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Standard for Internal Validation of Human Short Tandem Repeat Profiling on Capillary Electrophoresis Platforms

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Foreword

This document outlines the requirements for internal validation of short tandem repeat DNA profiling using STR kits on capillary electrophoresis platforms utilized in forensic laboratories.

The validation of capillary electrophoresis is one part of the process of generating a DNA result and there are steps prior to and after that need to be considered.

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 Human Forensic Biology 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.

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Standard for Internal Validation of Human Short Tandem Repeat Profiling on Capillary Electrophoresis Platforms

1 Scope

This document details requirements for performing an internal validation of a human short tandem repeat (STR) multiplex kit using capillary electrophoresis (CE) in forensic laboratories.

2 Normative References

The following normative reference is indispensable for the application of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments applies.

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

3 Terms and Definitions

For purposes of this document, the following definitions apply.

3.1
analytical threshold
The minimum height requirement at and above which detected peaks on a STR DNA profile electropherogram can be reliably distinguished from instrument background noise; peaks above this threshold are generally not considered noise and are either artifacts or true alleles.

3.2
artifact
A non-allelic product of the amplification process (e.g., stutter, non-templated nucleotide addition, or other non-specific product), an anomaly of the detection process (e.g., single or multichannel voltage spikes or "pull-up"), or a by-product of primer synthesis (e.g., "dye blob") that may be observed on an electropherogram.

3.3
contamination
Exogenous DNA or other biological material in a DNA sample, PCR reaction, or item of evidence; the exogenous DNA or biological material could be present before the sample is collected or introduced during collection or testing of the sample.

3.4
drop-in
Allelic peak(s) in an electropherogram that are not reproducible across multiple independent amplification events.

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3.5
drop-out
Failure of an otherwise amplifiable allele to produce a signal above analytical threshold because the allele was not present in sufficient quantity in the aliquot that underwent PCR amplification.

3.6
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.

3.7
peak height ratio
The relative ratio of two peaks at a given locus in a diploid heterozygous single-source sample.

3.8
precision
The degree of mutual agreement among a series of individual measurements, values and/or results.

3.9
repeatability studies
Studies to evaluate the degree of variability in multiple measurements (e.g., replicate samples) under constant conditions, such as studies by the same operator using the same equipment in the same laboratory within short intervals of time.

3.10
reproducibility studies
Studies to evaluate the degree of variability in multiple measurements (e.g., replicate samples) under varying conditions, such as studies using the same methods with different operators or different equipment.

3.11
sensitivity studies
Studies performed during developmental and/or internal validation of DNA or other test methods designed to define the lower and upper limits/bounds of an assay to accurately detect an analyte.

3.12
stochastic threshold
The peak height value in a DNA profile above which it is reasonable to assume that, at a given locus, allelic drop-out of a sister allele in a heterozygous pair has not occurred in a single source DNA sample: due to the possibility of shared alleles in mixed samples, the presence of allele peaks above the stochastic threshold is no guarantee that allele dropout did not occur in mixed DNA sample profiles.

3.13
stutter
An artifact of polymerase chain reaction (PCR) amplification typically observed one or more repeat units smaller or larger than a short tandem repeat (STR) allele in a DNA profile, may result from

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strand slippage during PCR amplification. A stutter peak is generally of lower relative fluorescence units (RFU) than the allele peak.

3.14
validation
The process of performing and evaluating a set of experiments that establishes 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. May include developmental and/or internal validation.

4 Requirements

4.1 The internal validation of STR test kits using capillary electrophoresis shall include the studies described in this section, in conjunction with ANSI/ASB Standard 038 and supported by relevant developmental validation studies. This work shall also be supplemented with published scientific literature or other appropriate scientific resources, where available. See additional materials available to include the ASB Best Practice Recommendation 129 *(not yet published, also out for public comment)* included in Annex A.

A single analysis software package (e.g., GeneMapper™[b] or GeneMarker®[c]), version and set of analysis parameters shall be used throughout the performance of studies described below. If changes are made to the analysis parameters, the impact of those changes shall be described and where needed the data reanalyzed.

4.1.1 The laboratory shall conduct an STR genotyping concordance study.

4.1.1.1 STR genotypes obtained from the new STR test kit shall be compared to genotypes obtained from the previous kit for all overlapping loci, if applicable.

4.1.1.2 This study shall include the evaluation of concordance of the new STR test kit utilizing an appropriate certified reference material for STR genotyping.

4.1.1.3 In the event that a discordant genotype is observed when comparing identical genetic markers, this discordant data shall be noted in the final validation document, and a potential explanation for the discordant data supported by data or other research studies shall be included in the final validation document.

4.1.2 The laboratory shall perform sensitivity studies that will include replicates for each set of assay parameters (e.g., PCR cycle number, injection time, injection voltage, and PCR reaction volume) that the laboratory utilizes with the new STR kit.

4.1.2.1 This study shall include a range of DNA inputs anticipated to be amplified as well as lower and higher quantities to empirically define the upper and lower limits of DNA inputs to be used for testing.

[b] This term is used as an example only, and does not constitute an endorsement of this product by the AAFS Standards Board.
[c] This term is used as an example only, and does not constitute an endorsement of this product by the AAFS Standards Board.

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4.1.2.2 DNA extracts spanning a range of DNA quality shall be included (e.g., synthetically and/or naturally degraded as well as inhibited DNA templates of known types expected to be encountered by the testing laboratory).

