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

Human Forensic Biology Subcommittee
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Keywords: internal validation, DNA extraction

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1 Scope

This document provides best practice recommendations when performing an internal validation of DNA extraction methods, and 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

ASB 038 Standard for Internal Validation of Forensic DNA Analysis Methods
ASB XXX Standard for Internal Validation of DNA Extraction Methods

3 Terms and Definitions

3.1
Concordance
Agreement between DNA typing results.

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

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

3.5
Developmental Validation
Accumulation of test data and determination of conditions and limitations of a new methodology; this generally occurs while the conditions and parameters are being worked out prior to the establishment of a defined assay, procedure or product.

3.6
DNA extraction
A technique used to release DNA from cells in a biological sample.

3.7
Inhibition
Prevention of DNA synthesis during polymerase chain reaction by any substance that either directly interacts with DNA or interferes with the DNA polymerase.

3.8
Internal validation
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
DNA purification
A process by which DNA is isolated from other cellular and non-cellular components using a combination of physical and/or chemical methods.

3.10
Polymerase chain reaction (PCR)
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.

3.11
Repeatability
Precision and accuracy of results (e.g. quantitative and/or qualitative) of the same operator and/or detection instrument.

3.12
Reproducibility
Precision and accuracy of results (e.g. quantitative and/or qualitative) among different operators and/or detection instruments.

3.13
Sensitivity studies
Studies performed during validation of DNA or other test methods designed to define the limits of the method.

4 General Considerations

4.1 The laboratory shall evaluate the DNA extraction method using validated laboratory testing procedures or procedures intended for use in casework.

4.2 Reagent blank controls should be run with all sample sets throughout the validation process.

4.3 Applicable DNA quantitation and amplification controls should be run with all sample sets.

4.4 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.

4.5 The laboratory should monitor results for the possibility of sample switch error or similar sample mishandling errors.

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

4.7 Suitability of a DNA extraction method for its intended use may not be solely defined by sensitivity, yield, or quality.

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

4.9 Additional studies should be performed on any modifications made to consumables used during the DNA extraction method which could affect the results (e.g. introduction of lyse and spin tubes).

4.10 Differential extraction methods should be evaluated to determine the effectiveness of the method for minimizing carry over, and ensuring DNA yields are maintained as determined during sensitivity evaluation.

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

5 Best Practice Recommendations for Internal Validation of DNA Extraction Methods

5.1 Concordance

5.1.1 Standard: The laboratory shall demonstrate concordance of the new DNA extraction method to previously validated methods or published results.

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

5.1.3 Considerations:

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

5.1.3.2 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.

5.1.4 Experimental Method:

5.1.4.1 Quantify the amount of DNA and perform DNA typing on all samples.

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

5.1.5 Data Analysis and Results:

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

5.1.5.2 Observed discordance should be documented and if possible, a reason provided.

5.1.6 Implementation:

5.1.6.1 Observed discordance may not invalidate the concordance study. 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.

5.2 References and Casework-like Samples

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

5.2.2 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 or otherwise challenging samples.

5.2.3 Considerations:

5.2.3.1 This study may be used to assess relative DNA extraction performance through comparison with another validated DNA extraction method or developmental validation.

5.2.3.2 The DNA extraction method being validated should generate reproducible results for pristine samples and consistent results for challenged samples.

5.2.3.3 Preparation of inhibited and adulterated sample sets should include control samples prepared using the same source of biological material in order to ensure comparable data sets.

5.2.3.4 Mixture samples may be utilized to evaluate the ability of the method to recover DNA from minor contributors and to inform the laboratory on major/minor determination.

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

5.2.4 Experimental Method:

5.2.4.1 Quantify the amount of DNA and perform DNA typing on all samples.

5.2.4.2 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 may include:

5.2.4.2.1 Single source samples and samples containing more than one contributor in varying template amounts.
5.2.4.2.2 Diverse sample types (e.g. blood, saliva, semen, etc.).
5.2.4.2.3 Inhibited samples.
5.2.4.2.4 Adulterated samples (e.g. latent print processing reagents, gun oil, etc.).
5.2.4.2.5 Degraded samples, including differential degradation in mixed samples.
5.2.4.2.6 Different substrates.
5.2.4.2.7 Different DNA collection methods (e.g. swabbing, tape-lift, cuttings, etc.).

5.2.5 Data Analysis and Results:

5.2.5.1 Evaluate the data and determine if the extraction method is compatible with samples tested.

5.2.5.2 Compare the amount of DNA recovered to expected quantities or results from previous methods, if applicable.

5.2.5.3 DNA typing results from samples should be compared to any previous results, where applicable.

5.2.5.3.1 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.

