OSAC PROPOSED STANDARD

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Standard for Internal Validation of DNA Extraction Methods

Human Forensic Biology Subcommittee
Biology Scientific Area Committee (SAC)
Organization of Scientific Area Committees (OSAC) for Forensic Science

OSAC Proposed Standard

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Standard for Internal Validation of DNA Extraction Methods

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Human Forensic Biology Subcommittee
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Foreword

This standard details the requirements for internal validation of DNA extraction methods for use in forensic laboratories. The requirements in this document supplement the general requirements in ANSI/ASB Standard 038 *Standard for Internal Validation of Forensic DNA Analysis Methods* and are best utilized in coordination with XXX Best Practice Recommendations for Internal Validation of DNA Extraction Methods. The development of this document was determined to be necessary by the Human Forensic Biology Subcommittee of the Biology Scientific Area Committee. This standard is not a substitute for policies and procedures that guide forensic science service providers in their work and does not encompass training on forensic science service provider policies and procedures.

Note: the numbers of test samples recommended in the document are minimum values. Testing of additional samples may be needed in some situations, such as:

- novel DNA extraction method that has not been extensively tested in the community;
- starting up DNA testing in a laboratory for the first time;
- the laboratory experience of the individuals conducting the validation studies and previous validation studies conducted within the laboratory; and,
- the complexity of the extraction method being evaluated.

Keywords: *internal validation, DNA extraction, acceptance criteria*

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Standard for Internal Validation of DNA Extraction Methods

1 Scope

This document provides requirements for performing an internal validation of DNA extraction methods prior to implementation within a forensic DNA laboratory. It is intended to be used in conjunction with ANSI/ASB 038 *Standard for Internal Validation of Forensic DNA Analysis,* and the OSAC Proposed Standard 2022-S-0041*, Best Practice Recommendations for Internal Validation of DNA Extraction Methods.* This standard is not a substitute for policies and procedures that guide forensic science service providers in their work and does not encompass training on forensic science service provider policies and procedures.

2 Normative References

The following references are indispensable for the application of the standard. For dated references, only the edition cited applies. For updated references, the latest edition of the referenced document (including any amendments) applies.

2.1 ANSI/ASB Standard 038, *Standard for Internal Validation of Forensic DNA Analysis Methods*[1]

2.2 OSAC Proposed Standard 2022-S-0041, *Best Practice Recommendations for Internal Validation of DNA Extraction Methods*[2]

3 Terms and Definitions

3.1
concordance
Agreement between DNA typing results. Antonym: discordance.

3.2
controls
Samples of known type, run in parallel with experimental, reference, or evidence samples that are used to demonstrate that a procedure is working correctly.

3.3
degradation
Fragmentation of DNA by chemical, physical, or biological means.

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

[2] Available from: https://www.nist.gov/osac/registry

3.4
DNA extraction

A technique used to release DNA from cells in a biological sample.

3.5
inhibition

Prevention of DNA synthesis during polymerase chain reaction by any substance that either directly interacts with DNA or interferes with the DNA polymerase.

3.6
internal validation

The accumulation of test data within the laboratory for developing the laboratory standard operating procedures and determining the limits of the method(s). Internal validation demonstrates that the established protocols for the technical steps of the test and for data interpretation perform as expected in the laboratory.

3.7
reagent blank

An analytical control, which contains no intentionally added template DNA, used to monitor contamination throughout the DNA testing process from DNA extraction to DNA typing results.

3.8
repeatability studies

Experiments performed to verify the results of the assay by the same personnel and/or applicable instrumentation.

3.9
reproducibility studies

Experiments performed to assess the capability to obtain the same test results when an experiment is repeated between different operators and/or instruments.

3.10
sensitivity studies

Experiments performed to define the lower and upper limits/bounds of an assay to accurately detect an analyte.

4 Requirements

ANSI/ASB Standard 038, *Standard for Internal Validation of Forensic DNA Analysis Methods*, shall be used in conjunction with this document because ANSI/ASB Standard 038 provides the foundational validation requirements upon which additional specific requirements, such as this document, will be based.

NOTE 1: The recovered DNA yields, degradation indices, and autosomal/Y-chromosome ratios may be used to assess the performance of the DNA extraction method, which includes the comparison of a new method to a previous one. Statistically significant differences may not be

cause for rejection of a new extraction method since the new method could be faster, easier to use, automatable, or the resulting DNA extracts more efficiently amplified.

NOTE 2: Refer to Appendix A of the Best Practice Recommendations for a cross-function study table where data from different studies may be used to meet the requirements under another category.

NOTE 3: Some of the studies below may not be applicable based on the laboratory's validation plan or intended purpose for the extraction method. For example, a laboratory may not need to assess multiple biological sample types or substrates.

