Scientific Working Group on DNA Analysis Methods

Validation Guidelines for DNA Analysis Methods: Overview

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SWGDAM Validation Guidelines for DNA Analysis Methods: Overview Document

The Scientific Working Group on DNA Analysis Methods, better known by its acronym of SWGDAM, is a group of approximately 50 scientists representing Federal, State, and Local forensic DNA laboratories in the United States and Canada. During meetings, which are held twice a year, Committees discuss topics of interest to the forensic DNA community and often develop documents to provide direction and guidance for the community.

This document was presented to the full SWGDAM group and received approval by the membership on date. The SWGDAM Executive Board approved posting of the document, with minor revisions, on date.

In the forensic context, the term "validation" refers to the process by which a procedure is evaluated to determine its efficacy and reliability for forensic application. This document and subsequent modules provide guidelines for the validation of DNA analysis methods and supersedes the Scientific Working Group on DNA Analysis Methods (SWGDAM) revised Validation Guidelines (2016). These recommendations are intended to serve as a guide for laboratories in validating procedures consistent with the FBI Director's Quality Assurance Standards (QAS). Terms used in this document and subsequent modules are intended to be consistent with definitions provided in the QAS.

Because these are guidelines and not minimum standards, in the event of a conflict between the QAS and these guidelines, the QAS and the QAS Audit Documents have precedence over these guidelines. Additionally, to avoid any such conflict, use of the mandatory term 'shall' has been used when that term is similarly used in the QAS although the use of the term 'shall' is not intended to transform these guidelines into standards.

These guidelines are not intended to be applied retroactively. Laboratories are encouraged to review their standard operating procedures and validation protocols in light of these guidelines and to update their procedures as needed.

1. Introduction

The SWGDAM Validation Guidelines (2016) were updated to assist laboratories in establishing reliable methods for DNA analysis and identifying limitations of the procedures. Each laboratory seeking to evaluate a new system must determine which validation studies are relevant to the methodology, in the context of its application, and determine the experiments required to satisfy each study. These guidelines are applicable to most methods used in DNA analysis. Some studies described herein may also assist in conducting performance checks of procedural modifications to existing standard operating procedures.

Performing internal validation studies can be a time consuming and laborious process. Laboratories are encouraged to communicate and discuss plans and experiences regarding validation workflows, including similar methods, which may save time and resources.

Laboratories validating a new platform, kit, reagents and/or procedure are encouraged to publish validation studies in a peer-reviewed journal. Publication provides access to information other laboratories can use to guide their internal validation efforts. Utilization of published validation data from laboratories increases efficiency through shared experience, provides a valuable crosscheck of original validity and enables ongoing improvements, and as a result, is strongly encouraged to promote consistency and demonstrate concordance among laboratories.

These Validation Guidelines have been organized such that required elements of validation studies and a glossary are contained herein (referred to as the "Overview" document). The Overview document will be supplemented by modules intended to provide technology or methodology specific guidance. The studies in each module are not synchronized to the FBI QAS; instead, they are presented in a suggested order to conserve resources such as time, reagents, samples and consumables and streamline required testing. These modules will be continually added or edited as necessary and will be reflected in the SWGDAM Validation Guidelines for DNA Analysis Methods: Modules List which can be found here: SWGDAM.org/publications.

2. General Considerations

The purpose of validation is to demonstrate the reliability and potential limitations of a technology or methodology. There are two types of validation required to implement or modify technologies for forensic DNA analysis – developmental and internal. The application of existing technology to the analysis of forensic samples does not necessarily create a new technology or methodology. Published developmental validation studies in other fields may sufficiently address forensic applications.

2.1 Developmental validation shall precede the implementation of any new methods used for forensic DNA analysis.

2.1.1 Peer-reviewed publication of the underlying scientific principle(s) of a technology shall be required.

2.1.2 Peer-reviewed publication of developmental validation studies is strongly encouraged. However, validated technologies or procedures may be implemented without such publication.

2.1.3 A DNA laboratory may rely upon another laboratory's published developmental validation studies. The citations and/or publications referencing that validation should be available and accessible to support the underlying scientific basis.

2.2 Prior to using a method or procedure for forensic applications, a laboratory shall conduct internal validation studies on samples representative of those typically encountered by the end-user laboratory to demonstrate the reliability and potential limitations of the method.

