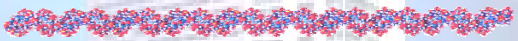
Validation:

What Is It, Why Does It Matter, and How Should It Be Done?



John M. Butler, PhD
National Institute of Standards and Technology
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301-975-4049
http://www.cstl.nist.gov/biotech/strbase/validation.htm

18th International Symposium on Human Identification
Hollywood, CA
October 4, 2007

Promega NIST

NIST and NIJ Disclaimer

Funding: Interagency Agreement 2003-IJ-R-029 between the National Institute of Justice and NIST Office of Law Enforcement Standards

Points of view are mine and do not necessarily represent the official position or policies of the US Department of Justice or the National Institute of Standards and Technology.

Certain commercial equipment, instruments and materials are identified in order to specify experimental procedures as completely as possible. In no case does such identification imply a recommendation or endorsement by the National Institute of Standards and Technology nor does it imply that any of the materials, instruments or equipment identified are necessarily the best available for the purpose.

My Background

- PhD **(Analytical Chemistry)** from University of Virginia Research conducted at FBI Academy under Bruce McCord doing CE for STR typing (May 1993 - Aug 1995)
- NIST Postdoc – developed STRBase website
- GeneTrace Systems – private sector experience validating assays and developing new technologies
- NIST Human Identity Project Leader since 1999
- Invited guest to FBI's Scientific Working Group on DNA Analysis Methods (SWGDAM) since 2000
- **Member of SWGDAM Validation Subcommittee** – resulting in Revised Validation Guidelines
- Served on WTC KADAP and helped evaluate and validate new miniSTR, mtDNA, and SNP assays

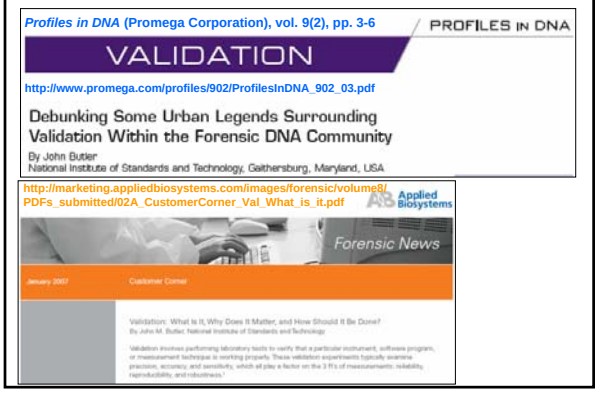
NIST Human Identity Project Team

John Butler, Margaret Kline, Pete Vallone, Jan Redman, Amy Decker, Becky Hill, Dave Duewer

Publications and presentations available on STRBase:
http://www.cstl.nist.gov/biotech/strbase/NISTpub.htm

- **26 publications** from Jan-Dec 2006
- **45 presentations** and **10 workshops** to the community from Jan-Dec 2006

Recent Articles I Have Written on Validation

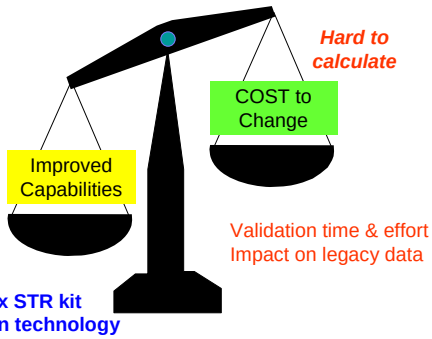


Stages of Technology for Forensic DNA Typing

- Idea
- Demonstration of feasibility
- Research and development
- Commercialization
- **Validation by forensic labs**
- Routine use by the community

TIME MONEY

Decision to Switch/Upgrade to New Technology



New multiplex STR kit
New detection technology
New DNA markers

Decisions about Changing Technologies

- Cost to change
- Comfort and experience levels
  - court approved methods must be used in forensic labs
- Capabilities…Enhancements
  - Are they really needed?
  - Will legacy data be impacted?

Where Is the Future Going for DNA Technology That Can Be Applied to Forensic DNA Typing?

Constant state of evolution (like computers)

- Higher levels of multiplexes
- More rapid DNA separations
- Better data analysis software
- New DNA Markers

Validating new technologies will always be important in progressive forensic DNA labs…

Validation Workshop Outline

Theoretical

- Importance of Validation
- History of Forensic Validation Guidelines
- **SWGDAM Revised Validation Guidelines**
- Validation Philosophy & Resources

BREAK

Practical

- Validation Survey Results
- Developmental Validation
- Internal Validation and Examples
- Documentation and Training

Background, Essentials and Importance of Validation

My Purpose in Teaching This Workshop

- I believe that many forensic laboratories, in an effort to be cautious, are taking too long to perform their validation studies and thereby delaying initiation of casework and contributing to backlogs in labs that are already overburdened
- Technology will continue to advance and thus validation of new methodologies will always be important in forensic DNA laboratories

There will always be something to "validate"…

Validation Workshop (Aug 24-26, 2005 at NFSTC)

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

COURSE CONTENTS

Day #1
- Validation Overview (John)
- Introduction to DAB Standards (Robyn & John)
- Developmental Validation (John)

Day #2
- Inconsistency in Validation between Labs (John)
- Internal Validation (Robyn)
- Method Modifications and Performance Checks (Robyn)

Day #3
- Practical Exercises (Robyn)

Was filmed and is being made into a training DVD as part of the President's DNA Initiative…

Importance of Validation

Questions to Keep in Mind…

- Why is validation important?
- How does validation help with quality assurance within a laboratory?
- What are the general goals of analytical validation?
- How is method validation performed in other fields such as the pharmaceutical industry?
- How do accuracy, precision, sensitivity, stability, reproducibility, and robustness impact measurements?

What is **Validation** and Why Should It Be Done?

- Part of overall quality assurance program in a laboratory
- **We want the correct answer when collecting data…**
  - We want **analytical measurements made in one location to be consistent with those made elsewhere** (without this guarantee there is no way that a national DNA database can be successful).
- 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…

Want no false negatives…

Why is Method Validation Necessary?

- It is an important element of quality control.
- Validation helps provide assurance that a measurement will be reliable.
- In some fields, validation of methods is a regulatory requirement.
- …
- The validation of methods is **good science**.

Roper, P., *et al.* (2001) *Applications of Reference Materials in Analytical Chemistry*. Royal Society of Chemistry, Cambridge, UK, pp. 107-108.

Definition of Validation

- **Validation** is confirmation by examination and provision of objective evidence that the particular requirements for a specified intended use are fulfilled.
- **Method validation** is the process of establishing the performance characteristics and limitations of a method and the identification of the influences which may change these characteristics and to what extent. It is also the process of verifying that a method is fit for purpose, i.e., for use for solving a particular analytical problem.