4.1 The laboratory shall evaluate the efficacy of the DNA extraction method using downstream validated laboratory testing procedures. If previously internally validated procedures are not available, then procedures intended for future use in casework should be used.

NOTE: For the assessment of the DNA extraction method using downstream procedures that have not been internally validated, the laboratory may rely on developmental validations, manufacturer recommended settings and methods, or validated procedures from other laboratories.

4.2 The laboratory shall design experiments and define acceptance criteria, where applicable, based, at a minimum, on the requirements listed in 4.3 through 4.8 below.

4.3 The laboratory shall demonstrate concordance by comparison to published developmental validation results or previously developed internal validation data.

4.3.1 DNA typing results of one sample from five different individuals obtained from the new DNA extraction chemistry shall be compared to results obtained from a previously internally or externally validated DNA extraction method.

4.3.2 Discordant typing results observed when comparing identical genetic markers using sample(s) from the same individual shall be documented in the final validation document.

NOTE: If possible, a potential explanation for the discordant typing results supported by data or other research studies should be included in the final validation document.

4.4 The laboratory shall evaluate the DNA extraction method using known references, casework-like samples, and substrates representative of those typically analyzed by the testing laboratory.

4.4.1 The laboratory shall assess if the DNA extraction method is fit for purpose by using different biological sample types, substrates, and sample conditions (e.g., inhibited samples, degraded samples). At a minimum, the assessment shall include the requirements stated in 4.4.1.1 through 4.4.1.4.

4.4.1.1 For each identified biological sample type [e.g., blood, semen, saliva, trace (epidermal cells), hairs, bones, tissue], a minimum of one sample from two different individuals shall be

tested in triplicate (for a total of six extractions for each biological sample type). Average yield of recovered DNA and standard deviations shall be calculated.

4.4.1.2 For a substrate study, at least one of the biological sample types shall be placed on a variety of different substrates expected to be encountered by the laboratory (minimum of five different substrates) and tested in triplicate. Average yield of recovered DNA and standard deviations shall be calculated.

4.4.1.3 For sample condition studies, the following tests shall be performed:

a) At least one of the biological sample types shall contain a known inhibitory substance(s) expected to be encountered by the laboratory [e.g., heme, humic acid (dirt)]. The inhibited sample(s) shall be tested in triplicate, at a minimum, and a qualitative assessment of the presence of inhibition shall be performed.

b) At least one of the biological sample types shall be compromised and demonstrated to contain degraded DNA. The biological sample(s) shall be tested in triplicate, at a minimum, and qualitatively assessed.

4.4.1.4 If the results from biological samples fail to meet defined laboratory acceptance criteria, then additional samples shall be tested. The number of additional samples tested depends on the outcome of the initial tests and the evaluation of possible reasons the data were outside the acceptance criteria.

4.4.2 If the laboratory tests samples containing semen, the following tests shall be performed:

a) At least one neat semen sample tested in triplicate.

b) At least one semen sample mixed with at least two other biological sources (e.g., vaginal swab with semen, blood with semen, saliva with semen) expected to be encountered by the laboratory, tested in triplicate.

4.4.3 The laboratory shall compare the results of known and casework-like samples extracted using the method being validated to the results from previously validated DNA extraction methods, where applicable.

4.5 The laboratory shall determine the susceptibility of the DNA extraction method to cause degradation or to inhibit PCR amplification.

4.5.1 At least two different biological sample types shall be tested in triplicate and the extracts taken through the complete DNA typing process. The resulting DNA profile data shall be qualitatively evaluated for apparent degradation and inhibition as compared to other extraction procedure(s).

4.5.2 The biological samples used shall not contain any known endogenous PCR inhibitors and shall be expected not to be degraded prior to extraction.

4.6 The laboratory shall conduct a sensitivity study to determine the limits of the DNA extraction method for obtaining DNA sufficient for analysis.

4.6.1 A dilution series of at least one biological sample being extracted consisting of three or more dilutions (e.g., neat, 1:10, 1:100), shall be performed and tested in triplicate.

4.6.2 Average yield of recovered DNA and standard deviations shall be calculated.

4.6.3 The samples shall be taken through the complete DNA typing process and the profiles quantitatively assessed.

4.7 The laboratory shall determine the precision (i.e., the reproducibility and repeatability) of the DNA extraction method by calculating the average DNA yields or concentrations and standard deviations.

NOTE: Precision studies can use data obtained from multiple studies within the validation.

4.8 The laboratory shall determine the susceptibility of the DNA extraction method to the introduction of exogenous DNA during the extraction process. The samples shall be taken through the complete DNA typing process.

4.8.1 A laboratory shall define what constitutes DNA contamination.

4.8.2 A laboratory shall calculate the DNA contamination rate using reagent blanks and samples containing DNA and use these data to define the expected rate of the method in casework.

Annex A
(informative)

Bibliography

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