OSAC PROPOSED STANDARD 2022-S-0041

Best Practice Recommendations 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

OSAC 2022-S-0041
Best Practice Recommendations for Internal Validation of DNA Extraction Methods

Prepared by
Human Forensic Biology Subcommittee
Version: 2.0
August 2026

Disclaimer:

This OSAC Proposed Standard was developed by the Organization of Scientific Area Committees (OSAC) for Forensic Science. It is a completed work product of the OSAC and has been approved for listing on the OSAC Registry. This OSAC Proposed Standard will be submitted to a standard developing organization and is subject to change.

This OSAC Proposed Standard has completed an open comment period and a rigorous technical and quality review by the originating OSAC unit (e.g., subcommittee), their parent body (e.g., scientific area committee), and the OSAC Standards Review Panel.

OSAC encourages the forensic science community to implement this OSAC Proposed Standard into practice as it represents the best guidance available as approved by the OSAC.

There may be references in this OSAC Proposed Standard to other publications under development by OSAC. This OSAC Proposed Standard may be used by the forensic-science community before the completion of such companion publications.

Any identification of commercial equipment, instruments, or materials in the OSAC Proposed Standard is not a recommendation or endorsement by the U.S. Government and does not imply that the equipment, instruments, or materials are necessarily the best available for the purpose.

Visit OSAC's website for more information about the Registry approval process.

Foreword

This best practices document functions in conjunction with the (standard #) *Standard for the Internal Validation of DNA Extraction Methods* and the general requirements in ANSI/ASB Standard 038 *Standard for Internal Validation of Forensic DNA Analysis Methods.* The development of this document was determined to be necessary by the Human Forensic Biology Subcommittee of the Biology Scientific Area Committee.

All hyperlinks and web addresses shown in this document are current as of the publication date of this standard.

Keywords: *internal validation, DNA extraction*

Table of Contents

Best Practice Recommendations for Internal Validation of DNA Extraction Methods

1 Scope

This document provides best practice recommendations when performing an internal validation of DNA extraction methods. It is intended to be a companion document to ASB 038 *Standard for Internal Validation of Forensic DNA Analysis Methods* and ASB XXX *Standard for Internal Validation of DNA Extraction Methods*. This level of detail is intended to provide process experts a roadmap to bring DNA extraction methods online.

2 Normative References

There are no normative reference documents. Annex A, informative reference, and Annex B, Bibliography, contain informative references.

3 Terms and Definitions

For purposes of this document, the following definitions apply.

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.

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

3.10
sensitivity studies
Experiments performed to define the lower and upper limits/bounds of an assay to accurately detect an analyte.

4 Recommendations

4.1 Considerations for Validation studies

4.1.1 The experiments designed to characterize a new extraction method should include defining the range of samples for which it is suitable (including concordance evaluation), the limits of yield produced from different sample types, and if the susceptibility to contamination is within a range deemed acceptable by the laboratory.

4.1.2 If the experiments are designed to compare a new extraction method with an existing method in use by the laboratory, then comparative studies should be performed using statistical means to evaluate DNA yields for specific sample types expected to be extracted using the new chemistry, the ranges of DNA yields for different biological quantities, and if the susceptibility to contamination is similar to the current method.

4.1.3 Reagent blank controls should be extracted concurrently and tested with all sample sets throughout the validation process.

4.1.4 Applicable DNA quantitation and amplification controls should be tested with all sample sets.

4.1.5 Calibration and/or maintenance status of the laboratory instrumentation used should be verified prior to sample analysis to ensure the systems are operating optimally and within manufacturer's parameters.

4.1.6 Biological samples with known genotypes should be used where possible.

4.1.7 The suitability of a DNA extraction method for its intended use need not be solely defined by sensitivity, yield, or quality. For example, the extraction method used for buccal reference samples may not optimize yield or DNA quality; however, it may perform sufficiently well for that specific application to be deemed acceptable.