EURACHEM Guide (1998) *The Fitness for Purpose of Analytical Methods: A Laboratory Guide to Method Validation and Related Topics*; available at http://www.eurachem.ul.pt/guides/valid.pdf

More Validation Definitions

ISO 17025

5.4.5.1 Validation is the **confirmation by examination** and the provision of objective evidence that the particular requirements for a specific intended use are fulfilled

DAB Quality Assurance Standards for Forensic DNA Testing Laboratories

2 (ff) Validation is a **process by which a procedure is evaluated** to determine its efficacy and reliability for forensic casework analysis and includes:

To demonstrate that a method is suitable for its intended purpose…

Definitions

J.M. Butler (2005) *Forensic DNA Typing*, 2nd Edition, p. 389, 391

- **Quality assurance** (QA) – planned or systematic actions necessary to provide adequate confidence that a product or service will satisfy given requirements for quality
- **Quality control** (QC) – day-to-day operational techniques and activities used to fulfill requirements of quality
- **Validation** – the process of demonstrating that a laboratory procedure is **robust**, **reliable**, and **reproducible** in the hands of the personnel performing the test in that laboratory

Definitions

J.M. Butler (2005) *Forensic DNA Typing*, 2nd Edition, p. 391

- **Robust method** – successful results are obtained a high percentage of the time and few, if any, samples need to be repeated
- **Reliable method** – the obtained results are accurate and correctly reflect the sample being tested
- **Reproducible method** – the same or very similar results are obtained each time a sample is tested

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** – can be performed with every run (set of samples)

Historical Perspective

Brief Historical Overview

Profiles in DNA (Sept 1999) 3(2): 10-11


CURRENT EVENTS

The Evolution of Quality Standards for Forensic DNA Analyses in the United States

By Special Agent Lawrence A. Presley, MS, MA
Federal Bureau of Investigation Laboratory, Washington, DC
lpresley@fbi.gov

Quality problems in late 1980s with DNA testing
TWGDAM established under FBI Lab sponsorship in 1988
NRC I (1992) and NRC II (1996) issued reports recommending formal QA programs
DNA Identification Act of 1994 lead to formation of DNA Advisory Board (DAB)
DAB Standards issued in Oct 1998 and Apr 1999
When DAB was dissolved in 2000, SWGDAM assumed leadership role

DNA Identification Act (1994)

Public Law 103-322

42 § 14131. Quality assurance and proficiency testing standards

(a) Publication of quality assurance and proficiency testing standards

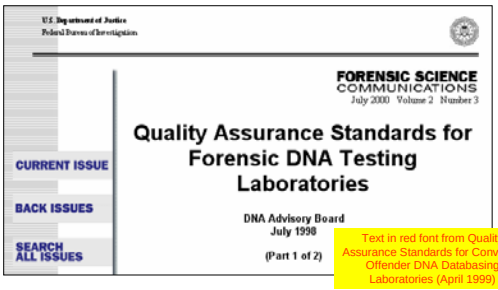
(1) (A) Not later than 180 days after September 13, 1994, the Director of the Federal Bureau of Investigation shall appoint an advisory board on DNA quality assurance methods from among nominations proposed by the head of the National Academy of Sciences and professional societies of crime laboratory officials.

(B) The advisory board shall include as members scientists from State, local, and private forensic laboratories, molecular geneticists and population geneticists not affiliated with a forensic laboratory, and a representative from the National Institute of Standards and Technology.

(C) **The advisory board shall develop, and if appropriate, periodically revise, recommended standards for quality assurance**, including standards for testing the proficiency of forensic laboratories, and forensic analysts, in conducting analyses of DNA.

DNA Advisory Board (DAB)

DAB Standards


U.S. Department of Justice
Federal Bureau of Investigation

FORENSIC SCIENCE COMMUNICATIONS
July 2000 Volume 2 Number 3

CURRENT ISSUE
BACK ISSUES
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Quality Assurance Standards for Forensic DNA Testing Laboratories

DNA Advisory Board
July 1998

(Part 1 of 2)

Text in red font from Quality Assurance Standards for Convicted Offender DNA Databasing Laboratories (April 1999)

http://www.fbi.gov/hq/lab/fsc/backissu/july2000/codis2a.htm

http://www.fbi.gov/hq/lab/fsc/backissu/july2000/codis1a.htm

Outline of DAB Standards

Quality Assurance Standards (QAS)

1. SCOPE
2. DEFINITIONS
3. QUALITY ASSURANCE PROGRAM
4. ORGANIZATION AND MANAGEMENT
5. PERSONNEL
6. FACILITIES
7. EVIDENCE *(SAMPLE)* CONTROL
8. **VALIDATION**
9. ANALYTICAL PROCEDURES
10. EQUIPMENT CALIBRATION AND MAINTENANCE
11. REPORTS
12. REVIEW
13. PROFICIENCY TESTING
14. CORRECTIVE ACTION
15. AUDITS
16. SAFETY
17. SUBCONTRACTOR OF ANALYTICAL TESTING FOR WHICH VALIDATED PROCEDURES EXIST

http://www.fbi.gov/hq/lab/fsc/backissu/july2000/codis2a.htm

http://www.fbi.gov/hq/lab/fsc/backissu/july2000/codis1a.htm

Validation Section of the DNA Advisory Board Standards

issued October 1, 1998 and April 1999; published in *Forensic Sci. Comm.* July 2000

STANDARD 8.1 The laboratory shall use validated methods and procedures for forensic casework analyses *(DNA analyses)*.

8.1.1 Developmental validation that is conducted shall be appropriately documented.

8.1.2 Novel forensic DNA methodologies shall undergo developmental validation to ensure the accuracy, precision and reproducibility of the procedure. The developmental validation shall include the following:

8.1.2.1 Documentation exists and is available which defines and characterizes the locus.

8.1.2.2 Species specificity, sensitivity, stability and mixture studies are conducted.

8.1.2.3 Population distribution data are documented and available.

8.1.2.3.1 The population distribution data would include the allele and genotype distributions for the locus or loci obtained from relevant populations. Where appropriate, databases should be tested for independence expectations.

8.1.3 Internal validation shall be performed and documented by the laboratory.

8.1.3.1 The procedure shall be tested using known and non-probative evidence samples *(known samples only)*. The laboratory shall monitor and document the reproducibility and precision of the procedure using human DNA control(s).

8.1.3.2 The laboratory shall establish and document match criteria based on empirical data.

8.1.3.3 Before the introduction of a procedure into forensic casework *(database sample analysis)*, the analyst or examination team shall successfully complete a qualifying test.

8.1.3.4 Material modifications made to analytical procedures shall be documented and subject to validation testing.

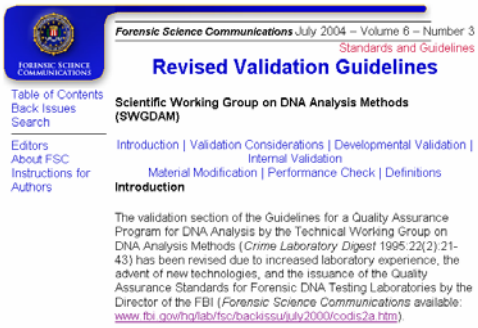
8.1.4 Where methods are not specified, the laboratory shall, wherever possible, select methods that have been published by reputable technical organizations or in relevant scientific texts or journals, or have been appropriately evaluated for a specific or unique application.

FORENSIC SCIENCE COMMUNICATIONS JULY 2000 VOLUME 2 NUMBER 3

Revised SWGDAM Validation Guidelines

(July 2004)

http://www.fbi.gov/hq/lab/fsc/backissu/july2004/standards/2004_03_standards02.htm



The document provides validation guidelines and definitions approved by SWGDAM July 10, 2003.

