Scientific Working Group on DNA Analysis Methods

Validation Guidelines for DNA Analysis Methods

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SWGDAM Validation Guidelines for DNA Analysis Methods

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 revised in November 2016 to address Next Generation Sequencing (NGS) technologies. The SWGDAM Executive Board approved posting of this document, with the minor revisions, in December 2016.

This document provides guidelines for the validation of DNA analysis methods and supersedes the Scientific Working Group on DNA Analysis Methods (SWGDAM) Revised Validation Guidelines (2012). These recommendations are intended to serve as

a guide for laboratories in validating procedures consistent with the FBI Director's Quality Assurance Standards (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 and 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. It is anticipated that these guidelines will evolve further as future technologies emerge.

Introduction

The SWGDAM Revised Validation Guidelines (July 2012) 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 number of samples required to satisfy each study. These guidelines are applicable to most methods used in DNA analysis. Some studies herein described may also assist in conducting performance checks of material modifications to existing procedures.

1. **Definitions**

   Accuracy is the degree of conformity of a measured quantity to its actual (true) value. Accuracy of a measuring instrument is the ability of a measuring instrument to give responses close to a true value.

   Analytical procedure is an orderly step-by-step procedure designed to ensure operational uniformity and minimize analytical drift.

Bioinformatics is a method to process and analyze biological data produced by a next generation sequencing instrument using combined tools such as computer science, statistics and mathematics.

Contamination is the unintentional introduction of exogenous DNA into a DNA sample or PCR reaction.

Critical Instrument is an instrument requiring calibration or a performance check prior to use and periodically thereafter.

Index is a molecular barcode, typically consisting of DNA sequence(s) covalently bound to genetic material from a sequencing library that provides sample identity and allows for multiple samples to be analyzed simultaneously.

Library (or sequencing library) is a work product consisting of genetic material prepared for analysis on a next generation sequencing instrument.

Material modification is an alteration of an existing analytical procedure that may have a consequential effect(s) on analytical results.

Methodology is used to describe the analytical processes and procedures used to support a DNA-typing technology: for example, extraction methods (manual vs. automated), quantitation methods (slot blot, fluorometry, real-time), typing test kit, and platform (capillary electrophoresis, real-time gel and end-point gel systems, next generation sequencing).

Next Generation Sequencing (NGS; also known as massively parallel sequencing, deep sequencing and high throughput sequencing) is a term used to describe modern sequencing technologies other than Sanger sequencing.

Performance check is a quality assurance measure to assess the functionality of laboratory instruments and equipment that affect the accuracy and/or validity of forensic, database, known or casework reference sample analysis.

Polymorphism (genetic) is the occurrence in a population of two or more alleles at a

genetic locus.

Precision characterizes the degree of mutual agreement among a series of individual measurements, values and/or results. 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.

Technology is used to describe the type of forensic DNA analysis performed in the laboratory, such as RFLP, STR, YSTR, XSTR, SNP, Microhaplotypes or mitochondrial DNA.

2. **General Considerations**

   2.1 **Validation** is a process by which a procedure is evaluated to determine its efficacy and reliability for forensic casework and/or database analysis.

   2.2 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. Developmental validation studies in other fields may sufficiently address forensic applications.

   2.2.1 **Developmental validation** is the acquisition of test data and determination of conditions and limitations of a new or novel DNA methodology for use on forensic, database, known or casework reference samples.

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

   2.2.1.2 Peer-reviewed publication (or other means of dissemination to the scientific community, such as presentation at a scientific meeting) of developmental validation studies is encouraged. However,

validated technologies or procedures may be implemented without such publication.

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

2.2.2   **Internal validation** is an accumulation of test data within the laboratory to demonstrate that established methods and procedures perform as expected in the laboratory. Prior to using a procedure for forensic applications, a laboratory shall conduct internal validation studies.

