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Standard for Routine Internal Evaluation of a Laboratory's DNA Interpretation and Comparison Protocol

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2410 North 21st Street
Colorado Springs, CO 80904

Foreword

This standard provides the laboratory and a laboratory system with a method for the annual evaluation of its developed, verified and implemented interpretation and comparison protocol as well as its consistent use by all individuals required to adhere to the protocol. An effective and continued evaluation of the application of the laboratory's interpretation and comparison protocol is essential for monitoring and addressing protocol clarity and drift, analyst-to-analyst variation and variation between laboratories within a larger laboratory system.

Compliance with this standard allows the laboratory to demonstrate reliable and reproducible DNA interpretation, comparison and reporting by each participant in the evaluation of profiles that range in complexity and are representative of those encountered in casework. Equally, it allows laboratory technical leaders, quality assurance managers or other appropriate staff to take the actions necessary to address any inconsistencies among staff and make modifications and improvements to the laboratory protocol in accordance with its quality system and other related standards.

Currently, external proficiency test providers do not offer DNA profiles or samples that allow for the evaluation of all components of the laboratory DNA interpretation and comparison protocol. Thus, DNA data for the annual evaluation and consistent use of a laboratory's DNA protocol may be best provided from within a laboratory or shared between laboratories using similar analytical processes. Compliance with this standard is independent of and separate from the compliance with external proficiency test requirements.

While this standard applies directly to capillary electrophoresis-based STR DNA testing, the requirements of this standard may be applied to the evaluation of the reliability and consistent use of any laboratory interpretation and comparison protocol regardless of the methodology employed.

The American Academy of Forensic Sciences established the Academy Standards Board (ASB) in 2015 with a vision of safeguarding Justice, Integrity and Fairness through Consensus Based American National Standards. To that end, the ASB develops consensus based forensic standards within a framework accredited by the American National Standards Institute (ANSI) and provides training to support those standards. ASB values integrity, scientific rigor, openness, due process, collaboration, excellence, diversity and inclusion. ASB is dedicated to developing and making freely accessible the highest quality documentary forensic science consensus Standards, Guidelines, Best Practices, and Technical Reports in a wide range of forensic science disciplines as a service to forensic practitioners and the legal system.

This document was revised, prepared, and finalized as a standard by the DNA Consensus Body of the AAFS Standards Board. The draft of this standard was developed by the Biology/DNA Interpretation and Reporting Subcommittee of the Organization of Scientific Area Committees (OSAC) for Forensic Science.

Questions, comments, and suggestions for the improvement of this document can be sent to AAFS-ASB Secretariat, asb@aafs.org or 401 N 21st Street, Colorado Springs, CO 80904.

All hyperlinks and web addresses shown in this document are current as of the publication date of this standard.

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Keywords: *DNA, mixtures, interpretation, comparison, protocol, internal evaluation, intra-laboratory consistency, inter-laboratory consistency.*

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Standard for Routine Internal Evaluation of a Laboratory's DNA Interpretation and Comparison Protocol

1 Scope

This standard provides the requirements for the technical leader (or appropriate personnel) to: 1) routinely evaluate the consistent application of the developed, verified and implemented DNA interpretation and comparison protocol within a laboratory and laboratory system; and 2) assess whether the DNA interpretation and comparison protocol is appropriately and consistently used to produce reliable and reproducible interpretations and comparisons. This standard addresses the development of an internal evaluation system, including proper format of data, types of data to use, frequency of evaluation, and how to assess results.

This standard applies directly to capillary electrophoresis-based STR DNA testing, but may also be applied as appropriate to laboratories using other DNA testing methods. This standard applies to manual/binary interpretation and comparison methods as well as methods using software as part of the analysis, interpretation, comparison and/or for generation of statistical statements.

2 Normative References

There are no normative references. Annex A (Bibliography) contains informative references.

3 Terms and Definitions

For purposes of this document, the following definitions apply.

3.1
administrator
The individual who oversees, but does not participate in, the evaluation; this will usually be, but is not restricted to, the technical leader.