4.1.8 Additional experiments should be performed if any modifications are made to the DNA extraction method, which could affect results (e.g., addition of chemicals beyond what is included in vendor kits, addition of bovine serum albumin (BSA), change in incubation times, change to the number of wash steps, etc.).

4.1.9 Additional experiments should be performed if any modifications are made to consumables used during the DNA extraction method, which could affect the results (e.g., introduction of lyse and spin tubes).

4.1.10 Differential extraction methods should be evaluated to determine the effectiveness of the method for minimizing carryover between sperm and non-sperm fractions and ensuring DNA yields are maintained as determined during sensitivity evaluation.

4.1.11 Data should be compared with previously internally or externally validated methods or published results (e.g., proficiency test consensus profile, NIST Standard Reference Material (SRM), or purchased sample with published results), when possible.

4.1.12 Consistent preparation of sample replicates is needed to minimize variation.

4.2 Defining Acceptance Criteria

4.2.1 Requirement from Standard

NOTE: Refer to section 4.2 of the Standard for Internal Validation of DNA Extraction Methods.

> "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."

4.2.2 Objective

The purpose of defining acceptance criteria is to establish a list of objectives in validating a particular procedure and determining if that procedure fulfills the objectives and is suitable for use. Ideally this should be established prior to the study being performed, however objectives can be changed based on findings.

4.2.3 Considerations

4.2.3.1 Failing to meet a pre-defined objective may not be grounds for rejecting an extraction method.

4.2.3.2 A brief justification should be provided as to why the extraction method itself should not be rejected.

4.2.3.3 Objectives may include procedural improvements such as efficiency, use of resources, cost, time savings, ease of use or application, and others, rather than simply DNA yield.

4.2.4 Implementation

4.2.4.1 Objectives are defined for each validation task, if applicable.

4.2.5 An evaluation of the results should be performed upon completion of each task/study to determine if the pre-defined acceptance criteria were met (if applicable). This evaluation may differ according to each task/study performed.

4.3 Concordance

4.3.1 Requirement from Standard

NOTE: Refer to section 4.3 and its sub-sections of the Standard for Internal Validation of DNA Extraction Methods.

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

4.3.2 Objective

The purpose of concordance testing is to demonstrate agreement between the DNA typing results obtained from the DNA extraction method compared to previous results.

4.3.3 Considerations

4.3.3.1 Concordance may be confirmed through a stand-alone experiment or the use of data generated from another experiment within the internal validation.

4.3.3.2 Concurrent comparison of the new extraction method to previously validated methods can inform the laboratory if differences in results are due to the extraction procedures or downstream variables and its general applicability.

4.3.4 Experimental Method

4.3.4.1 Quantify the amount of DNA purified using the new extraction method and perform DNA typing on all samples.

4.3.4.2 Concordance should be evaluated by comparing the DNA typing results of the DNA extraction method to the results from a previously internally or externally validated method or published results (e.g., proficiency test consensus profile, NIST Standard Reference Material (SRM) or purchased sample with published results).

4.3.5 Data Analysis and Results

4.3.5.1 A comparison of observed DNA typing results to the known values should be performed.

4.3.5.2 Observed discordance should be documented and if possible, a reason provided. Discordance might be the production of a partial profile instead of the expected complete profile for a sample with sufficient DNA quantity.

4.3.6 Implementation

4.3.6.1 Observed discordance may not invalidate the concordance study.

NOTE: Common reasons for discordance may include analysis of samples in the stochastic range, a null allele resulting from a primer binding site mutation, a difference in allele call due to different PCR primer sets, or a different method of fragment separation affecting resolution or migration.

4.4 References and Casework-like Samples

4.4.1 Requirement from Standard

NOTE: Refer to section 4.4 and its sub-sections of the Standard for Internal Validation of DNA Extraction Methods.

> "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."

4.4.2 Objective

The purpose of testing representative samples is to assess the performance of the extraction method using different sample types, collection methods, and substrates to reflect those commonly submitted for laboratory analysis, including inhibited, degraded, or otherwise challenging samples.