2.2.1 Internal validation studies including the approval of the technical leader, shall be retained and available for review. Documentation, at a minimum, should include:

2.2.1.1 Summary of each study conducted

2.2.1.2 Results of each study, including generated data

2.2.1.3 Approval of the technical leader for implementation

2.2.2 Standard operating procedures, quality assurance parameters, and interpretation guidelines shall be derived from internal validation studies. For example, lower template DNA may cause extreme heterozygote imbalance; as such, empirical heterozygote peak-height ratio data could be used to formulate mixture interpretation guidelines and determine the appropriate ratio by which two peaks are determined to be heterozygotes. In addition to establishing an analytical threshold, results from sensitivity studies could be used to determine the extent and parameters of quality

control tests that reagents or instruments require prior to their being used in actual casework.

2.2.3 For laboratory systems that consist of more than one laboratory, each of the laboratories shall complete, document, and maintain studies which may be impacted by site-specific factors (such as precision, sensitivity, contamination, etc.). Studies that are not location-specific may be shared among locations and the summary of the shared validation data shall be available at each site.

2.2.4 Control samples (e.g., HL60, 2800M, 9947A, SRM, 007, and others) are *NOT* to be considered known samples. These positive controls are not subjected to the same laboratory processes and are therefore known to behave differently. Control samples can be included but are not to be considered the 'known' samples for these experiments.

2.2.4.1 It is important to utilize samples as part of the internal validation studies that are extracted DNA obtained from the laboratory's validated procedures.

3. Developmental Validation

The developmental validation process shall include, where applicable, the following studies using samples that are representative of those typically encountered by the end user laboratory:

3.1 **Characterization of genetic markers:** The basic characteristics (described below) of a genetic marker shall be determined and documented.

3.1.1 Inheritance: The mode of inheritance of DNA markers demonstrated through family studies.

3.1.2 Mapping: The genomic location of the genetic marker.

3.1.3 Detection: Technological basis for identifying the genetic marker (e.g., capillary electrophoresis, DNA sequencing, hybridization assays).

3.1.4 Polymorphism: Type of variation (e.g., sequence and/or length variants).

3.2 **Species specificity:** The ability to detect genetic information from non-targeted species (e.g., detection of microbial DNA in a human assay) shall be determined through laboratory studies and/or sequence homology searches against genomic databases (e.g., a BLAST search). The detection of genetic information from non-targeted species does not necessarily invalidate the use of the assay, but may help define the limits of the assay. Species cross-reactivity may be demonstrated by using a number of commercially available non-human DNA at quantities similar to that of the targeted application.

3.3 **Sensitivity studies:** The ability to obtain reliable results from a range of DNA quantities, to include the upper and lower limits of the assay, shall be evaluated.

3.4 **Stability studies:** The ability to obtain results from DNA recovered from biological samples deposited on various substrates and subjected to various environmental and chemical insults should be evaluated. In most instances, assessment of the effects of these factors on new forensic DNA procedures is not required. However, if substrates and/or environmental and/or chemical insults could potentially affect the analytical process, then the process shall be evaluated to determine the effects of such factors.

3.4.1 For database samples, stability studies may include samples on various substrates and subjected to potential PCR inhibitors or various storage conditions.

3.5 **Precision and accuracy studies:** The ability of the assay to obtain repeatable and/or reproducible results must be determined, when practicable. The measure of precision is usually expressed in terms of imprecision and computed as a standard deviation of the test results while the measure of accuracy can be accomplished by checking results against an appropriate and available certified reference material.

3.6 **Case-type samples:** The ability to obtain reliable results should be evaluated using samples that are representative of those typically encountered by the end-user laboratory. Where appropriate, consistency of typing results should be demonstrated by comparing results from the previous procedures to those obtained using the new procedure.

3.7 **Population studies:** The distribution of genetic markers in populations (i.e., frequencies) should be determined in relevant population groups. When appropriate, (e.g., novel markers and/or alleles), databases should be tested for independence expectations (e.g., Hardy Weinberg Equilibrium and Linkage Equilibrium).

3.8 **Mixture studies:** The ability to obtain reliable results from mixed-source samples shall be determined. These studies will assist the laboratory in establishing guidelines for mixture interpretation, which may include determination of the number of contributors to the mixture, determination of the major and minor contributor profiles, and contributor ratios or proportions in addition to male:autosomal or male:female DNA quantification determination. These are best achieved by varying the number of contributors, mixture ratios, and overall template amounts.

