ASB Standard 039, First Edition
202X

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

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

ASB Approved Xxxxx 202X

ANSI Approved Xxxxxx 202X

410 North 21st Street
Colorado Springs, CO 80904

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 STR profiling kits is one part of the process of generating a DNA result. There are steps prior to and after this amplification step and their impact on the STR profiling kit validation studies 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 410 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.

ASB procedures are publicly available, free of cost, at www.aafs.org/academy-standards-board.

Keywords: *internal validation, DNA, short tandem repeat profiling, capillary electrophoresis.*

Table of Contents *(to be finalized prior to publication)*

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*[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 in an 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.

[a] Available from: https://www.aafs.org/academy-standards-board

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
probabilistic genotyping
The use of biological modeling (i.e., statistical modeling informed by biological data), statistical theory, computer algorithms, and/or probability distributions to infer genotypes and/or calculate likelihood ratios.

3.10
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.

3.11
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.

3.12
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.

3.13
stochastic threshold
The peak height value in a DNA electrophoretic profile above which it is reasonable to assume, based on validation studies, 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 drop-out did not occur in mixed DNA sample profiles.

3.14
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 strand slippage during PCR amplification. A stutter peak is generally of lower relative fluorescence units (RFU) than the allele peak.

3.15
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 shall be supported by relevant developmental validation studies. The validation results may also be supplemented with published scientific literature or other appropriate scientific resources that support or add to the validation findings (e.g., manufacturer's user manual), 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.

4.1.1 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.

4.1.2 The laboratory shall conduct an STR genotyping concordance study.

4.1.2.1 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.

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

4.1.2.3 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.

[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.

4.1.3 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.

4.1.3.1 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.

4.1.3.2 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.

4.1.4 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.

4.1.5 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.

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

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

4.1.8 The laboratory shall conduct studies utilizing mixed DNA samples having a range of DNA ratios, DNA template input quantities, numbers of contributors, and degrees of allele sharing (including mixtures of first-degree biological relationships i.e., parent and child; siblings), expected to be interpreted by the testing laboratory.

4.1.8.1 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, the development of interpretation and comparison protocols, and probabilistic genotyping systems can be found in ANSI/ASB Standard 020, ANSI/ASB Standard 040, and ANSI/ASB Standard 018, respectively.

4.1.9 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.

4.1.10 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.

4.1.10.1 The laboratory shall document contamination events and calculate drop-in rates in accordance with its quality system.

4.1.11 The validation study results shall be used to determine the standard operating procedure of the laboratory for this kit.

4.1.11.1 Evaluation of the laboratory's newly established standard operating procedures using the new STR test kit shall be performed with a range of sample types.

4.1.11.2 The evaluation shall include studies utilizing known and casework-like samples with a range of sample types representative of those expected to be encountered by the testing laboratory.

4.1.11.3 These studies shall 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.

4.1.11.4 The results of the evaluation shall be used to assess the thresholds and other parameters that have been established during previous studies.

4.1.11.5 The evaluation shall address the limitations of the protocols to be included in the standard operating procedures for this kit.

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 of the documents listed in 5(a) and 5(b) by the DNA Technical Leader or other appropriate personnel and communication, with requisite training as needed, to all analysts prior to implementation in the laboratory.

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 test protocols and parameters).

Annex A
(informative)

Bibliography

The following bibliography is not intended to be an all-inclusive list, review, or endorsement of literature on this topic. The goal of the bibliography is to provide publications cited informationally, and publications relevant to the standard.

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

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

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

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

5] ASB Standard 123, Standard for Routine Internal Evaluation of a Laboratory's DNA Interpretation and Comparison Protocol[d].

6] ANSI/ASB Standard 171, Best Practice Recommendations for the Management and Use of Quality Assurance DNA Elimination Databases in Forensic DNA Analysis[d].

7] ASB BPR 129, *Best Practice Recommendations for Internal Validation of Human Short Tandem Repeat Profiling on Capillary Electrophoresis Platforms*[d] *(also out for public comment).*

8] Australia New Zealand Policing Advisory Agency (ANZPAA) National Institute of Forensic Science (NIFS) *Validation Working Group Guideline for the Validation of Forensic Science Methods*. 2025.