Previous Guidelines Regarding Validation

Technical Working Group on DNA Analysis Methods (TWGDAM)

- TWGDAM (1995) – *Crime Lab Digest* 22(2):20-43
  - Budowle *et al.* "Guidelines for a quality assurance program for DNA analysis"
- TWGDAM (1991) – *Crime Lab Digest* 18(2):44-75
  - Kearney *et al.* "Guidelines for a quality assurance program for DNA analysis"
- TWGDAM (1989) – *Crime Lab Digest* 16(2):40-59
  - Kearney *et al.* "Guidelines for a quality assurance program for DNA restriction fragment length polymorphism analysis"

AABB standards (1989) – first standards adopted by an organization dealing with DNA testing impacting human identification; the standards are not intended to provide the details of a technique but rather to give an overview of general policies that when followed will help guarantee reliable results…

From more information on American Association of Blood Banks (AABB) – see http://www.aabb.org

Differences between 1991 and 1995 TWGDAM Guidelines

Crime Lab Digest 1991; 18(2):44-75
Crime Lab Digest 1995; 22(2):20-43

Validation
4.1.3 Expanded upon RFLP and added information on STRs
4.1.5.10 Added "where appropriate"
4.4.2.1 Added (b) "when a PCR product is characterized by direct sequencing…"

Equipment, Materials, and Facilities
5.3.2 Added "an extraction area for samples containing low DNA levels…"

Analytical Procedures
7.2.2 Changed "regular use" to "periodic use" and removed "cellular"
7.3 Added "where appropriate"
7.5.1.3 Removed "substrate" and "(e.g. unstained areas adjacent…)"
7.5.1.4 Deleted original 7.5.1.4 and moved 7.5.1.5 (1991) to 7.4.1.4 (1995)

Audits
10.1 Changed from "annually" to "at least once every 2 years"

At that time, it was not possible to quantify DNA down to the level where DNA could be amplified. Sections 4.4.2.1 and 5.3.2 were added to accommodate mtDNA sequencing needs.

Differences between 1991 and 1995 TWGDAM Guidelines

Crime Lab Digest 1991; 18(2):44-75
Crime Lab Digest 1995; 22(2):20-43

(1991) 4.1.3 Each locus to be used must go through the necessary validation.

(1995) 4.1.3 Once an RFLP procedure has been validated, **appropriate studies of limited scope** (*e.g.*, population studies, human DNA control value determination) must be available for each new locus used. A similar standard should be maintained when adding new loci to the different PCR-based techniques (*e.g.*, addition of short tandem (STR) locus to a validated STR procedure).

Comparison of DAB Standards and Previous Validation Guidelines

DNA Loci

TWGDAM 1989	TWGDAM 1991/1995	DAB (1998)	SWGDAM 2004
Inheritance	Inheritance (4.2.1)	Defined	Inheritance (2.1.1)
Gene mapping	Gene mapping (4.2.2)	Characterized	Mapping (2.1.2)
Polymorphism type	Polymorphism type (4.2.4)		Polymorphism type (2.1.4)
Probe available	Primers known (4.4.1.1)		Primer publication not required (2.10)
	Detection basis (4.2.3)		Detection basis (2.1.3)

PCR Considerations

TWGDAM 1989	TWGDAM 1991/1995	DAB (1998)	SWGDAM 2004
(Not discussed)	Minimum sample (4.1.5.10)	Sensitivity	Sensitivity studies (2.3)
	Primer sequence (4.4.1.1)		Primer publication not required (2.10)
	Contamination control (4.4.1.2)		
	PCR conditions (4.4.1.3)		PCR conditions (2.10.1)
	PCR cycle # (4.4.1.4)		
	Differential PCR (4.4.1.5)		Differential PCR (2.10.2)
	Positive & negative controls (4.4.2)		Positive & negative controls (2.10.4)
			Coamplification assessed (2.10.3)

Comparison of DAB Standards and Previous Validation Guidelines

Developmental Validation

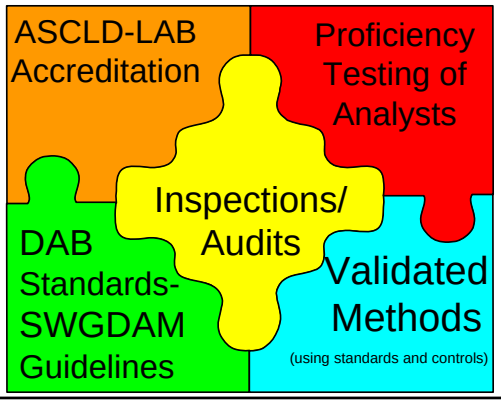
TWGDAM 1989	TWGDAM 1991/1995	DAB (1998)	SWGDAM 2004
Standard specimens	Standard specimens	Standard specimens	
Different tissues	Different tissues		Sensitivity (2.3)
Consistency	Consistency		
Population studies	Population studies	Population studies	Population studies (2.7)
Reproducibility	Reproducibility	Reproducibility	Reproducibility (2.5)
Time/Temp	Environmental	Stability	Stability studies (2.4)
Degradation/Matrix	Degradation/Matrix		
Non-probative	Non-probative		Case-type samples (2.6)
Non-human	Non-human	Species specificity	Species specificity (2.2)
On-site (alpha/beta)	On-site (alpha/beta)		
	Mixed specimens	Mixture	Mixture studies (2.8)
		Accuracy	Precision & accuracy (2.9)
		Precision	
			PCR based procedures (2.10)

Comparison of DAB Standards and Previous Validation Guidelines

Internal Validation

TWGDAM 1989	TWGDAM 1991/1995	DAB (1998)	SWGDAM 2004
Known samples	Known samples	Known samples	Known & non-probative (3.1)
Proficiency tests	Proficiency tests		
Precision	Precision		
	Contamination control		
		Reproducibility	Reproducibility & precision (3.2)
		Non-probative	
		Match criteria	Match criteria (3.3)
			Sensitivity & stochastic effects (3.4)
			Mixture studies (3.5)
			Contamination (3.6)
			Qualifying test (3.7)

Ensuring Accurate Forensic DNA Results

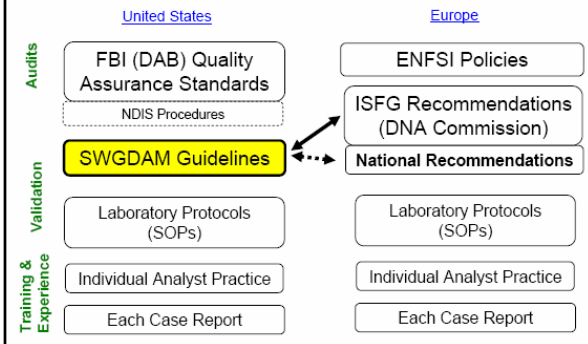


Checks and Controls on DNA Results

Community	FBI DNA Advisory Board's Quality Assurance Standards *(also interlaboratory studies)* ISO17025
Laboratory	ASCLD/LAB Accreditation and Audits
Analyst	Proficiency Tests & Continuing Education
Method/Instrument	**Validation of Performance** ***(along with traceable standard sample)***
Protocol	Standard Operating Procedure is followed
Data Sets	Allelic ladders, positive and negative amplification controls, and reagent blanks are used
Individual Sample	Internal size standard present in every sample
Interpretation of Result	Second review by qualified analyst/supervisor
Court Presentation of Evidence	Defense attorneys and experts with power of discovery requests

My perspective…

Hierarchy of Rules for Forensic DNA Labs



Hopefully each conforms to the levels above it…

Validation Philosophy

When is Validation Needed?