3.9 **PCR-based studies**

3.9.1 Publication of the sequence of individual primers is not required in order to appropriately demonstrate the reliability and limitations of PCR-based technologies. However, availability of the primer sequences is encouraged in order to aid in the identification of potential primer binding site variants and troubleshooting. PCR-based studies should include:

3.9.1.1 The reaction conditions needed to provide the required degree of specificity and robustness shall be determined. These include, but are

not limited to, thermal cycling parameters, the concentration of primers, buffers, magnesium chloride, dNTPs and DNA polymerase.

3.9.1.2 The potential for differential amplification among loci, preferential amplification of alleles within a locus, and stochastic amplification should be assessed to measure the specificity and robustness of the PCR reaction and the impact on peak height balance between and within a genetic marker.

3.9.1.3 The effects of multiplexing should be assessed to measure the specificity and robustness of the PCR reaction.

3.9.1.4 Appropriate controls should be assessed to ensure that the method works correctly and ensure the data are valid.

3.9.1.5 Criteria for detection of amplified product should be determined based on the platform and/or method used and instrument baseline noise should be defined for quantitative and capillary electrophoresis typing methods.

3.9.1.6 Appropriate measurement standards (qualitative and/or quantitative) for characterizing the alleles or resulting DNA product shall be established.

4. Internal Validation

4.1 The internal validation process shall include the applicable studies detailed below and outlined in the relevant module(s). If conducted within the same laboratory, developmental validation studies may satisfy some elements of the internal validation. A laboratory's internal validation can be used to supplement any elements in which the developmental validation is insufficient.

4.2 The laboratory should evaluate the suitability of each study based on the methodology and/or application. If the lab determines that a study is not applicable, the reason(s) shall be documented in the validation summary. Using

the specific module(s) as guidance, the laboratory should determine the appropriate number of samples and the type of samples required for each study to demonstrate the potential limitations and reliability of the method. It is recognized that a validation study cannot account for all potential casework scenarios; however, samples representing the range of forensic sample types expected to be routinely encountered by the end-user laboratory should be selected for evaluation.

4.3 At the time of validating new DNA methods (from amplification through characterization), typing test kit, or platform instrument model, the laboratory shall check results from the new method/kit/platform for concordance with an appropriate and available certified reference material (or sample made traceable to the certified reference material) prior to the implementation of the method for forensic analysis.

Internal validation data may be shared by all locations in a multi-laboratory system. The summary of the shared validation data shall be available at each site. At a minimum, each laboratory in a multi-laboratory system shall complete, document, and maintain applicable site-specific precision, sensitivity, and contamination assessment studies.

4.4 Internal validation studies shall be documented and summarized. Internal validation studies shall be reviewed by the technical leader and approval documented prior to implementing a procedure for forensic applications.

4.5 The internal validation process shall include the studies detailed below, as applicable:

4.5.1 **Known and non-probative evidence samples or mock evidence samples:**

4.5.1.1 Methods intended for casework samples shall be evaluated and tested using known samples (e.g., reference blood or buccal samples) and case type samples. Methods intended for database samples should be evaluated and tested using known samples collected on the substrates routinely encountered by the laboratory.

4.5.1.2 The known samples selected for the studies should exhibit a high level of heterozygosity. The use of heterozygous samples will help establish intra-locus balance metrics and aid in the determination of appropriate interpretation thresholds.

4.5.1.3 Known and non-probative sample studies may be used to assess the concordance of a method and therefore the degree of accuracy of the system. The data generated can also help establish appropriate stutter filters, supplement the noise and threshold calculations, and assess potential contamination events associated with the method. Results from these studies should be compared to the previous results of known samples and/or non-probative evidence or mock case samples to assess concordance. Observed discordance(s) should be documented and, where possible, explained. The potential impact of any discordance should be assessed.

4.5.1.4 Case type samples may include non-human DNA at template levels similar to those expected to be routinely encountered during casework analysis (e.g., mold, bacteria). Results of these studies can be used to determine how non-human artifacts can be recognized and how their presence will affect the interpretation of the DNA profile.