NOTE Optimal input ranges for single source DNA samples can be determined using the studies described in 4.1.2.1 and 4.1.2.2.

4.1.3 The laboratory shall determine the analytical threshold for each dye channel of the new STR test kit using a range of sample types and DNA input quantities across multiple analyses.

4.1.4 The laboratory shall characterize peak height ratio variation observed for each locus of the new STR test kit utilizing single source samples amplified over a range of DNA input amounts.

4.1.5 The laboratory shall characterize PCR stutter artifacts observed for each STR locus of the new STR test kit.

4.1.6 The laboratory shall determine stochastic threshold(s) for any manual binary method used to interpret the new STR test kit data.

4.1.7 The laboratory shall conduct studies utilizing mixed DNA samples having a range of DNA ratios, DNA template input quantities, and numbers of contributors with varied degrees of allele sharing expected to be interpreted by the testing laboratory.

The results of these studies shall be used to define the limits of mixture interpretation and to establish the protocols for assessing the number of contributors to be used in the interpretation, the use of mixture ratios, and distinguishing major and minor components of mixtures.

NOTE Depending on the interpretation method chosen, additional studies may need to be performed (e.g., specificity testing within probabilistic genotyping). Additional information on mixture studies and the development of interpretation protocols can be found in ASB Standard 020.

4.1.8 The laboratory shall demonstrate allelic sizing precision and calling accuracy of the new STR test kit and capillary electrophoresis instrument through repeatability and reproducibility studies.

4.1.9 The laboratory shall determine the susceptibility of the genotyping process to the introduction and detection of exogenous DNA by documenting allelic drop-in and contamination through the evaluation of controls (i.e., reagent blanks, negative and positive amplification controls) and samples with known genotypes. The laboratory shall document contamination events and calculate drop-in rates in accordance with its quality system.

4.1.9.1 The laboratory shall conduct studies utilizing known and casework-like samples with a range of sample types representative of those expected to be encountered by the testing laboratory. This study should minimally include processing a set of samples using all DNA extraction and quantification chemistries utilized by the laboratory to verify compatibility with the new STR test kit.

4.1.9.2 The results of these studies shall be used to evaluate the thresholds and other parameters that have been established during previous studies and where possible, address the limitations of the protocols to be included in the standard operating procedures for this kit.

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5 Conformance

In order to demonstrate conformance with this standard, the laboratory shall have the following:
a) documentation of all internal validation studies as listed in Section 4;
b) documented quality assurance parameters, interpretation protocols, and analytical procedures derived from internal validation studies; and
c) documented approval by the DNA Technical Leader or other appropriate personnel prior to implementation and communicated to all analysts.

All documentation and data from studies performed shall be made readily available for review (e.g., by auditors or inspectors, stakeholders who use reports generated by the DNA mixture test protocols and parameters).

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Annex A
(informative)

Bibliography

The following information provides a list of the literature resources that may assist the DNA technical leader in defining the breadth and scope of the validation materials that may be useful. This list is not meant to be all inclusive. The laboratory shall develop a list tailored to its specific needs. Updated references shall be added to the laboratory's list as new methods or technologies are incorporated into the laboratory's protocols.

1] ANSI/ASB Standard 018, Standard for Validation of Probabilistic Genotyping Systems, First Edition, 2020[d].

2] ANSI/ASB Standard 020, *Standard for Validation Studies of DNA Mixtures, and Development and Verification of a Laboratory's Mixture Interpretation Protocol*, First Edition 2018[a]

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

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

5] ASB Std 129 Reference to be added upon publication.

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10] FBI, *Quality Assurance Standards for DNA Databasing Laboratories*. Effective July 1, 2020[f].

11] FBI, *Quality Assurance Standards for Forensic DNA Testing Laboratories*. Effective July 1, 2020[g].

[d] Available from: https://www.aafs.org/academy-standards-board
[e] Available from: http://enfsi.eu/wp-content/uploads/2016/09/minimum_validation_guidelines_in_dna_profiling_-_v2010_0.pdf
[f] Available from: https://docs.wixstatic.com/ugd/4344b0_809d01b3e9f9451cb9edd9a85f2c2e5b.pdf
[g] Available from: https://docs.wixstatic.com/ugd/4344b0_6782472e073442ec877085584aaffa36.pdf

12] SWGDAM. *SWGDAM Guidelines for STR Enhanced Detection Methods*[h].

13] SWGDAM. *SWGDAM Interpretation Guidelines for Autosomal STR Typing by Forensic DNA Testing Laboratories*[i].

14] SWGDAM. *SWGDAM Interpretation Guidelines for Y-Chromosome Testing*[j].

15] SWGDAM. *SWGDAM Validation Guidelines for Forensic DNA Analysis Methods*[k].

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[h] Available from: http://media.wix.com/ugd/4344b0_29feed748e3742a5a7112467cccec8dd.pdf
[i] Available from: https://docs.wixstatic.com/ugd/4344b0_50e2749756a242528e6285a5bb478f4c.pdf
[j] Available from: http://media.wix.com/ugd/4344b0_da25419ba2dd4363bc4e5e8fe7025882.pdf
[k] Available from: https://docs.wixstatic.com/ugd/4344b0_813b241e8944497e99b9c45b163b76bd.pdf

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