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

DRAFT



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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 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.

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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.

^a Available from: <https://www.aafs.org/academy-standards-board>

- 35 **3.5**
36 **drop-out**
37 Failure of an otherwise amplifiable allele to produce a signal above analytical threshold because the
38 allele was not present in sufficient quantity in the aliquot that underwent PCR amplification.
- 39 **3.6**
40 **interpretation**
41 The process of evaluating DNA data for purposes including, but not limited to, defining assumptions
42 related to mixtures and single source profiles, distinguishing between alleles and artifacts,
43 assessing the possibility of degradation, inhibition, and stochastic effects, and determining whether
44 the data are suitable for comparison.
- 45 **3.7**
46 **peak height ratio**
47 The relative ratio of two peaks at a given locus in a diploid heterozygous single-source sample.
- 48 **3.8**
49 **precision**
50 The degree of mutual agreement among a series of individual measurements, values and/or results.
- 51 **3.9**
52 **probabilistic genotyping**
53 The use of biological modeling (i.e., statistical modeling informed by biological data), statistical
54 theory, computer algorithms, and/or probability distributions to infer genotypes and/or calculate
55 likelihood ratios.
- 56 **3.10**
57 **repeatability studies**
58 Studies to evaluate the degree of variability in multiple measurements (e.g., replicate samples)
59 under constant conditions, such as studies by the same operator using the same equipment in the
60 same laboratory within short intervals of time.
- 61 **3.11**
62 **reproducibility studies**
63 Studies to evaluate the degree of variability in multiple measurements (e.g., replicate samples)
64 under varying conditions, such as studies using the same methods with different operators or
65 different equipment.
- 66 **3.12**
67 **sensitivity studies**
68 Studies performed during developmental and/or internal validation of DNA or other test methods
69 designed to define the lower and upper limits/bounds of an assay to accurately detect an analyte.
- 70 **3.13**
71 **stochastic threshold**
72 The peak height value in a DNA profile above which the results of validation studies show that it is
73 statistically justified to assume that, at a given locus, allelic drop-out of a sister allele in a
74 heterozygous pair has not occurred in a single source DNA sample: due to the possibility of shared
75 alleles in mixed samples, the presence of allele peaks above the stochastic threshold is no guarantee
76 that allele dropout did not occur in mixed DNA sample profiles.

77 **3.14**
78 **stutter**

79 An artifact of polymerase chain reaction (PCR) amplification typically observed one or more repeat
80 units smaller or larger than a short tandem repeat (STR) allele in a DNA profile, may result from
81 strand slippage during PCR amplification. A stutter peak is generally of lower relative fluorescence
82 units (RFU) than the allele peak.

83 **3.15**
84 **validation**

85 The process of performing and evaluating a set of experiments that establishes the efficacy,
86 reliability, and limitations of a method, procedure or modification thereof; establishing recorded
87 documentation that provides a high degree of assurance that a specific process will consistently
88 produce an outcome meeting its predetermined specifications and quality attributes. May include
89 developmental and/or internal validation.

90 **4 Requirements**

91 **4.1** The internal validation of STR test kits using capillary electrophoresis shall include the
92 studies described in this section, in conjunction with ANSI/ASB Standard 038, and should be
93 supported by relevant developmental validation studies. The validation results may also be
94 supplemented with published scientific literature or other appropriate scientific resources that
95 support or add to the validation findings, where available. See additional materials available to
96 include the ASB Best Practice Recommendation 129 (*not yet published, also out for public comment*)
97 included in Annex A.

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

102 **4.1.1** The laboratory shall conduct an STR genotyping concordance study.

103 **4.1.1.1** STR genotypes obtained from the new STR test kit shall be compared to genotypes
104 obtained from the previous kit for all overlapping loci, if applicable.

105 **4.1.1.2** This study shall include the evaluation of concordance of the new STR test kit utilizing an
106 appropriate certified reference material for STR genotyping.

107 **4.1.1.3** In the event that a discordant genotype is observed when comparing identical genetic
108 markers, this discordant data shall be noted in the final validation document, and a potential
109 explanation for the discordant data supported by data or other research studies shall be included in
110 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.