4.5.2 **Sensitivity and Stochastic Studies:**

4.5.2.1 The laboratory shall determine the sensitivity levels of the assay or procedure. The known samples selected for the studies should exhibit a high level of heterozygosity. The use of heterozygous samples will help establish intra-locus balance metrics. Sensitivity studies are performed to assess the ability to obtain reliable results from a range of

DNA quantities, to include the upper and lower limits of the assay, and to determine the dynamic range, ideal target range, limit of detection, heterozygote balance (e.g., peak height ratio), and the signal-to-noise ratio associated with the assay. Sensitivity studies can also be used to evaluate excessive random (stochastic) effects generally resulting from low quantity and/or low quality samples.

4.5.3 **Precision and Accuracy Studies:**

4.5.3.1 Precision and accuracy of the assay/instrument shall demonstrate that it is achieving the expected result and must also address repeatability and/or reproducibility when practicable.

4.5.3.2 Precision depends only on the distribution of random errors and does not relate to the true value or specified value. The measure of precision is usually expressed in terms of imprecision and computed as a standard deviation of the test results.

4.5.3.3 Accuracy of a measuring instrument is the ability of a measuring instrument to give responses close to a true value. This can be accomplished by checking results against an appropriate and available certified reference material.

4.5.3.3.1 **Repeatability**: Precision and accuracy of results (e.g., quantitative and/or qualitative) of the same operator and/or detection instrument should be evaluated.

4.5.3.3.2 **Reproducibility:** Precision and accuracy of results (e.g., quantitative and/or qualitative) among different operators and/or detection instruments should be evaluated.

4.5.4 **Mixture Studies:**

4.5.4.1 Mixture studies consisting of samples that are representative of those typically encountered by the end-user laboratory shall be performed. For example, forensic DNA mixture studies should use known samples that represent the number of contributors and the range of general mixture types for which the procedure will be used in casework (e.g.,

mixture proportions and template quantities). These studies will help establish interpretation guidelines to include: determination of the number of contributors to the mixture, determination of the major and minor contributor profiles, and contributor ratios/proportions. As an additional example, laboratories validating a new extraction method should include in the mixture studies the body fluids, and combinations thereof, that are typically encountered by the end-user laboratory.

4.5.5 **Contamination Assessment:**

4.5.5.1 Contamination studies shall be performed to evaluate and measure the potential for the introduction of exogenous DNA at any point during sample processing. Based on these studies, the laboratory should/can determine ways to mitigate contamination and/or develop a policy for data interpretation when contamination has been identified.

4.5.5.2 These studies also serve to assess the presence of potential contaminants in the reagents used throughout the various sample processes in the laboratory as well as assessing the efficacy of personal protective equipment and cleaning protocols. The laboratory shall evaluate, using negative controls and known samples, the detection of exogenous DNA originating from reagents, consumables, other samples, operator(s) and/or the laboratory environment.

4.5.5.3 Should contamination be encountered during the contamination assessment studies, the origin of the event must be explored and should be characterized, when possible. The validation should establish procedures that will minimize the occurrence of a contamination event. Technical procedures should detail how to address contamination should it occur in casework analyses.

5. Procedure Modification

A procedure modification is an alteration of an existing analytical procedure that may have a consequential effect(s) on analytical results and the change is outside the initial scope of the original validation. Examples of a procedure modification include a decrease in reaction volume of an amplification kit or a change in injection time for a genetic analyzer.

5.1 A procedure modification must be evaluated prior to use with forensic samples. The modified procedure must be evaluated by comparing the original procedure to the modified procedure using similar samples to ensure concordance. The laboratory should define the appropriate sample number, sample type, and the studies necessary to evaluate the modification. The evaluation shall be documented, reviewed by the technical leader and approval documented prior to the implementation of the modified procedure into forensic applications.

5.2 If the procedure modification is determined to have an impact on the efficacy or reliability of the forensic analysis (such as modifications that impact the efficacy of the PCR process or the detection of DNA types), additional internal validation studies (such as sensitivity and stochastic studies) may be necessary to demonstrate the continued reliability and potential limitations of the method.

6. Performance Check

A performance check is a quality assurance measure to assess the functionality of laboratory critical equipment and instruments that affect the accuracy and/or validity of forensic sample analysis.

6.1 A laboratory shall have and follow a documented program for conducting performance checks of critical instruments and equipment.

6.1.1 This program will document the laboratory protocol, the performance characteristics and acceptance limits.

6.1.2 The laboratory should evaluate the appropriate sample number and type to demonstrate the reliability of the instrument or equipment.

6.1.3 If the laboratory determines that a performance check study is not necessary, the justification should be documented.

