NIST
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NIST DNA Analyst Webinar Series:
Validation Concepts and Resources – Part 1
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Validation Overview

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Validation Webinar Overview

John

- **Why** perform validation studies?

Robin

- **What** does validation entail?

Catherine
Mark

- **How** can validation be performed?

Becky/Mike

  - What tools are available to help?

Why Perform Validation Studies?

Reliability

1. Validation is part of a good quality system and is required as part of ISO 17025 accreditation

Reproducibility

2. Validated methods lead to more reliable results that in turn enable obtained results to be comparable between laboratories

Robustness

3. We want the correct answer when collecting data and we want no false negatives (if we fail to get a result from a sample, we want to have confidence that the sample contains no DNA rather than there might have been something wrong with the detection method)

Method validation is good science!

Purpose of Validation Studies

- "The purpose of validation studies is **to observe, document, and understand variation in the data generated under specific laboratory conditions**. **Validation helps define the scope or range of conditions under which reliable results may be obtained**. … By operating within validated ranges, uncertainty in measurements made on evidentiary samples with the technique can be accurately conveyed in laboratory reports."

Butler, J.M. (2015) *Advanced Topics in Forensic DNA Typing: Interpretation*, p. 10

There are many laboratory activities to validate…

- New STR kits
- CE instruments
- Quantitation kits or assays
- Genotyping software
- Rapid DNA instrument
- DNA extraction robotic process
- Probabilistic genotyping software

General Levels of Validation

- **Developmental Validation** – commonly performed by commercial manufacturer of a novel method or technology (more extensive than internal validation)
- **Internal Validation** – performed by individual lab when new method is introduced
- **Performance Checks** – verification of instrument or method reliability
  - With capillary electrophoresis methods, a lab can effectively do a performance check with every set of samples using the allelic ladder and internal size standard results

Validation Guidance for Forensic DNA

November 2010 ENFSI DNA Working Group Guidelines

http://www.enfsi.eu/sites/default/files/documents/minimum_validation_guidelines_in_dna_profiling_-_v2010_0.pdf

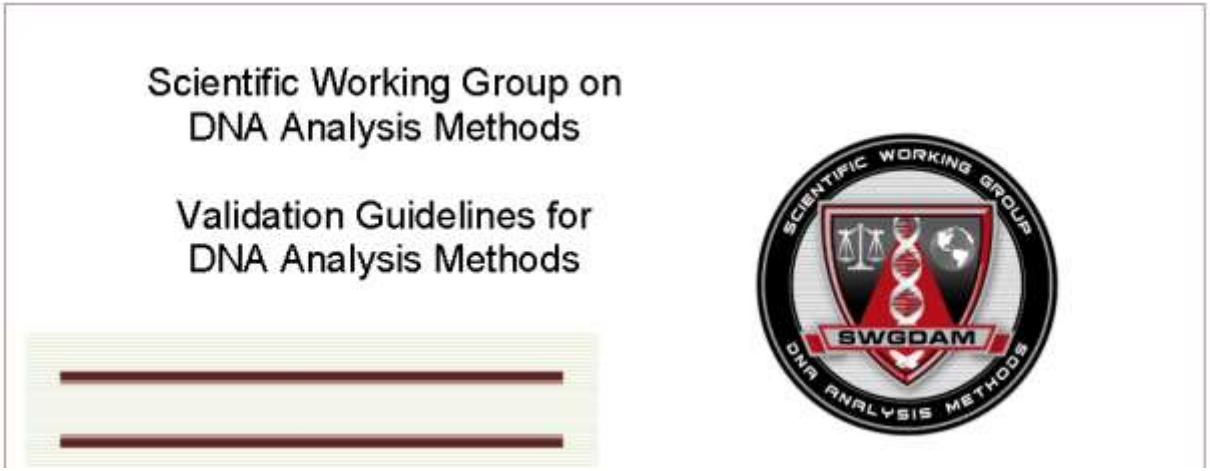
Recommended Minimum Criteria for the Validation of Various Aspects of the DNA Profiling Process			
DOCUMENT TYPE :	REF. CODE:	ISSUE NO:	ISSUE DATE:
POLICY	ENFSI DNA WORKING GROUP	001	November 2010

ISO 17025 Section 5.4.5 *discusses validation of methods*

December 2012 SWGDAM Guidelines

http://swgdam.org/SWGDAM_Validation_Guidelines_APPROVED_Dec_2012.pdf

Supersedes 2004 SWGDAM Revised Validation Guidelines *and builds on FBI Quality Assurance Standards (QAS) Section 8*



SWGDAM 2012 Validation Guidelines

- From p. 2: “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.”

What do the FBI Quality Assurance Standards (QAS) state regarding validation?

http://swgdam.org/SWGDAM_Validation_Guidelines_APPROVED_Dec_2012.pdf

FBI Quality Assurance Standards **Section 8 on Validation**

http://www.fbi.gov/about-us/lab/biometric-analysis/codis/qas-standards-for-forensic-dna-testing-laboratories-effective-9-1-2011

Standard 8.1 The laboratory shall use validated methodologies for DNA analyses. There are two types of validations: developmental and internal.

Standard 8.2 Developmental validation shall precede the use of a novel methodology for forensic DNA analysis.

8.2.1 **Developmental validation studies** shall include, where applicable, characterization of the genetic marker, species specificity, sensitivity studies, stability studies, reproducibility, case-type samples, population studies, mixture studies, precision and accuracy studies, and PCR-based studies. PCR-based studies include reaction conditions, assessment of differential and preferential amplification, effects of multiplexing, assessment of appropriate controls, and product detection studies. **All validation studies shall be documented**.