4.4.3 Considerations

4.4.3.1 This experiment should be used to assess whether the new DNA extraction method is fit for purpose through comparison with another validated DNA extraction method or developmental validation.

4.4.3.1.1 When a laboratory performs a statistical assessment, then quantitative metrics such as DNA yield and number of alleles obtained may be used for comparative analyses with the current extraction method. Statistical assessment may involve comparative tests such as Student's t-test, standard deviation, or %CV. A Bonferroni correction may be performed.

4.4.3.1.2 If a laboratory performs a qualitative assessment, then it may be solely assessed based on the DNA profile obtained (i.e., completeness of the profile, peaks above stochastic threshold, etc.), or a DNA concentration threshold, etc.

4.4.3.2 The DNA extraction method being validated should generate reproducible results for pristine samples and consistent results for challenged samples. For example, if a sample with known inhibitors present (a challenged sample) is extracted with the new extraction method, yet PCR inhibition is still observed, the experiment will have demonstrated that the new method does not remove that inhibitor efficiently.

4.4.3.3 Preparation of inhibited and adulterated sample sets should include control samples prepared using the same source of biological material without the inhibitor/adulterant to ensure comparable data sets.

4.4.3.4 This experiment can assist in identifying optimal extraction volumes, weights, inputs, and/or appropriate sampling strategies for some extraction substrates.

4.4.4 Experimental Method

4.4.4.1 The laboratory should quantify the amount of DNA and perform DNA typing on all samples.

4.4.4.2 The testing laboratory should analyze known and case-type samples representing those expected to be encountered by the laboratory for casework using the DNA extraction method. Samples should include the following:

a) single source samples;

b) samples containing more than one contributor in varying template amounts;

c) diverse biological sample types (e.g., blood, saliva, semen);

d) inhibited samples (e.g., heme and humic acid) where the concentration of the inhibitor, if known, should be used at quantities known to cause inhibition;

e) adulterated samples (e.g., latent print processing reagents, gun oil);

f) degraded samples, including differential degradation in mixed samples;

g) different substrates (e.g., cotton, denim, synthetic fabrics, cotton swabs, paper);

h) different DNA collection methods (e.g., swabbing, tape-lift, cuttings).

4.4.5 Data Analysis and Results

4.4.5.1 The amount of DNA recovered should be compared to expected quantities from previous methods, if applicable, or purchased standards that provide an expected range of yields.

4.4.5.2 DNA results from samples should be compared to any previous results, where applicable (e.g., the numbers of alleles, other quantitative profile comparisons).

4.4.5.3 A laboratory may define that the new extraction method must produce yields for specific sample types within a range of that produced by the current method.

4.4.5.4 If comparison with a previous method and using the same samples is not possible, then comparison with validation data from the previous method using similar samples should be performed.

4.4.5.5 The method's ability to remove or mitigate PCR inhibition should be determined through the evaluation of the DNA quantitation and typing results.

4.4.6 Implementation

The results from this experiment should be used to develop laboratory procedures for use in casework or databank operations.

4.5 Inhibition and Degradation

4.5.1 Requirement from Standard

NOTE: Refer to section 4.5 and its sub-sections of the Standard for Internal Validation of DNA Extraction Methods.

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

4.5.2 Objective

The purpose of this experiment is to determine if the DNA extraction method introduces inhibition or causes degradation.

4.5.3 Considerations

4.5.3.1 Quantitation methods that provide information related to sample degradation or inhibition may assist in sample evaluation.

4.5.3.2 While an extraction method may lead to degradation or inhibition, this may be variable based on the extraction method being evaluated and thus degradation and inhibition may not always be detected.

4.5.4 Experimental Method

4.5.4.1 The laboratory should quantify the amount of DNA and perform DNA typing on all samples.

4.5.4.2 Samples should be single-source DNA samples of good quality and not limited in concentration, i.e., pristine samples.