5.2.5.4 The method's ability to remove or mitigate PCR inhibition should be determined.

5.2.6 Implementation:

5.2.6.1 The results from this study should be used to inform laboratory procedures and workflow.

5.3 Inhibition and Degradation

5.3.1 Standard: The laboratory shall determine the susceptibility of the DNA extraction method to the introduction of inhibition or degradation.

5.3.2 Objective: The purpose of this study is to determine if the DNA extraction method introduces inhibition or causes degradation.

5.3.3 Considerations:

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

5.3.4 Experimental Method:

5.3.4.1 Quantify and perform DNA typing on all samples.

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

5.3.5 Data Analysis and Results:

5.3.5.1 DNA 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, etc.).

5.3.6 Implementation:

5.3.6.1 The data from this study shall inform the laboratory as to the applicability of this extraction method to case samples.

5.4 Sensitivity:

5.4.1 Standard: The laboratory shall determine the sensitivity of the DNA extraction method.

5.4.2 Objective: Sensitivity studies are used to assess the ability to obtain reliable results from a range of DNA quantities.

5.4.3 Experimental Method:

5.4.3.1 Quantify and perform DNA typing on all samples.

5.4.3.2 Dilutions of single source biological material should be extracted in triplicate.

5.4.4 Data Analysis and Results:

5.4.4.1 Analyze the resulting data using the laboratory's analysis parameters.

5.4.4.2 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.

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

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

5.4.4.4.1 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.

5.4.5 Implementation:

5.4.5.1 The laboratory should evaluate the DNA yields to determine the suitability for use with casework.

5.5 Reproducibility and Repeatability:

5.5.1 Standard: The laboratory shall demonstrate the precision and accuracy of the DNA extraction method through repeatability and reproducibility studies.

5.5.2 Objective: The method should consistently yield comparable quantities of DNA with minimal operator-to-operator variability, when used for the same type and amount of sample, and when performed using the same equipment.

5.5.3 Considerations:

5.5.3.1 Samples should be homogeneous to the extent possible.

5.5.4 Experimental Method:

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

5.5.4.2 Quantify all samples.

5.5.4.3 The laboratory should determine the reproducibility of the method through repeated tests by different individuals, when practicable.

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

5.5.5 Data Analysis and Results:

5.5.5.1 A comparison of DNA quantitation values should be performed.

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

5.5.5.1.2 The laboratory should evaluate inconsistencies in DNA concentrations.

5.5.6 Implementation:

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

5.6 Contamination:

5.6.1 Standard: The laboratory shall determine the susceptibility of the DNA extraction method to the introduction of exogenous DNA.

5.6.2 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.

5.6.3 Considerations:

5.6.3.1 The following should be considered for the origin of contamination:

5.6.3.1.1 Consumables (e.g. tubes, reagents, pipette tips, etc.)
5.6.3.1.2 Laboratory personnel (e.g. appropriate personal protective equipment (PPE), training, sample handling, etc.)
5.6.3.1.3 Laboratory environment (e.g. possible new cleaning schedule/procedure, HVAC systems, workflow, dedicated lab space, etc.)
5.6.3.1.4 Laboratory workflow (e.g. automation equipment, hoods, centrifuges, etc.)
5.6.3.1.5 DNA sampling method (e.g. M-vac, swabs, tape lift, etc.)

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

5.6.4 Experimental Method: Not applicable

5.6.5 Data Analysis and Results:

5.6.5.1 All validation samples, including the extraction (reagent) blank controls, should be evaluated for the presence of exogenous DNA.

5.6.5.2 If observed, the source of the contamination should be characterized, attribution attempted and the rate of occurrence estimated.

5.6.6 Implementation:

5.6.6.1 The results of this study will identify the point(s) in the laboratory process where contamination event(s) occur and should be used to establish laboratory policies concerning the following:

5.6.6.1.1 Laboratory procedures to prevent and detect contamination.
5.6.6.1.2 Control measures (e.g. PPE).
5.6.6.1.3 Procedural requirements concerning controls (e.g. placement and number).
5.6.6.1.4 Corrective measures required when contamination is detected.
5.6.6.1.5 Systemic problems that may require further improvements.
5.6.6.1.6 Contamination tolerance interpretation guidelines.
5.6.6.1.7 Laboratory environmental requirements (e.g. design, workflow, cleaning, instrument maintenance, etc.).

Annex

Appendix A

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 x-axis is a category list of the samples in process and the y-axis is the listed outline of each study described in this document.

Studies as outlined in Guidelines		Concordance	Known, Non-Probative, and Mock	Inhibition Degradation	Sensitivity	Reproducibility and Repeatability
		Sample Series Generated				
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

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