9] Barwick, V. (ed.) *Planning and Reporting Method Validation Studies: Supplement to Eurachem Guide on the Fitness for Purpose of Analytical Methods*. 1st Ed. 2019.

10] Bregu, J., D. Conklin, E. Coronado, M. Terrill, R.W. Cotton, and C.M. Grgicak, Analytical "Thresholds and Sensitivity: Establishing RFU Thresholds for Forensic DNA Analysis." *Journal of Forensic Science.* 2013, 58, pp. 120–129.

11] Butler, J.M. "Quality Assurance and Validation." *In: Advanced Topics in Forensic DNA Typing: Methodology.* Elsevier, 2011.

12] Butler, J.M., Iyer, H., Press, R., Taylor, M.K., Vallone, P.M., Willis, S. (2024) DNA Mixture Interpretation: A NIST Scientific Foundation Review. (National Institute of Standards and Technology, Gaithersburg, MD), NIST Series NIST IR 8351.

[d] Available from: https://www.aafs.org/academy-standards-board

13] Cantwell, H. (ed.) *Eurachem Guide – The Fitness for Purpose of Analytical Methods: A Laboratory Guide to Method Validation and Related Topics.* 3rd Ed. 2025.

14] ENSFI. *Recommended Minimum Criteria for the Validation of Various Aspects of the DNA Profiling Process*. 2010.

15] Expert Working Group on Human Factors in Forensic DNA Interpretation. *Forensic DNA Interpretation and Human Factors: Improving Practice Through a Systems Approach*. National Institute of Standards and Technology, Gaithersburg, MD, NIST IR 8503. 2024.

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

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

18] Forensic Science Regulator. *Guidance - Forensic Science Providers: Validation*. FSR-G-201, Issue 2. 2024.

19] Gilder, J. R., T.E. Doom, K. Inman, and D.E. Krane, "Run-Specific Limits of Detection and Quantitation for STR-based DNA Testing." *Journal of Forensic Sciences*, 2007, 52, pp. 97–101. doi:10.1111/j.1556-4029.2006.00318.x

20] Jones, J.P., L.J. Farrell, L.M. Benson. (eds.). *Informational Scientific Primers for Officers of the Court: Intended to Strengthen Use of Forensic Science Evidence*. National Institute of Standards and Technology, Gaithersburg, MD, NIST Special Publication (SP) NIST SP 1500-28. [see Section 1D "Method Validation and Method Verification." 2025.

21] Organization of Scientific Area Committees (OSAC) for Forensic Science. *Human Factors in Validation and Performance Testing of Forensic Science*. OSAC Technical Series 0004. 2020.

22] OSAC Human Biology Subcommittee. *Process Map for Human Forensic DNA Analysis* (Current Practice). 2022.

23] Swofford, H., S. Lund, B. Guttman, E.L. Romsos, J. Soon, M. Taylor, H. Iyer, P.M. Vallone, J.P. Jones, V. Desiderio, J.M. Butler, S. Koch, R.M. Thompson, G. Fiumara, E. Sisco, K. Sauerwein, A. Getz, R. Ramotowski. *Validation in Forensic Science: Guiding Principles for the Collection and Use of Validation Data.* National Institute of Standards and Technology, Gaithersburg, MD, NISTIR 8589. 2025.

SWGDAM Documents are available from: www.swgdam.org/publications

24] SWGDAM. *SWGDAM Guidelines for STR Enhanced Detection Methods*.

25] SWGDAM. *SWGDAM Interpretation Guidelines for Autosomal STR Typing by Forensic DNA Testing Laboratories.*

26] SWGDAM. *SWGDAM Interpretation Guidelines for Y-Chromosome STR Testing.*

27] SWGDAM. *SWGDAM Validation Guidelines for Forensic DNA Analysis Methods*.

Academy Standards Board
410 North 21st Street
Colorado Springs, CO 80904

www.aafs.org/academy-standards-board