4.5.4.3 DNA extracts that appear to show inhibition may be re-amplified and the new amplification reaction spiked with high-quality DNA, such as a commercial positive control, to demonstrate that the high quality, exogenous DNA is also inhibited by the DNA extract. Also, a dilution of the inhibited DNA extract may be performed to demonstrate that it can be successfully amplified.

4.5.5 Data Analysis and Results

DNA quantitation and/or typing results should be evaluated to determine if any adverse effect is observed that may be attributable to the extraction chemistry (e.g., signal reduction, partial or complete inhibition, allelic or locus dropout). Either quantitative or qualitative analyses can be performed.

4.5.6 Implementation

The data from this experiment should inform the laboratory as to the applicability of this extraction method to case samples and also if modifications to the extraction may need to be implemented.

4.6 Sensitivity

4.6.1 Requirement from Standard

NOTE: Refer to section 4.6 of the Standard for Internal Validation of DNA Extraction Methods.

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

4.6.2 Objective

The purpose of sensitivity studies is to assess the ability of the extraction method to yield reliable results from biological samples containing a range of DNA quantities.

4.6.3 Considerations

High-quality samples should be used (i.e., samples that are not degraded or inhibited).

4.6.4 Experimental Method

4.6.4.1 Serial dilutions of single source biological material consisting of at least three concentrations should be extracted in triplicate.

4.6.4.2 At least one of the biological sample types targeted for use with the new method shall be tested from a minimum of two different individuals.

4.6.5 Data Analysis and Results

4.6.5.1 Evaluate the DNA typing results from replicate extractions to determine at what sample dilution a full DNA profile is no longer obtained and when the DNA profile obtained is no longer interpretable.

4.6.5.2 Compare the DNA typing results from replicate extractions to determine consistency of the DNA extraction method.

4.6.5.2.1 Compare recovered quantities with previous methods, if possible, to determine if the method has comparable performance.

4.6.5.2.2 If comparison with a previous method using the same samples is not possible, then comparison with validation data from the previous method using similar samples should be performed.

4.6.6 Implementation

The laboratory should evaluate the DNA yields to determine the suitability for use with casework for the sample types tested. For example, if the new extraction method fails to generate sufficient DNA for a specific type of sample (e.g., blood), but appears to perform efficiently for other samples, that information should inform the laboratory about the new method's suitability for the variety of sample types for which it is intended.

4.7 Reproducibility and Repeatability

4.7.1 Requirement from standard

NOTE: Refer to section 4.7 of the Standard for Internal Validation of DNA Extraction Methods.

> "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."

4.7.2 Objective

The purpose of this study is to demonstrate that the method consistently yields comparable quantities of DNA with minimal operator-to-operator and/or instrument-to-instrument variability, if applicable, when used for the same type and amount of sample, and when performed using the same equipment.

4.7.3 Considerations

Samples should be homogeneous to the extent possible.

4.7.4 Experimental Method

4.7.4.1 Samples should be single-source DNA of good quality and not limited in concentration.

4.7.4.2 The laboratory should quantify all samples.

4.7.4.3 The laboratory should determine the reproducibility of the method through repeated tests by different individuals and instruments, where applicable.

4.7.4.4 The laboratory should determine the repeatability of the method through repeated tests by the same individual.

4.7.5 Data Analysis and Results

4.7.5.1 A comparison of DNA quantitation values should be performed.

4.7.5.2 The range, mean and standard deviation values for the concentration of DNA in the extracted product should be calculated.

4.7.5.3 The laboratory should evaluate inconsistencies in DNA concentrations, such as when a DNA concentration is greater than two standard deviations from the mean.

4.7.6 Implementation

The laboratory should evaluate the data and determine if the method produces reproducible results and suitability for use with casework.

4.8 Contamination

4.8.1 Requirement from standard

NOTE: Refer to section 4.8 and its sub-sections of the Standard for Internal Validation of DNA Extraction Methods.

> "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."

4.8.2 Objective

The purpose of the contamination study is to inform the laboratory as to the susceptibility of the DNA extraction method to the introduction of exogenous DNA originating from reagents, consumables, laboratory personnel, instrumentation, workflow, and environment.