6.1.4 A laboratory's evaluation may also determine that additional performance check studies are necessary.

6.2 If service is performed on a critical instrument or equipment, a performance check is required before returning it to use for forensic analysis.

6.3 If the physical location or the environment of the instrument has been changed (e.g., instrument moved to another room, significant remodeling of the room), a performance check should be completed before returning it to use for casework analysis.

6.4 After an internal validation has been performed on a critical instrument, each additional critical instrument of the same make and model shall require a performance check.

6.4.1 The performance check should demonstrate that results are reproducible on the new critical instrument and that testing results associated with new critical instrumentation is comparable to testing results generated during the internal validation and acceptable for use within the laboratory.

6.4.2 If the laboratory determines that the new critical instrument is not within acceptable parameters, then the laboratory must address the instrument and/or procedure to minimize or mitigate the difference.

7. Software

7.1 Software or software tools used in a forensic laboratory that may have an impact on the analytical procedure, interpretation or statistical calculations shall be validated to ensure the software fulfills its intended purpose and is suitable for use in the laboratory. This includes the following software uses:

- Software used as a component of instrumentation
- Software used for the analysis and/or interpretation of DNA data
- Software used for statistical calculations for forensic genetic applications
- Software tools (e.g., macros, workbooks, LIMS) used for analytical workflow

7.1.1 Software shall be evaluated to assess the suitability of the software for its intended use in the laboratory and to determine the necessity of validation studies and/or software testing. This evaluation shall include the determination of which studies will and will not be conducted and shall be documented.

Software validations including the summary and results shall be reviewed by the laboratory's technical leader and approval documented prior to implementation.

7.2 Modifications to software or a software upgrade that will have an impact on the analytical process, interpretation or statistical calculations shall require validation. For software upgrades or modifications, the laboratory should require a software developer to provide written documentation, such as release notes, to explain the purpose and scope of the modification.

7.2.1 The validation required for each software change is determined by the type of change and the impact of the change on the operation of the software.

7.2.1.1 A major revision to software or software tools that impact the analytical process, interpretation or statistical calculations shall require validation prior to implementation.

7.2.1.2 A minor revision to software or software tools that does not impact the analytical process, interpretation or statistical calculations shall require a (functional testing) performance check.

References and Suggested Readings

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

Federal Bureau of Investigation. (2020) *Quality Assurance Standards for Forensic DNA Testing Laboratories;* available at https://www.fbi.gov/about-us/lab/codis/qas-standards-for-forensic-dna-testing-laboratories-effective-07-01-2020.

Scientific Working Group on DNA Analysis Methods (SWGDAM). (2017) *SWGDAM Interpretation Guidelines for Autosomal STR Typing by Forensic DNA Testing Laboratories;* available at https://1ecb9588-ea6f-4feb-971a-73265dbf079c.filesusr.com/ugd/4344b0_50e2749756a242528e6285a5bb478f4c.pdf.

Informational Web Site: Additional information may be obtained from the following web site:

https://strbase.nist.gov/

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Document Version	Revision History
July 2003	Original. (Published in Forensic Science Communications in July 2004; available at http://www.fbi.gov/about-us/lab/forensic-science-communications/fsc/july2004/index.htm/standards/2004_03_standards02.htm)
November 2012	The document was revised to update the guidelines to incorporate changes to the FBI Director's Quality Assurance Standards (QAS). The revisions include: addition of a preface that describes the QAS have precedence over these guidelines; definitions added to Section 1 for critical instrument, methodology, precision and technology; revised description of developmental and internal validation in Section 2; added Table of recommended studies for internal validation in Section 4; and References and Suggested Reading added in a new Section 8.
November 2012	Approved by the SWGDAM membership.
December 2012	Approved by the SWGDAM Executive Board, with minor revisions, for posting on swgdam.org.
November 2016	The document was revised to address Next Generation Sequencing (NGS) technologies. Revisions include: new definitions in Section 1 for bioinformatics, index, library and next generation sequencing; revisions to the definitions in Section 1 for methodology and technology; the addition of NGS-specific studies to both Sections 3 and 4; and revisions to Section 7.
December 2016	Approved by the SWGDAM Executive Board, with minor revisions, for posting on www.swgdam.org.
July, 2021	This document was reformatted to a Validation Overview document with general information about validation testing. Specific technology or methodology validation information has been moved to a separate Module format for each topic.

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