2.2.2.1 Internal validation studies should be sufficiently documented and summarized.

2.2.2.2 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 require prior to their being used in actual casework.

2.2.2.3 For laboratory systems that consist of more than one laboratory, each of the laboratories shall perform, document and maintain studies which may be impacted by location-specific factors (such as precision, sensitivity, contamination, etc.). Studies that are not location-specific may be shared among all locations.

3. **Developmental Validation**

   The developmental validation process shall include, where applicable, the following studies:

   3.1 **Characterization of genetic markers:** The basic characteristics (described below) of a genetic marker should 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, etc.).

       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) should be determined. 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.

   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, should 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 should be evaluated to determine the effects of such factors.

3.5 **Precision and accuracy** of the assay should be demonstrated:

Precision characterizes the degree of mutual agreement among a series of individual measurements, values and/or results. 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.

Accuracy is the degree of conformity of a measured quantity to its actual (true) value. Accuracy of a measuring instrument is the ability of a measuring instrument to give responses close to a true value.

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

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

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 testing 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 should be determined in relevant population groups. When appropriate, databases should be tested for independence expectations.

3.8 **Mixture studies:** The ability to obtain reliable results from mixed-source samples should be determined. These studies will assist the laboratory to establish 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.

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.

3.9.2 The reaction conditions needed to provide the required degree of specificity and robustness should be determined. These include, but are not limited to, thermal cycling parameters, the concentration of primers, magnesium chloride, DNA polymerase, and other critical reagents.

3.9.3 The potential for differential amplification among loci, preferential amplification of alleles in a locus, and stochastic amplification (i.e., excessive allelic signal imbalances due to the random sampling and amplification of low template quantities) should be assessed.

3.9.4 The effects of multiplexing should be assessed.

3.9.5 Appropriate controls should be assessed.

3.9.6 Criteria for detection of amplified product should be determined based on the platform and/or method.

3.9.7 Appropriate measurement standards (qualitative and/or quantitative) for

characterizing the alleles or resulting DNA product should be established.

3.10 **NGS-specific studies**

3.10.1 The effects of barcoding/indexing samples and subsequent bioinformatic sample separation should be assessed, to include, when appropriate, the effects of novel barcodes/indices that have not otherwise undergone developmental validation.

3.10.2 Sensitivity studies should address limit of detection as it relates to both starting DNA input as well as the extent of varying the quantity (extent of sample multiplexing in sequencing) and/or quality of libraries pooled in the sequencing reaction.

3.10.3 NGS instrumentation should be assessed for the possibility of signal cross talk during sequencing, and sample carryover between runs.

4. Internal Validation

The internal validation process shall include the studies detailed below. If conducted within the same laboratory, developmental validation studies may satisfy some elements of the internal validation guidelines. The laboratory should evaluate the appropriate sample number and type, based on the methodology and/or application necessary to demonstrate the potential limitations and reliability. The laboratory should determine the suitability of each study based on the methodology and may determine that a study is not necessary. The recommended internal validation studies are summarized in Table 1.

4.1 **Known and nonprobative evidence samples or mock evidence samples:**
Methods intended for casework samples should be evaluated and tested using known samples and nonprobative evidence samples or mock case samples.

Methods intended for database samples should be evaluated and tested using known samples. Results from these studies should be compared to the previous results of known samples and/or nonprobative evidence or mock case samples to ensure concordance.

4.2 **Sensitivity and Stochastic Studies:** The laboratory should demonstrate sensitivity levels of the test. Sensitivity studies are used to determine the dynamic range, ideal target range, limit of detection, limit of quantitation, 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.

4.3 **Precision and accuracy** of the assay should be demonstrated:

Precision characterizes the degree of mutual agreement among a series of individual measurements, values and/or results. 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.

Accuracy is the degree of conformity of a measured quantity to its actual (true) value. Accuracy of a measuring instrument is the ability of a measuring instrument to give responses close to a true value.