4.8.3 Considerations

4.8.3.1 The following is a non-exhaustive list of possible sources for the origin of contamination:

a) consumables (e.g., tubes, reagents, pipette tips);

b) laboratory personnel (e.g., use of appropriate personal protective equipment (PPE), training, sample handling);

c) laboratory environment (e.g., possible new cleaning schedule/procedure, HVAC systems, workflow, dedicated lab space);

d) laboratory workflow (e.g., automation equipment, hoods, centrifuges);

e) DNA sampling method (e.g., M-vac, swabs, tape lift)

4.8.3.2 Additional controls may be processed to test the system based on the variables described in the considerations above.

NOTE: For example, an effective contamination susceptibility test utilizes an alternating arrangement of reagent blank samples and those containing an abundant source of biological material during the DNA extraction process.

4.8.4 Experimental Method

4.8.4.1 All validation samples where a DNA profile has been developed should be evaluated for the presence of exogenous DNA.

4.8.4.2 The laboratory should quantify and perform DNA typing on all reagent blank controls that are run with each sample set.

4.8.5 Data Analysis and Results

If observed, the source of the contamination should be characterized (i.e., differentiating between a drop-in allele event and extensive contamination), attribution attempted, and the rate of occurrence estimated.

4.8.6 Implementation

The results of this study may identify the point(s) in the laboratory extraction process and related quality assurance/quality control processes where contamination event(s) occur and should be used to establish laboratory policies concerning *the* following:

a) laboratory procedures to prevent and detect contamination;

b) control measures (e.g., use of appropriate PPE);

c) procedural requirements concerning controls (e.g., placement and number);

d) corrective measures required when contamination is detected;

e) systemic problems that may require further improvements;

f) contamination tolerance interpretation guidelines;

g) laboratory environmental requirements (e.g., design, workflow, cleaning, instrument maintenance).

Annex A
(informative)

***Cross function study*—**This table is intended to assist laboratories with efficiently utilizing sample data generated across the multiple studies outlined in this document. The columns correspond to a category list of the samples in process, and the rows are the listed outline of each study described in this document.

Sample Series Generated

Studies as outlined in Guidelines		Concordance	Known, Non-Probative, and Mock	Inhibition Degradation	Sensitivity	Reproducibility and Repeatability
5.1	Concordance	X	X			X
5.2	Known, Non-Probative, and Mock Samples		X			
5.3	Inhibition Degradation			X		
5.4	Sensitivity				X	
5.5	Reproducibility and Repeatability		X		X	X
5.6	Contamination	X	X	X	X	X

Annex B

(informative)

Bibliography

1) ENSFI. Recommended Minimum Criteria for the Validation of Various Aspects of the DNA Profiling Process. https://enfsi.eu/wp-content/uploads/2016/09/minimum_validation_guidelines_in_dna_profiling_-_v2010_0.pdf. Accessed July 27, 2022.

2) "Guidelines and Considerations for Evaluating or Validating Extraction Chemistry. Applied Biosystems." *Forensic News*, July, 2010. https://assets.thermofisher.com/TFS-Assets/LSG/Specification-Sheets/cms_086052.pdfAccessed July 27, 2022.

3) Lee, G.M., Thornthwaite, J.T., and Rasch, E.M. "Picogram per Cell Determination of DNA by Flow Cytofluorometry." *Analytical Biochemistry*, 1984; 19137:22 l-6.

4) Lee, S.B., and Shewale, J.G. *DNA Extraction Methods in Forensic Analysis. In Encyclopedia of Analytical Chemistry*, editors John Wiley & Sons, Ltd., 2017. DOI: 10.1002/9780470027318.a1104m.pub2.

5) SWGDAM Validation Guidelines for Forensic DNA Analysis Methods. https://www.swgdam.org/_files/ugd/4344b0_813b241e8944497e99b9c45b163b76bd.pdf. Accessed July 27, 2022.