1]

[1] Note: this paragraph will need modification at the ASB level once the other document has been moved through the process.

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1. Scope

This document provides requirements for a laboratory's DNA interpretation and comparison protocol.

2. Normative References

There are no normative references for these standards.

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.
drop-in
(1) Allelic peak(s) in an electropherogram that are not reproducible across multiple independent amplification events. (2) A hypothesis/postulate for the observation of one or more allelic peaks in an electropherogram that are inconsistent with the assumed/known contributor(s) to a sample.

3.3
drop-out
(1) Failure of an otherwise amplifiable allele to produce a signal above analytical threshold because the allele was not present or was not present in sufficient quantity in the aliquot that underwent PCR amplification. Also known as allelic dropout. (2) A hypothesis/postulate for the failure to observe one or more allelic peaks in an electropherogram that are expected for the assumed contributor(s) to a sample.

3.4
evidentiary data
Data derived from biological specimens of unknown source.

3.5
inconclusive comparison
A comparison for which there is insufficient support for inclusion or exclusion.

3.6
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.

3.7
interpretation
The process of evaluating DNA data for purposes including, but not limited to, determining whether the data are suitable for comparison, the possible presence of a mixture, the probable number of contributors, mixture ratios, distinguishing between alleles and artifacts, and assessing the possibility of degradation, inhibition, and stochastic effects.

3.8
mixture
DNA typing results originating from two or more individuals.

3.9
protocol
A specified way to carry out an activity or a process.

3.10
reference data
Data derived from biological specimens of known identity.

3.11
stochastic effects
Stochastic effects are observed in a PCR-based DNA electrophoretic profile at one or more loci; potentially a result of sampling variation (e.g. pipetting) of the target DNA that goes into the PCR as well as random events between primers and target DNA during PCR amplification. Stochastic effects generally occur when suboptimal or limiting quantities of DNA are tested. The observed effects 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.

3.12
unsuitable for comparison (uninterpretable)
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 shall be developed from and supported by internal validation studies and may be supplemented with published scientific literature or other appropriate scientific resources, where available.

4.2 The laboratory shall maintain and follow documented DNA interpretation protocols that address:

4.2.1 When DNA data should be interpreted as originating from a single source versus multiple sources;

4.2.2 When 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 known contributors;

4.2.3 The evaluation of 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, 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 (e.g., characterizing and defining the maximum number of contributors, issues associated with low-level data and low-level contributors, tolerance for contamination, etc.);

4.2.5 What constitutes interpretable data versus data that are unsuitable for comparison/uninterpretable.

4.3 The laboratory shall have a documented policy requiring the interpretation of evidentiary data 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 occur independently of comparison to the previously generated reference data.

4.3.3 When an assumption of a known contributor is used for interpretation, it shall be documented in the case record.

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.

4.4.1.1 Criteria for drawing conclusions from comparisons between evidentiary data and reference (or other evidentiary) data shall be based on the laboratory's internal validation studies and may be supplemented with published scientific literature or other appropriate scientific resources, where available.

4.4.1.2 If a comparison is deemed inconclusive, the reason(s) shall be documented in the case record.

4.4.2 The laboratory shall have protocols that address re-evaluation of evidentiary data after the comparison to reference (or other evidentiary) data has been performed.

4.4.2.1 All re-evaluations of and changes to the original evidentiary data interpretation shall be thoroughly documented within the case record.

5. Conformance

Documentation demonstrating conformance with the standards described here will be approved by the laboratory's DNA Technical Leader or other appropriate personnel and will be made readily available in hard copy and/or electronic format for review.

Annex A
(informative)

Foundational Principles

The evaluation and interpretation of DNA data, from single individuals as well as from complex DNA mixtures, and the comparison of that data to other DNA data are critical components of 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.

A.1 Requirements – Additional Information (informative)

It is the intent of these standards that any DNA data: 1) that falls 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 standards 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. While each of the standards 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.

These standards are organized in a manner intended to mirror the chronology of DNA data interpretation. First, DNA data interpretation protocols are developed from validation data (Standard 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 to determine whether the data (either in part or as a whole) are interpretable, or unsuitable for comparison (Standard 4.2). This assessment shall be performed independent of any comparisons to reference data (Standard 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 (Standard 4.4). When comparisons are made to reference data, one of three conclusions may be drawn: (1)The source of the reference data is included as a possible contributor to the DNA data; (2) the source of the reference data is excluded as a contributor to the DNA data; or (3) no conclusions can be drawn as to whether the source of the reference data is or is not a possible contributor to the DNA data (termed an inconclusive comparison). Additional details pertaining to specific standards are described below.

Standard 4.1 - These standards are intended for use in conjunction with "Standards for Validation Studies of DNA Mixtures and the Development and Verification of a Laboratory's Mixture Interpretation Protocol" and the "Quality Assurance Standards for Forensic DNA Testing Laboratories." Additional guidance is available in the SWGDAM "Interpretation Guidelines for Autosomal STR Typing by Forensic DNA Testing Laboratories" (see Bibliography).

Standard 4.2.5 - 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 be determined to be uninterpretable and therefore not suitable for comparison.

Standard 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 profile 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 contamination or control failure.

Standard 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 and independent of comparison to the previously generated reference data.

Standard 4.4.1.2 – The ambiguity of whether reference data is represented in the evidentiary data may result in an inconclusive 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.

Standard 4.4.2 – After completion of the initial evaluation and 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 after comparison shall be documented in the case record, to include the reasons for the re-interpretation.

Annex B
(informative)

Bibliography

Federal Bureau of Investigation, (2011) *Quality Assurance Standards for Forensic DNA Testing Laboratories*, available at https://www.fbi.gov/file-repository/qas-audit-for-forensic-dna-testing-laboratories.pdf/view

SWGDAM. *Interpretation Guidelines for Autosomal STR Typing by Forensic DNA Testing Laboratories.* It is available at https://www.fbi.gov/file-repository/codis_swgdam.pdf/view