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

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

4.4 **Mixture studies:** Mixed DNA samples that are representative of those typically encountered by the testing laboratory should be evaluated. These studies will assist a casework laboratory to establish 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. A simplified mixture study may also assist a databasing laboratory to recognize mixtures and/or contamination.

4.5 **Contamination assessment:** The laboratory should evaluate, using both controls and known samples, the detection of exogenous DNA (including allele drop-in and heteroplasmy) originating from reagents, consumables, other samples, operator and/or laboratory environment.

4.6 **NGS-specific studies**

4.6.1 Sensitivity studies should address limit of detection as it relates to both starting DNA input as well as the extent of varying the quantity (extent of sample multiplexing in sequencing) and/or quality of libraries pooled in the sequencing reaction.

TABLE 1 – Summary of Recommended Studies for Internal Validation

	Extraction System	Quantitation System	Amplification System/Reaction Conditions	Detection System
Known / Non-Probative Samples	X	X	X	X
Precision and Accuracy: Repeatability	X	X	X	X
Precision and Accuracy: Reproducibility	X	X	X	X
Sensitivity Studies	X	X	X	X
Stochastic Studies		X	X	X
Mixture Studies	X*	X*	X	X
Contamination Assessment	X	X	X	X

"System" includes methodology, chemistries, and instrumentation.

* Mixture studies will be required if the assay is intended to distinguish different contributors (male/female, major/minor, etc.).

5. **Material Modification**

A material modification is an alteration of an existing analytical procedure that may have a consequential effect(s) on analytical results; for example, a decrease in reaction volume of an amplification test kit that is already in use by the laboratory or a change in injection time for a genetic analyzer. A material modification shall be evaluated by comparing the results from the original procedure to the results of the modified procedure to ensure concordance. The laboratory should evaluate the appropriate sample number, sample type, and the studies necessary to demonstrate this.

6. **Performance Check**

A performance check is a quality assurance measure to assess the functionality of laboratory instruments and equipment that affect the accuracy and/or validity of forensic, database, known or casework reference sample analysis. This may be required after repairs and/or scheduled maintenance. The laboratory should evaluate the appropriate sample number and type to demonstrate the reliability of the instrument or equipment. The laboratory should also determine the suitability of each study and may determine that a study is not necessary.

6.1   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, etc.), a performance check should be completed.

6.2   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. The performance check should demonstrate that results are reproducible on the new critical instrument and that values from the internal validation can still be obtained. For example, the performance check of a new critical instrument should demonstrate that the sensitivity level is consistent with the sensitivity level obtained from an internal validation, but need not demonstrate whether or not the new critical instrument is more sensitive.

7. **Software**

7.1   New software or significant software changes (including bioinformatics tools) that may impact interpretation, the analytical process, or sizing algorithms shall require a validation prior to implementation. Depending on the function and application of the software, the laboratory should determine the appropriate validation studies to identify its reliability and limitations.

7.2   A software upgrade that would not impact interpretation, the analytical process,

or sizing algorithms shall require a performance check.

8. **References and Suggested Readings**

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

FBI. Quality Assurance Standards for Forensic DNA Testing Laboratories
(September 1, 2011) available at http://www.fbi.gov/about-us/lab/codis/qas-
standards-for-forensic-dna-testing-laboratories-effective-9-1-2011

Scientific Working Group on DNA Analysis Methods. *Interpretation Guidelines for
Autosomal STR Typing by Forensic DNA Testing Laboratories*. Approved at the
Scientific Working Group on DNA Analysis Methods meeting, Fredericksburg,
Virginia, January 2010. Available at
http://www.swgdam.org/Interpretation_Guidelines_January_2010.pdf

Informational Web Sites: Additional information may be obtained from the following
web sites:

www.cstl.nist.gov/strbase

http://www.cstl.nist.gov/strbase/validation.htm

http://www.cstl.nist.gov/strbase/training.htm

Revision History

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.