- 111 **4.1.2** The laboratory shall perform sensitivity studies that will include replicates for each set of
112 assay parameters (e.g., PCR cycle number, injection time, injection voltage, and PCR reaction
113 volume) that the laboratory utilizes with the new STR kit.
- 114 **4.1.2.1** This study shall include a range of DNA inputs anticipated to be amplified as well as lower
115 and higher quantities to empirically define the upper and lower limits of DNA inputs to be used for
116 testing.
- 117 **4.1.2.2** DNA extracts spanning a range of DNA quality shall be included (e.g., synthetically and/or
118 naturally degraded as well as inhibited DNA templates of known types expected to be encountered
119 by the testing laboratory).
- 120 **NOTE** Optimal input ranges for single source DNA samples can be determined using the studies described in
121 4.1.2.1 and 4.1.2.2.
- 122 **4.1.3** The laboratory shall determine the analytical threshold for each dye channel of the new STR
123 test kit using a range of sample types and DNA input quantities across multiple analyses.
- 124 **4.1.4** The laboratory shall characterize peak height ratio variation observed for each locus of the
125 new STR test kit utilizing single source samples amplified over a range of DNA input amounts.
- 126 **4.1.5** The laboratory shall characterize PCR stutter artifacts observed for each STR locus of the
127 new STR test kit.
- 128 **4.1.6** The laboratory shall determine stochastic threshold(s) for any manual binary method used
129 to interpret the new STR test kit data.
- 130 **4.1.7** The laboratory shall conduct studies utilizing mixed DNA samples having a range of DNA
131 ratios, DNA template input quantities, and numbers of contributors with varied degrees of allele
132 sharing expected to be interpreted by the testing laboratory.
- 133 The results of these studies shall be used to define the limits of mixture interpretation and to
134 establish the protocols for assessing the number of contributors to be used in the interpretation,
135 the use of mixture ratios, and distinguishing major and minor components of mixtures.
- 136 **NOTE** Depending on the interpretation method chosen, additional studies may need to be performed (e.g.,
137 specificity testing within probabilistic genotyping). Additional information on mixture studies and the
138 development of interpretation protocols can be found in ANSI/ASB Standard 020 and ANSI/ASB Standard
139 040.
- 140 **4.1.8** The laboratory shall demonstrate allelic sizing precision and calling accuracy of the new
141 STR test kit and capillary electrophoresis instrument through repeatability and reproducibility
142 studies.
- 143 **4.1.9** The laboratory shall determine the susceptibility of the genotyping process to the
144 introduction and detection of exogenous DNA by documenting allelic drop-in and contamination
145 through the evaluation of controls (i.e., reagent blanks, negative and positive amplification controls)
146 and samples with known genotypes. The laboratory shall document contamination events and
147 calculate drop-in rates in accordance with its quality system.

148 **4.1.10** The validation study results shall be used to determine the standard operating procedure
149 of the laboratory for this kit. Assessment of the laboratory's newly established standard operating
150 procedures using the new STR test kit shall be performed with a range of sample types.

151 **4.1.10.1** The assessment shall include studies utilizing known and casework-like samples with a
152 range of sample types representative of those expected to be encountered by the testing laboratory.
153 These studies shall minimally include processing a set of samples using all DNA extraction and
154 quantification chemistries utilized by the laboratory to verify compatibility with the new STR test
155 kit.

156 **4.1.10.2** The results of the assessment shall be used to evaluate the thresholds and other
157 parameters that have been established during previous studies. The evaluation shall address the
158 limitations of the protocols to be included in the standard operating procedures for this kit.

159 **5 Conformance**

160 In order to demonstrate conformance with this standard, the laboratory shall have the following:

- 161 a) documentation of all internal validation studies as listed in Section 4;
- 162 b) documented quality assurance parameters, interpretation protocols, and analytical procedures
163 derived from internal validation studies; and
- 164 c) documented approval of the documents listed in 5(a) and 5(b) by the DNA Technical Leader or
165 other appropriate personnel and communication to all analysts prior to implementation in the
166 laboratory.

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

170 **Annex A**
171 **(informative)**

172 **Bibliography**

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

- 178 1] ANSI/ASB Standard 018, Standard for Validation of Probabilistic Genotyping Systems, First
179 Edition, 2020^d.
- 180 2] ANSI/ASB Standard 020, *Standard for Validation Studies of DNA Mixtures, and Development and*
181 *Verification of a Laboratory's Mixture Interpretation Protocol*, First Edition 2018^a
- 182 3] ANSI/ASB Standard 038, Standard for Internal Validation of Forensic DNA Analysis Methods,
183 First Edition 2020^d.
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- 198 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://ensfi.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

- 199 12] SWGDAM. *SWGDAM Guidelines for STR Enhanced Detection Methods*^h.
200 13] SWGDAM. *SWGDAM Interpretation Guidelines for Autosomal STR Typing by Forensic DNA Testing*
201 *Laboratories*ⁱ.
202 14] SWGDAM. *SWGDAM Interpretation Guidelines for Y-Chromosome Testing*^j.
203 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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