- Before introduction of a new method into routine use
- Whenever the conditions change for which a method has been validated, e.g., instrument with different characteristics
- Whenever the method is changed, and the change is outside the original scope of the method

L. Huber (2001) Validation of Analytical Methods: Review and Strategy. Supplied by www.labcompliance.com

Costs/Benefits of Validation and Quality Assurance

Costs

- Direct
  - Test materials
  - Standards
  - Quality assurance equipment
  - Analysis of QA/QC samples
  - Quality assurance official
  - Committee Work
  - Interlab Studies
  - Travel to meetings

Benefits

- More efficient outputs
- Fewer replicates for same reliability
- Fewer do-overs
- Greater confidence of:
  - Staff
  - Laboratory
  - Customers

Table 26.2 in J.K. Taylor (1987) *Quality Assurance of Chemical Measurements*. Lewis Publishers: Chelsea, MI.

Some Purposes of Validation

- To accept an individual sample as a member of a population under study
- To admit samples to the measurement process
- To minimize later questions on sample authenticity
- To provide an opportunity for resampling when needed

Sample validation should be based on objective criteria to eliminate subjective decisions…

J.K. Taylor (1987) *Quality Assurance of Chemical Measurements*. Lewis Publishers: Chelsea, MI, p. 193

The VAM Principles

VAM = Valid Analytical Measurement

1. Analytical measurements should be made to satisfy an agreed requirement.
2. Analytical measurements should be made using methods and equipment that have been tested to ensure they are fit for their purpose.
3. **Staff making analytical measurements should be both qualified and competent to undertake the task**.
4. There should be a regular and independent assessment of the technical performance of a laboratory.
5. Analytical measurements made in one location should be consistent with those made elsewhere.
6. Organizations making analytical measurements should have well defined quality control and quality assurance procedures.

Roper P *et al.* (2001) *Applications of Reference Materials in Analytical Chemistry*. Royal Society of Chemistry: Cambridge UK, p. 2

The Community Benefits from Training

- To better understand what validation entails and how it should be performed (why a particular data set is sufficient)
- Many labs already treat DNA as a "black box" and therefore simply want a "recipe" to follow
- People are currently driven by fear of auditors and courts rather than scientific reasoning
- Many different opinions exist and complete consensus is probably impossible

How do you validate a method?

- Decide on analytical requirements
  - Sensitivity, resolution, precision, etc.
- **Plan a suite of experiments**
- **Carry out experiments**
- Use data to assess fitness for purpose
- Produce a statement of validation
  - Scope of the method

Roper, P., *et al.* (2001) *Applications of Reference Materials in Analytical Chemistry*. Royal Society of Chemistry, Cambridge, UK, pp. 108-109.

Assumptions When Performing Validation

- The equipment on which the work is being done is broadly suited to the application. It is clean, well-maintained and within calibration.
- The staff carrying out the validation are competent in the type of work involved.
- There are no unusual fluctuations in laboratory conditions and there is no work being carried out in the immediate vicinity that is likely to cause interferences.
- The samples being used in the validation study are known to be sufficiently stable.

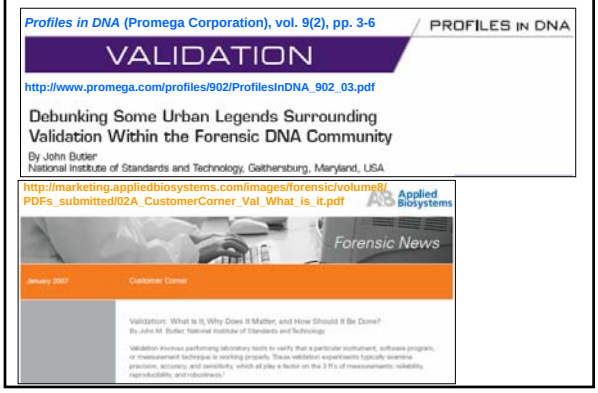
Roper, P., *et al.* (2001) *Applications of Reference Materials in Analytical Chemistry*. Royal Society of Chemistry, Cambridge, UK, pp. 110-111.

Tools of Method Validation

- Standard samples
  - positive controls
  - NIST SRMs
- Blanks
- Reference materials prepared in-house and spikes
- Existing samples
- Statistics
- **Common sense**

Roper, P., *et al.* (2001) *Applications of Reference Materials in Analytical Chemistry*. Royal Society of Chemistry, Cambridge, UK, p. 110.

Recent Articles I Have Written on Validation



Urban Legends of Validation…

Butler, J.M. (2006) *Profiles in DNA* vol. 9(2), pp. 3-6

#1: HUNDREDS OR THOUSANDS OF SAMPLES ARE REQUIRED TO FULLY VALIDATE AN INSTRUMENT OR METHOD

#2: VALIDATION IS UNIFORMLY PERFORMED THROUGHOUT THE COMMUNITY

#3: EACH COMPONENT OF A DNA TEST OR PROCESS MUST BE VALIDATED SEPARATELY

#4: VALIDATION SHOULD SEEK TO UNDERSTAND EVERYTHING THAT COULD POTENTIALLY GO WRONG WITH AN INSTRUMENT OR TECHNIQUE

#5: LEARNING THE TECHNIQUE AND TRAINING OTHER ANALYSTS ARE PART OF VALIDATION

#6: VALIDATION IS BORING AND SHOULD BE PERFORMED BY SUMMER INTERNS SINCE IT IS BENEATH THE DIGNITY OF A QUALIFIED ANALYST

#7: DOCUMENTING VALIDATION IS DIFFICULT AND SHOULD BE EXTENSIVE

#8: ONCE A VALIDATION STUDY IS COMPLETED YOU NEVER HAVE TO REVISIT IT

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?

Common Perceptions of Validation

The goal is not to experience every possible scenario during validation…

"You cannot mimic casework because every case is different."

Effort

Lots of experiments are required

Many labs are examining far too many samples in validation and thus delaying application of casework and contributing to backlogs…

Significant time is required to perform studies

Time

Number of Samples Needed

Relationship between a sample and a population of data

How do you relate these two values?

Data collected in your lab as part of validation studies → All potential data that will be collected in the future in your lab

Student's *t*-Test associates a sample to a population

"Sample" of Typical Data

"Population" of All Data Obtained

Student's *t*-Tests

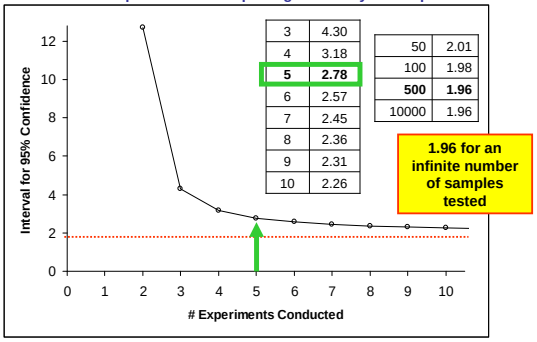
"Student" (real name: W. S. Gossett [1876-1937]) developed statistical methods to solve problems stemming from his employment in a brewery.