3.2
comparison
The process of examining two or more DNA data sets to assess the degree of similarity or difference.

3.3
consistent
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.

3.4
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
stochastic effects
Changes in a DNA profile that generally occur when suboptimal or limiting quantities of DNA are tested.

NOTE 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).

3.6
technical leader
An employee who is accountable for the technical operations of the laboratory and who is authorized to initiate, suspend, and resume laboratory operations.

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 shall administer this internal evaluation program annually.

All proficiency-tested individuals in the laboratory who are performing mixed DNA data interpretation and/or comparisons including analysts, technical reviewers, and the technical leader, shall fulfill the requirements of this standard a minimum of once every two years. A minimum of half of these individuals need to fulfill this requirement every year in addition to the annual proficiency testing requirements.

NOTE If the number of examiners (n) is odd, then (n + 1)/ 2 and (n - 1)/ 2 will be tested in alternate years [(e.g., if there are 7 examiners, then 4 examiners will be tested in year one, and 3 will be tested in year two (or vice versa)].

4.1.1 The laboratory shall define, by written laboratory protocol, the overall goals of the evaluation program and the frequency with which each technology, kit, and protocol used within the laboratory will be evaluated using this standard.

4.1.2 The administrator of each internal evaluation shall define which technology, kit, and protocol(s) are included in each evaluation.

When the technical leader (or equivalent) is participating in the internal evaluation, the technical leader shall assign another person to take on the administrator role.

4.1.3 Prior to each evaluation, the laboratory shall document the scope of the evaluation, including the number and types of profiles to be included based on the specific criteria being evaluated.

NOTE While this standard generally applies to mixed DNA data, the laboratory is encouraged to also include single source DNA profiles exhibiting stochastic effects in the evaluation.

4.2 Mixed DNA data under evaluation shall be provided to each individual participating in the evaluation, and shall meet the following criteria.

a) Provided in electronic format as raw data files.

b) Developed by the laboratory and/or accepted from another laboratory using the same amplification kit, analytical instrument, and parameters as the recipient laboratory.

c) Previously generated or specifically produced for the purpose of evaluating the protocol; however, the data used shall not have been previously interpreted by the individual(s) participating in this evaluation.

d) Of known origin generated from mixed DNA samples having a known number of contributors and profile of each contributor. This information can be used to determine the range of acceptable answers given the appropriate application of the laboratory's interpretation and comparison protocol (see 4.4).

e) Interpreted and compared using the current laboratory protocol for interpretation and comparison.

f) Representative of the types of DNA profiles encountered in casework in the laboratory performing the evaluation. Specifically, mixture complexity shall include variations in template amount, contributor ratio, and number of contributors. Periodically, where the period is defined by written laboratory protocol, one or more profiles exceeding the parameters and limitations of the laboratory protocol shall be included in the internal evaluation. These variations shall span the range of the types and complexities of mixtures typically encountered by the laboratory in casework with a focus on the limitations of the testing procedures and interpretation of data, including high quality and poor quality profiles. These test profiles shall not be duplicative of the types of profiles currently resulting from commercially-available proficiency tests and shall include profiles which exhibit stochastic effects. The administrator should also include profiles that meet certain criteria and/or require the use of certain steps of the protocol for more in depth investigation within the laboratory.

   These may include DNA profiles:

   — exhibiting various stochastic effects (e.g., drop-out, drop-in, elevated stutter, peak height imbalance);

   — with major and minor contributors (e.g., single vs. multiple major contributors, multiple minor contributors);

   — where all contributors are exhibiting stochastic effects;

   — with related individuals (e.g., father/daughter);

   — requiring different propositions/conditioning (assuming);

   — that are uninterpretable or unsuitable for comparison;

— that are mixtures requiring subjective decisions and/or analyst/individual discretion, if any exist in the protocol;

— that exceed the parameters and limitations of the laboratory protocol.

NOTE Based on the types of profiles routinely interpreted and compared, level of training, types of samples and profiles expected to be encountered in casework, areas of interest in the protocol for evaluation, etc. different profiles may be provided to different groups of analysts and/or individuals on various teams.