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

Standard 8.3 Except as provided in Standard 8.3.1.1, internal validation of all manual and robotic methods shall be conducted by each laboratory and reviewed and approved by the laboratory's technical leader prior to using a procedure for forensic applications.

8.3.1 **Internal validation studies** conducted after the date of this revision shall include as applicable: known and non-probative evidence samples or mock evidence samples, reproducibility and precision, sensitivity and stochastic studies, mixture studies, and contamination assessment. Internal validation studies shall be documented and summarized. **The technical leader shall approve the internal validation studies**.

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

8.3.2 **Internal validation shall define quality assurance parameters and interpretation guidelines**, including as applicable, guidelines for mixture interpretation.

8.3.3 A complete change of detection platform or test kit (or laboratory assembled equivalent) shall require internal validation studies.

Standard 8.4 Before the introduction of a methodology into the laboratory, the analyst or examination team shall successfully complete a competency test to the extent of his/her/their participation in casework analyses.

Standard 8.5 The performance of a modified procedure shall be evaluated by comparison with the original procedure using similar DNA samples.

Standard 8.6 Each additional critical instrument shall require a performance check. Modifications to an instrument, such as a detection platform, that do not affect the analytical portion of the instrument shall require a performance check.

Standard 8.7 Modifications to software, such as an upgrade, shall require a performance check prior to implementation. New software or significant software changes that may impact interpretation or the analytical process shall require a validation prior to implementation.

SWGDAM 2012 Validation Guidelines

- From p. 2: “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**.”
  - **Removes the 50 sample minimum requirement** of the SWGDAM Revised Validation Guidelines published in 2004
  - Laboratory must now determine appropriate numbers of samples for validation studies

http://swgdam.org/SWGDAM_Validation_Guidelines_APPROVED_Dec_2012.pdf

Points of Emphasis (1) from the SWGDAM 2012 Validation Guidelines

- 2.2.1.3: "A DNA laboratory may rely upon another laboratory's developmental validation studies."
- 2.2.2.2: "**Quality assurance parameters and interpretation guidelines shall be derived from internal validation studies**."
- 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." [e.g., >2-person mixtures]
- 3.8: "**Mixture studies**: The **ability to obtain reliable results** from mixed-source samples should be determined." [e.g., have >2-person mixtures been examined with lab protocols?]

http://swgdam.org/SWGDAM_Validation_Guidelines_APPROVED_Dec_2012.pdf

SWGDAM 2012 Validation Guidelines

- Section 4. Internal Validation
  - "…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**."

A primary purpose for validation studies then is to push the system until it fails in order to understand the potential limitations – to define the scope and range of method (and interpretation) reliability

http://swgdam.org/SWGDAM_Validation_Guidelines_APPROVED_Dec_2012.pdf

Points of Emphasis (2) from the SWGDAM 2012 Validation Guidelines

- 4.2: "**Sensitivity and Stochastic Studies**: The laboratory should demonstrate sensitivity levels of the test. … Sensitivity studies can also be used **to evaluate excessive random (stochastic) effects** generally resulting from low quantity and/or low quality samples." [i.e., you need to test low quantity and quality samples]

- 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…" [e.g., have >2-person mixtures been examined with your lab protocols?]

http://swgdam.org/SWGDAM_Validation_Guidelines_APPROVED_Dec_2012.pdf

My 2006 Urban Legends of Validation

http://www.promega.com/resources/profiles-in-dna/2006/debunking-some-urban-legends-surrounding-validation-within-the-forensic-dna-community/

A review of these urban legends with some more recent perspectives is available in Butler (2012) *Advanced Topics in Forensic DNA Typing: Methodology*, p. 190.

From Butler 2012, p. 190: “Validation requires common sense and is best performed…with well-characterized samples through concordance to results produced from previous methods. Some aspects of validation can be achieved with a minimal amount of DNA samples while other aspects will require more extensive studies…”

My Philosophy towards Validation

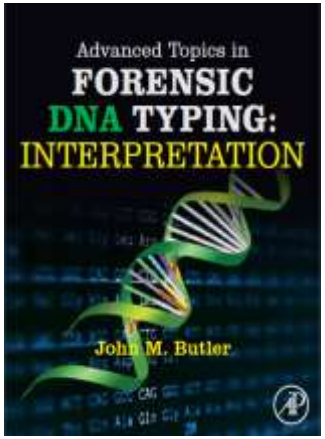
Ask first: Does the new method improve your capability?

- **C**oncordance – are the same typing results obtained with the new technique as with an older one?
- **C**onstant Monitoring – check multiple allelic ladders in a batch against one another to confirm precision and consistent lab temperature
- **C**ommon Sense – are replicate tests repeatable?

Is validation simply something your laboratory does in order to pass an audit or do the results impact your SOPs & daily work?

Validation data should inform laboratory interpretation protocols developed and utilized

- Analytical threshold
- Stochastic threshold
  - (not needed if using a probabilistic genotyping method)
- Stutter threshold
  - general, locus-specific, or allele-specific
- Peak height ratios
  - to define potential genotype combinations



Depending on the sensitivity and specificity of interpretation desired, more validation data may be needed…

New *Interpretation* book
Available September 2014

Thank you for your attention

STRBase validation information available at:
http://www.cstl.nist.gov/strbase/validation.htm

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