Student's *t*-test deals with the problems associated with inference based on "small" samples: the calculated mean (X_{avg}) and standard deviation (σ) may by chance deviate from the "real" mean and standard deviation (i.e., **what you'd measure if you had many more data items: a "large" sample**).

http://www.physics.csbsju.edu/stats/t-test.html

Student's t-Test Curve

Impact of Number of Experiments on Capturing Variability in a Population of Data



3	4.30
4	3.18
5	**2.78**
6	2.57
7	2.45
8	2.36
9	2.31
10	2.26

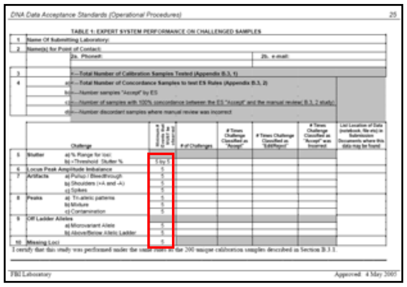
50	2.01
100	1.98
500	**1.96**
10000	1.96

1.96 for an infinite number of samples tested

The Number “5” in Forensic Validation

NDIS Appendix B Expert System Validation Requirements

- At least **5 challenge events** must be observed for each issue (e.g., pullup, shoulders, spikes, tri-allelic patterns, mixtures, contamination, variant alleles)



DNA Data Acceptance Standards (Operational Procedures) 25

TABLE 1: EXPERT SYSTEM PERFORMANCE ON CHALLENGED SAMPLES

1	Name Of Submitting Laboratory:	
2	Name(s) for Point of Contact:	
	2a. Phone#:	2b. e-mail:
3	Total Number of Calibration Samples Tested (Appendix B.3.1)	
4	a) Total Number of Concordance Samples to test ES Rules (Appendix B.3.2)	
	b) Number samples "Accept" by ES	
	c) Number of samples with 100% concordance between the ES "Accept" and the manual review (B.3.2 study)	
	d) Number discordant samples where manual review was incorrect	

	Challenge		Minimum # Events that would be observed	# of Challenges	# Times Challenge Classified as "Accept"	# Times Challenge Classified as "Edit/Reject"	# Times Challenge Classified as "Accept" was Incorrect	List Location of Data (notebook, file etc) in Submission Documents where this data may be found
5	Stutter	a) % Range for loci:						
		b) >Threshold Stutter %	5 by 5					
6	Locus Peak Amplitude Imbalance		5					
7	Artifacts	a) Pullup / Bleedthrough	5					
		b) Shoulders (+A and -A)	5					
		c) Spikes	5					
8	Peaks	a) Tri-allelic patterns	5					
		b) Mixture	5					
		c) Contamination	5					
9	Off Ladder Alleles							
		a) Microvariant Allele	5					
		b) Above/Below Allelic Ladder	5					
10	Missing Loci		5					

I certify that this study was performed under the same rules as the 200 unique calibration samples described in Section B.3.1.

FBI Laboratory Approved: 4 May 2005

Allele Frequency Tables

Allele frequencies denoted with an asterisk (*) are **below the 5/2N minimum allele threshold** recommended by the National Research Council report (NRCII) *The Evaluation of Forensic DNA Evidence* published in 1996.

D3S1358		Butler *et al.* (2003) *JFS* 48(4):908-911 **Caucasian** *N= 302*	Einum *et al.* (2004) *JFS* 49(6): 1381-1385 **Caucasian** ***N= 7,636***
	Allele		
	11	0.0017*	0.0009
	12	0.0017*	0.0007
	13	--	0.0031
	14	0.1027	0.1240
Most common allele	**15**	**0.2616**	**0.2690**
	15.2	--	--
	16	0.2533	0.2430
	17	0.2152	0.2000
	18	0.15232	0.1460
	19	0.01160	0.0125
	20	0.0017*	0.0001*

Minimum Allele Frequency = **5**/2N

Want to sample at least 5 chromosomes to provide a somewhat reliable estimate of an allele's frequency in a population

Validation in Other Fields
(Besides Forensic DNA Testing)

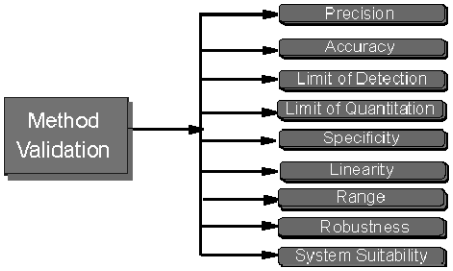
Pharmaceutical Industry and FDA Follows ICH Validation Documents

- ICH (**International Conference on Harmonization** of Technical Requirements for Registration of Pharmaceuticals for Human Use)
  - http://www.ich.org
  - **Q2A**: Text on Validation of Analytical Procedures (1994)
    - http://www.fda.gov/cder/guidance/ichq2a.pdf
  - **Q2B**: Validation of Analytical Procedures : Methodology (1996)
    - http://www.fda.gov/cder/guidance/1320fnl.pdf

- From Q2B:
  - "For the establishment of linearity, **a minimum of five concentrations is recommended**"
  - "Repeatability should be assessed using (1) **a minimum of 9 determinations covering the specified range for the procedure** (e.g., 3 concentrations/3 replicates each); or (2) a minimum of 6 determinations at 100 percent of the test concentration."

ICH Method Validation Parameters

http://www.waters.com/watersdivision/contentd.asp?watersit=JDRS-5LT6WZ



Method validation provides an assurance of reliability during normal use, and is sometime referred to as "the process of providing documented evidence that the method does what it is intended to do."

Precision

- "The closeness of agreement between independent test results obtained under stipulated conditions."
- "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."
- "A measure for the reproducibility of measurements within a set, that is, of the scatter or dispersion of a set about its central value."

EURACHEM Guide (1998) *The Fitness for Purpose of Analytical Methods: A Laboratory Guide to Method Validation and Related Topics*, p. 45; available at http://www.eurachem.ul.pt/guides/valid.pdf

Accuracy

- "The closeness of agreement between a test result and the accepted reference value."
- "Accuracy of a measuring instrument is the ability of a measuring instrument to give responses close to a true value."

EURACHEM Guide (1998) *The Fitness for Purpose of Analytical Methods: A Laboratory Guide to Method Validation and Related Topics*, pp. 39, 41; available at http://www.eurachem.ul.pt/guides/valid.pdf

Sensitivity

- **Limit of detection** (**LOD**) – "the lowest content that can be measured with reasonable statistical certainty."
- **Limit of quantitative measurement** (**LOQ**) – "the lowest concentration of an analyte that can be determined with acceptable precision (repeatability) and accuracy under the stated conditions of the test."
- How low can you go?

