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

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1. SCOPE

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

2. NORMATIVE REFERENCES

The following referenced documents are the foundation of these requirements. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document applies.

FBI, *Quality Assurance Standards for DNA Databasing Laboratories.*

https://www.swgdam.org/#!publications/c1mix

FBI, *Quality Assurance Standards for Forensic DNA Testing Laboratories.*

https://www.swgdam.org/#!publications/c1mix

SWGDAM. *SWGDAM Validation Guidelines for Forensic DNA Analysis Methods.*
https://www.swgdam.org/#!publications/c1mix

3. TERMS and DEFINITIONS

3.1 **Analytical threshold** is 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** is 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 **Capillary electrophoresis** (CE) is an electrophoretic technique for separating DNA molecules by their relative size based on their differential migration through a capillary tube filled with a liquid polymer.

3.4 **Controls** are samples of known types, run in parallel with experimental, reference, or evidence samples that are used to demonstrate that a procedure is working correctly.

3.5 **Deoxyribonucleic acid** (DNA) is a genetic material of organisms, usually double-stranded, a biopolymer composed of nucleic acids, identified by the

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presence of deoxyribose, a sugar, and the four nucleobases. DNA is a stable molecule.

3.6 **Drop-in** is allelic peak(s) in an electropherogram that are not reproducible across multiple independent amplification events.

3.7 **Drop-out** is 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.

3.8 **Internal Validation** is 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.

3.9 **Locus** (loci) is a unique physical location of a gene (or specific sequence of DNA) on a chromosome.

3.10 **Peak height ratio** is the relative ratio of two peaks at a given locus.

3.11 **Polymerase chain reaction** (PCR) is an enzymatic process by which a specific region of DNA is replicated during repetitive cycles that consist of the following: denaturation of the template; annealing of primers to complementary sequences at an empirically determined temperature; and extension of the bound primers by a DNA polymerase.

3.12 **Precision studies** are studies performed to evaluate the degree of mutual agreement among a series of individual measurements, values and/or results.

3.13 **Probabilistic genotyping** is 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.

3.14 **Sensitivity studies** are a set of critical 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.

3.15 **Short tandem repeats** (STR) are multiple copies of an identical (or similar) DNA sequence arranged in direct succession where the repeat sequence unit is 2 base pairs (bp) to 6 bp in length.

3.16 **Stochastic threshold** is the peak height value in a DNA electrophoretic 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.

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3.17 **Stutter** is an artifact of PCR amplification typically observed one or more repeat units smaller or larger than an STR allele in a DNA electrophoretic profile, may result from strand slippage during PCR amplification. A stutter peak is generally of lower RFU than the allele peak.

3.18 **Validation** is the process of performing and evaluating a set of experiments that establish 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 laboratory shall conduct an STR genotyping concordance study.

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

4.1.2 This study shall include the evaluation of concordance of the new STR test kit utilizing the appropriate NIST Standard Reference Material for STR genotyping.

4.1.3 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 if possible a potential explanation for the discordant data supported by data or other research studies shall be included in the final validation document.

4.2 The laboratory shall determine the sensitivity level and optimal DNA input range for each set of assay conditions/parameters (i.e. PCR cycle number, injection time/voltage, PCR reaction volume, etc.) with the new STR test kit.

4.2.1 A range of DNA input spanning lower and higher quantities than typically analyzed by the testing laboratory shall be utilized.

4.3 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 runs.

4.4 The laboratory shall characterize expected 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.

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

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4.6 The laboratory shall determine a stochastic threshold for any manual binary interpretation method used to interpret the new STR test kit data or employ a probabilistic genotyping method.

4.7 The laboratory shall conduct a study utilizing mixed DNA samples that are representative of the number of contributors, DNA ratios, and DNA template input quantities expected to be interpreted by the testing laboratory.

4.7.1 The results of this study shall be used to establish mixture interpretation protocols for determining number of contributors, mixture ratios, and distinguishing major and minor contributors.

4.8 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.

4.9 The laboratory shall determine the susceptibility of the genotyping process to the introduction and detection of exogenous DNA through the evaluation of controls (i.e. reagent blanks, negative and positive controls) and samples with known genotypes.

4.9.1 The laboratory shall document observed allelic drop-in.

4.10 The laboratory shall evaluate the new STR test kit using the laboratory's testing procedures, and samples representative of those regularly analyzed by the testing laboratory.

4.10.1 The laboratory shall compare the results to previous results of known and case-type samples, where applicable.

4.10.2 This study shall include samples using all DNA extraction chemistries utilized by the laboratory.

5. CONFORMANCE

In order to demonstrate conformance with this standard, the laboratory shall have completed all applicable validation studies as described above:

5.1 Concordance

5.2 Sensitivity

5.3 Analytical threshold(s)

5.4 Peak height ratio variation

5.5 PCR Stutter artifacts

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5.6 Stochastic threshold(s)

5.7 Mixed DNA samples

5.8 Precision and accuracy

5.9 Exogenous DNA and drop-in

5.10 Known and non-probative samples

6. ANNEX

Annex A – Bibliography

Bregu, J., Conklin, D., Coronado, E., Terrill, M., Cotton, R. W. and Grgicak, C. M. (2013), Analytical Thresholds and Sensitivity: Establishing RFU Thresholds for Forensic DNA Analysis,. J Forensic Sci, 58: 120–129. doi:10.1111/1556-4029.12008

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

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

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

Scientific Working Group on DNA Analysis Methods. *Interpretation Guidelines for Autosomal STR Typing by Forensic DNA Testing Laboratories*.
https://www.swgdam.org/#!publications/c1mix

Informational Web Sites: Additional information may be obtained from the following websites:

www.cstl.nist.gov/strbase

http://www.cstl.nist.gov/strbase/validation.htm

http://www.cstl.nist.gov/strbase/training.htm

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