4.3 Known reference data shall be provided for interpretation and comparison (e.g., assumed contributors for proposition building and/or deducing second contributors; contributors and non-contributors for comparison). The known reference data profiles shall be provided as electronic data or as typed profiles as appropriate based on the laboratory's protocol and the scenario being evaluated.

4.4 The administrator of the evaluation shall clearly define the acceptable range of variability in the interpretation of the data based on the known genotypes and ratios of contributors to the DNA profile provided. The administrator shall also clearly define the correct assumptions and the acceptable outcomes of the comparisons prior to the start of the evaluation. The evaluation shall ensure the loci and data chosen for interpretation, comparison and statistical analysis are correct and consistent with the laboratory's protocol and that any other relevant portions of the protocol were appropriately applied during the evaluation.

4.4.1 The administrator shall ensure the data were appropriately interpreted, and that the assumptions and propositions made by the participants are consistent with the laboratory's protocol.

4.4.2 The responses from the participants shall include all assumptions and decisions made during the interpretation and comparison processes. These shall be reported based on the laboratory's DNA interpretation and comparison protocol and all other documentation required by the laboratory protocol.

4.5 Any element of the internal evaluation outside the acceptable range outlined in 4.4 shall be documented, evaluated to assess the root cause of the discrepancy, and addressed in accordance with the laboratory's quality system.

5 Conformance

5.1 Documentation demonstrating conformance with the requirements described here shall be approved by the laboratory's DNA technical leader or other appropriate personnel and retained by the laboratory, to include:

a) the scope of the annual evaluation;

b) the types of DNA profiles provided and the individual(s) who generated the DNA profiles;

c) the acceptable range of responses and the individual(s) who set the acceptable range of responses;

d) the protocols used during the evaluation;

e) the original documentation from each participant who performed the annual evaluation;

f) who performed the review of the annual evaluation;

g) assessment of any deviations;

h) root cause analysis performed;

i) all corrective actions implemented.

5.2 Documentation of the root cause analysis and any corrective actions implemented shall be maintained by the laboratory. Depending on the nature of the root cause, the discrepancy may necessitate one or more of the following corrective actions/remediations, such as re-training of the individual(s); modification of the protocol by providing additional detail of the procedures, clarity, and ease of use; additional validation work with subsequent modification to the protocol, protocol verification and training; audit of previous casework; suspending DNA casework; and any other appropriate measures resulting from the evaluation, depending on the laboratory's quality system.

Annex A
(informative)

Bibliography

The following information provides a list of the literature resources that may assist the DNA technical leader. This list is not meant to be all inclusive. A laboratory develops a list tailored to its specific needs. Updated references added to the laboratory's list as new methods or technologies are incorporated into the laboratory's protocols.

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2] ANSI/ASB Standard 022, *Standard for Forensic DNA Analysis Training Programs*. First Edition 2019.[1]

3] ANSI/ASB Standard 038, *Standard for Internal Validation of Forensic DNA Analysis Methods*, First Edition, 2020.[1]

4] ANSI/ASB Standard 040, *Standard for Forensic DNA Interpretation and Comparison Protocols*, First Edition, 2019.[1]

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6] FBI, *Quality Assurance Standards for Forensic DNA Testing Laboratories*.[2]

7] ISO/IEC 17025:2017. *General requirements for the competence of testing and calibration laboratories*.[3]

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[2] Available from: https://www.swgdam.org/publications

[3] Available from: https://www.iso.org/standard/66912.html

[4] Available from: https://strbase.nist.gov/interlab/MIX05/MIX05poster.pdf

12] SWGDAM. Scientific Working Group on DNA Analysis Methods, Interpretation Guidelines for Autosomal STR Typing by Forensic DNA Testing Laboratories. [5]

13] Butler, J., et al. "NIST Interlaboratory Studies Involving DNA Mixtures (MIX 05 and MIX13): Variation Observed and Lessons Learned." *Forensic Science International: Genetics*, 2018,37:81-94.

[5] Available from: https://www.swgdam.org/publications

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