EURACHEM Guide (1998) *The Fitness for Purpose of Analytical Methods: A Laboratory Guide to Method Validation and Related Topics*, p. 43; available at http://www.eurachem.ul.pt/guides/valid.pdf

Specificity

- "The ability of a method to measure only what it is intended to measure."
- "Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Typically these might include impurities, degradants, matrix, etc."
- The primers in PCR amplification provide specificity in forensic DNA testing.

EURACHEM Guide (1998) *The Fitness for Purpose of Analytical Methods: A Laboratory Guide to Method Validation and Related Topics*, p. 51; available at http://www.eurachem.ul.pt/guides/valid.pdf

Reproducibility

- "Precision under reproducibility conditions, i.e. conditions where test results are obtained with the same method on identical test items in different laboratories with different operators using different equipment."
- Will you get the same result each time you test a sample?
- Different from **repeatability**, which is the "precision under repeatability conditions, i.e. conditions where independent test results are obtained with the same method on identical test items in the same laboratory by the same operator using the same equipment within short intervals of time."

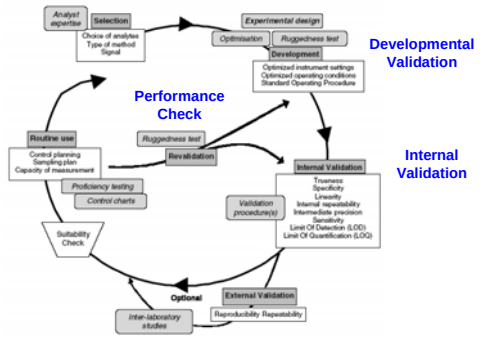
EURACHEM Guide (1998) *The Fitness for Purpose of Analytical Methods: A Laboratory Guide to Method Validation and Related Topics*, pp. 47-48; available at http://www.eurachem.ul.pt/guides/valid.pdf

Robustness (Ruggedness)

- "The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage."
- The method works routinely…
- You do not want the method to fail when you only have enough material for a single try.

EURACHEM Guide (1998) *The Fitness for Purpose of Analytical Methods: A Laboratory Guide to Method Validation and Related Topics*, p. 49; available at http://www.eurachem.ul.pt/guides/valid.pdf

The lifecycle of a method of analysis



Feinberg *et al.* (2004) *Anal. Bioanal. Chem.* 380: 502-514

Useful Resources on Validation

- Taylor JK. (1981) Quality assurance of chemical measurements. *Analytical Chemistry* 53(14): 1588A-1596A.
- Taylor JK. (1983) Validation of analytical methods. *Analytical Chemistry* 55(6): 600A-608A.
- Green JM. (1996) A practical guide to analytical method validation. *Analytical Chemistry* 68: 305A-309A.
- EURACHEM Guide (1998) *The Fitness for Purpose of Analytical Methods: A Laboratory Guide to Method Validation and Related Topics*; available at http://www.eurachem.ul.pt/guides/valid.pdf

See also STRBase Validation Section:
http://www.cstl.nist.gov/biotech/strbase/validation.htm

Summary of 2004 Validation Survey

Contacting the Community

- **Validation Standardization Questionnaire** handed out at NIJ DNA Grantees meeting (June 28-30, 2004)
- Emails sent to >200 scientists (July-Aug 2004)
  - Attendees from the NIJ DNA Grantees meeting
  - Participants in NIST interlaboratory studies
  - Contacts through STRBase website
- Responses from **52 scientists** were compiled
  - Covering 27 states + Puerto Rico, 4 companies, 2 outside US
- **Specific interviews were conducted** to gain perspectives from a small lab, a large lab, a private lab, and court testimony experience

Representative Labs Interviewed

- **Montgomery County Crime Lab** – small lab, 3 analysts, ~180 cases/year; using PP16 and ABI 310
- **Orchid Cellmark** – private contract lab, 40 analysts and technicians, ~5,000 cases/year; Profiler Plus/ COfiler and Identifiler with ABI 310 and ABI 3100; extensive court experience
- **AFDIL** – large federal lab, ~120 analysts/technicians, remains identification rather than strictly forensic cases, >1,000 cases/year (mtDNA & STRs); Profiler Plus/COfiler and PP16 with ABI 377 and ABI 3100

Information from interviews is included in the written report of this project…

Validation Standardization Questionnaire (conducted June-August 2004)

Review of Survey Questions

- What is validation?
- **How do you know when you are finished validating** a kit, instrument, software, or procedure?
- What steps are needed in internal validation and how many samples should be run at a minimum?
- **How many total samples do you think it takes to internally "validate" a new forensic kit?**
- How many different sets of samples are needed? Over what time period?
- Where do you look for guidance currently in terms of validation?
- **What are some kits, software, instruments that you are considering for validation in the next year?**
- How are validation, training, and proficiency testing related to one another?
- Do you think that the process of validation can be standardized?
- If a standard protocol or set of guidelines existed for validation, would you use it?
- If a standard set of samples existed for performing validation testing, would you use them?

Used to help define specific examples …

How I felt after taking on this project…

Me

Literature, Validation Data, Survey Responses

Validation Standardization Questionnaire (conducted June-August 2004)

How do you know when you are finished with a validation study? (1)

- "When you have demonstrated that it works as expected over a range of samples that is representative of what is seen in casework"
- "When repeat performance gave the same result"
- "**When you pull the toothpick out and it is dry?...** Meet at least minimum expectations and DAB guidelines"
- "You are very comfortable that you know how it works and your documentation will convince a reviewer you have put the kit thru a rigorous review/test."

Validation Standardization Questionnaire (conducted June-August 2004)

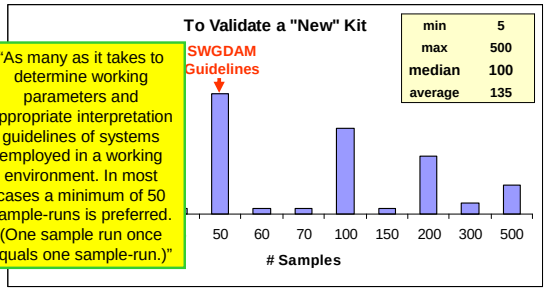
How do you know when you are finished with a validation study? (2)

- "Once a reasonable body of data has been assembled and analyzed, quirks have been revealed, and the upper and lower limits of the system have been challenged using a range of samples that one could expect to encounter in the everyday operation of the system"
- "When you achieve accuracy and precision to the desired statistical level of certainty"
- "You can never know…but it is always nice to have more samples!"
- "Validation is never complete"

Validation Standardization Questionnaire (conducted June-August 2004)

Survey Summary for Recommended **Total Number of Samples**

to Internally Validate a New Forensic Kit



"As many as it takes to determine working parameters and appropriate interpretation guidelines of systems employed in a working environment. In most cases a minimum of 50 sample-runs is preferred. (One sample run once equals one sample-run.)"

Choices in survey were: **10, 50, 500, or other ____**

Validation Standardization Questionnaire (conducted June-August 2004)

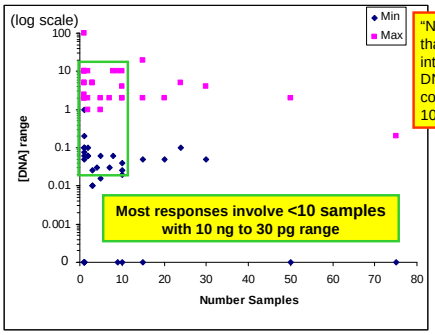
Survey Summary for Recommended **Precision Studies**

A few of the responses:

- "100 allelic ladder injections"
- "1 allelic ladder with 10 injections"
- "Depends upon the system being tested. For a databanking system, 50-100 runs of 50-100 specimens. Again, stats tell you when you've processed enough specimens to understand the system."
- "Minimum: Run one sample at least 8 times. Recommended: Run at least two samples plus allelic ladder at least 8 times." (24 sample-runs)

Validation Standardization Questionnaire (conducted June-August 2004)

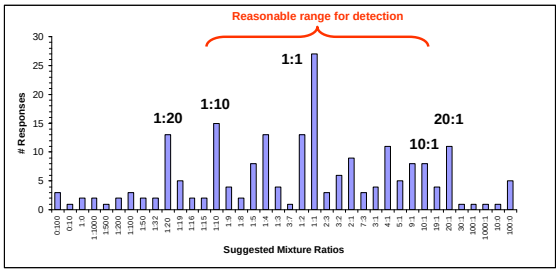
Survey Summary for Recommended **Sensitivity Studies**



"Need to run samples that challenge interpretation at high DNA and low DNA concentrations—e.g., 10 ng and <0.2 ng"

Validation Standardization Questionnaire (conducted June-August 2004)

Survey Summary for Recommended **Mixture Studies**



Some Recommended Numbers of Samples:

5 different 2-person mixtures
50 amplifications from at least 10 different mixtures
1 set of samples (ranging from 1:10 to 10:1)

Validation Standardization Questionnaire (conducted June-August 2004)

Survey Summary for Recommended **Non-Human Cases**

A few of the responses:

- "10-20 food animals, companion animals, local wildlife, ferrets"
- "I don't believe this is necessary in internal validation if external results are published. This would not be expected to vary in different analysts' hands."
- "I've trusted system manufacturers to handle this. Should I have?"
- "Minimum: Include information from developmental studies. If performing developmental studies, include at least bacterial and yeast/fungal example, plus mammalian and non-mammalian examples."

Validation Standardization Questionnaire (conducted June-August 2004)

Survey Summary for Recommended **Non-Probative Cases**

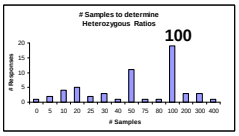
A few of the responses:

- Most responses were between 5-10 cases (range 3-25)
- "More important than the number of cases is the range of forensic samples that are typed during validation."
- "Complete cases are not required to test a system. Recommended: **Run at least 8 mock non-probative samples**. Note: Non-probative samples are not guaranteed to provide complete profiles. They are needed only to show that false results are not generated. Lack of results or incomplete results do not affect the validity of a validation."

Validation Standardization Questionnaire (conducted June-August 2004)

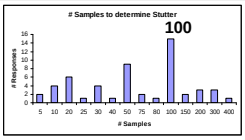
Survey Summary for Recommended **Numbers of Samples**

to Determine Heterozygote Peak Height Ratios and Stutter Values



min	0
max	400
median	50
average	85

Heterozygote Peak Height Ratios



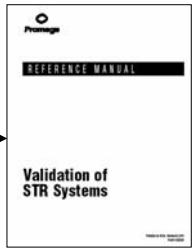
min	5
max	400
median	63
average	88

Stutter Values

Validation Standardization Questionnaire (conducted June-August 2004)

Where do you look for guidance currently in validation?

- SWGDAM
- DAB standards and ISO 17025
- Other scientists
- Literature publications
- Presentations at meetings
- Promega's validation guide →
- FBI studies and publications
- NIST studies and publications
- Previous scientific training
- Common sense



Published in March 2001

Validation Standardization Questionnaire (conducted June-August 2004)

Can Validation be Standardized?

Statements from survey responders…

Over 86% (45/52) said yes

Those who responded "no" said

- "to some degree it can be, however, validation is specific to the platform, kits, …",
- "a start-up lab should do much more than an experienced lab…",
- "validation builds on previous work by lab or published data",
- "parts of it can be standardized; I don't think the non-probative cases could be", and
- "only in a general way, as with the SWGDAM guidelines. The uniqueness of each new procedure would make standardization difficult."

Our Conclusion…

to a certain extent it can…but everyone will always have a different comfort level…and **inflexible, absolute numbers for defined studies will not likely be widely accepted**

Validation Standardization Questionnaire (conducted June-August 2004)

If a Standard Protocol or Set of Guidelines Existed for Validation, Would You Use It?

90% (47/52) said yes

Some responses

- "No-I would reference them. I may not completely abide by them but I would certainly review them",
- "No-but it would be taken into consideration",
- "Yes-we would have to or there would be problems in court",
- "Yes-as long as they remain updated, relevant and feasible guidelines and do not become dogma",
- "Yes-if it would pass an audit for validation", and
- "Yes-unless they were far less stringent than current practice."

A Thoughtful Comment from One Interviewee

Before a set of validation experiments is performed…

- The question should be asked "Do we already know the answer to this question from the literature or a previous study performed in-house?"
- If the answer is "yes" and we document how we know this answer, **then there is no need to perform that set of validation experiments**.

A good example of this scenario is non-human DNA studies.

Developmental Validation

DNA Advisory Board Quality Assurance Standards

Section 2. Definitions

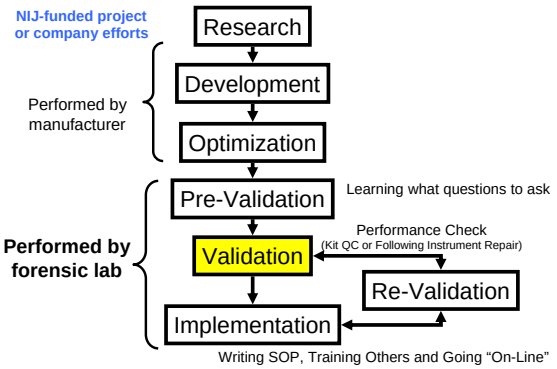
- (ff) Validation is a process by which a procedure is evaluated to determine its **efficacy and reliability** for forensic casework analysis *(DNA analysis)* and includes:

Manufacturer

  - (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 samples;
  - (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.

Forensic Lab

How an Assay Evolves



Validation Section of the DNA Advisory Board Standards

issued October 1, 1998 and April 1999; published in *Forensic Sci. Comm.* July 2000

STANDARD 8.1 The laboratory shall use validated methods and procedures for forensic casework analyses *(DNA analyses)*.

8.1.1 Developmental validation that is conducted shall be appropriately documented.

8.1.2 Novel forensic DNA methodologies shall undergo developmental validation to ensure the accuracy, precision and reproducibility of the procedure. The developmental validation shall include the following:

8.1.2.1 Documentation exists and is available which defines and characterizes the locus.

8.1.2.2 Species specificity, sensitivity, stability and mixture studies are conducted.

8.1.2.3 Population distribution data are documented and available.

8.1.2.3.1 The population distribution data would include the allele and genotype distributions for the locus or loci obtained from relevant populations. Where appropriate, databases should be tested for independence expectations.

8.1.3 Internal validation shall be performed and documented by the laboratory.

8.1.3.1 The procedure shall be tested using known and non-probative evidence samples *(known samples only)*. The laboratory shall monitor and document the reproducibility and precision of the procedure using human DNA control(s).

8.1.3.2 The laboratory shall establish and document match criteria based on empirical data.

8.1.3.3 Before the introduction of a procedure into forensic casework *(database sample analysis)*, the analyst or examination team shall successfully complete a qualifying test.

8.1.3.4 Material modifications made to analytical procedures shall be documented and subject to validation testing.

8.1.4 Where methods are not specified, the laboratory shall, wherever possible, select methods that have been published by reputable technical organizations or in relevant scientific texts or journals, or have been appropriately evaluated for a specific or unique application.

FORENSIC SCIENCE COMMUNICATIONS JULY 2000 VOLUME 2 NUMBER 3

Developmental Validation Overview

8.1.1 Developmental validation that is conducted shall be appropriately documented.

8.1.2 Novel forensic DNA methodologies shall undergo developmental validation **to ensure the accuracy, precision and reproducibility of the procedure**. The developmental validation shall include the following:

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8.1.2.3 Population distribution data are documented and available.

8.1.2.3.1 The population distribution data would include the allele and genotype distributions for the locus or loci obtained from relevant populations. Where appropriate, databases should be tested for independence expectations.

Validation Section of the DNA Advisory Board Standards

issued October 1, 1998 and April 1999; published in *Forensic Sci. Comm.* July 2000

Overview of Developmental Validation Studies

2. Developmental Validation: The developmental validation process may include the studies detailed below. Some studies may not be necessary for a particular method.

- 2.1 Characterization of genetic markers
- 2.2 Species specificity
- 2.3 Sensitivity studies
- 2.4 Stability studies
- 2.5 Reproducibility
- 2.6 Case-type samples
- 2.7 Population studies
- 2.8 Mixture studies
- 2.9 Precision and accuracy
- 2.10 PCR-based procedures

SWGDAM Revised Validation Guidelines
http://www.fbi.gov/hq/lab/fsc/backissu/july2004/standards/2004_03_standards02.htm

PowerPlex Y Developmental Validation Experiments

Study Completed (17 studies done)	Description of Samples Tested (performed in 7 labs and Promega)	# Run
Single Source (Concordance)	5 samples x 8 labs	40
Mixture Ratio (male:female)	6 labs x 2 M/F mixture series x 11 ratios (1:0,1:1,1:10,1:100,1:300,1:1000,0.5:300, 0.25:300,0.125:300, 0.0625:300, 0.03:300 ng M:F)	132
Mixture Ratio (male:male)	6 labs x 2 M/M mixtures series x 11 ratios (1:0, 19:1, 9:1, 5:1, 2:1, 1:1, 1:2, 1:5, 1:9, 1:19, 0:1)	132
Sensitivity	7 labs x 2 series x 6 amounts (1/0.5/0.25/0.125/0.06/0.03)	84
Non-Human	24 animals	24
NIST SRM	6 components of SRM 2395	6
Precision (ABI 3100 and ABI 377)	10 ladder replicates + 10 sample replicated + [8 ladders + 8 samples for 377]	36
Non-Probative Cases	65 cases with 102 samples	102
Stutter	412 males used	412
Peak Height Ratio	N/A (except for DYS385 but no studies were noted)	
Cycling Parameters	5 cycles (28/27/26/25/24) x 8 punch sizes x 2 samples	80
Annealing Temperature	5 labs x 5 temperatures (54/58/60/62/64) x 1 sample	25
Reaction volume	5 volumes (50/25/15/12.5/6.25) x [5 amounts + 5 concentrations]	50
Thermal cycler test	4 models (480/2400/9600/9700) x 1 sample + [3 models x 3 sets x 12 samples]	76
Male-specificity	2 females x 1 titration series (0-500 ng female DNA) x 5 amounts each	10
TaqGold polymerase titration	5 amounts (1.38/2.06/2.75/3.44/4.13 U) x 4 quantities (1/0.5/0.25/0.13 ng DNA)	20
Primer pair titration	5 amounts (0.5x/0.75x/1x/1.5x/2x) x 4 quantities (1/0.5/0.25/0.13 ng DNA)	20
Magnesium titration	5 amounts (1/1.25/1.5/1.75/2 mM Mg) x 4 quantities (1/0.5/0.25/0.13 ng DNA)	20

Krenke *et al.* (2005) *Forensic Sci. Int.* 148:1-14 TOTAL SAMPLES EXAMINED 1269

Other DAB Standards to Consider

9.1.1 The laboratory shall have an ***standard protocol*** for each analytical technique used.

9.1.2 The procedures shall include ***reagents, sample preparation, extraction, equipment and controls***, which are standard for DNA analysis and data interpretation.

9.2.3 The laboratory shall identify ***critical reagents*** (if any) and evaluate them prior to use in casework……

9.4 The laboratory shall monitor the analytical procedures using appropriate ***controls and standards***.

10.2 The laboratory shall identify ***critical equipment*** and shall have a documented program for calibration of instruments and equipment.

10.3 The laboratory shall have a ***documented program*** to ensure that instruments and equipment are properly maintained.

From Robyn Ragsdale (FDLE), Validation Workshop (Aug 24-26, 2005 at NFSTC)
http://www.cstl.nist.gov/biotech/strbase/validation/validationworkshop.htm

Instrument Calibration

STANDARD 10.2 The laboratory *(shall identify critical equipment and)* shall have a documented program for calibration of instruments and equipment.

10.2.1 Where available and appropriate, **standards traceable to national or international standards shall be used for the calibration**.

10.2.1.1 Where traceability to national standards of measurement is not applicable, the laboratory shall provide **satisfactory evidence of correlation of results**.

10.2.2 The frequency of the calibration shall be documented for each instrument requiring calibration. Such documentation shall be retained in accordance with applicable Federal or state law.

Validation Section of the DNA Advisory Board Standards
issued October 1, 1998 and April 1999; published in *Forensic Sci. Comm.* July 2000

Internal Validation

Internal Validation Overview

8.1.3 **Internal validation shall be performed and documented by the laboratory**.

8.1.3.1 The procedure shall be tested using known and non-probative evidence samples *(known samples only)*. The laboratory shall monitor and document the reproducibility and precision of the procedure using human DNA control(s).

8.1.3.2 The laboratory shall establish and document match criteria based on empirical data.

8.1.3.3 Before the introduction of a procedure into forensic casework *(database sample analysis)*, the analyst or examination team shall successfully complete a qualifying test.

8.1.3.4 **Material modifications** made to analytical procedures shall be documented and subject to validation testing.

8.1.4 Where methods are not specified, the laboratory shall, wherever possible, select methods that have been published by reputable technical organizations or in relevant scientific texts or journals, or have been appropriately evaluated for a specific or unique application.

Validation Section of the DNA Advisory Board Standards
issued October 1, 1998 and April 1999; published in *Forensic Sci. Comm.* July 2000

General Steps for Internal Validation

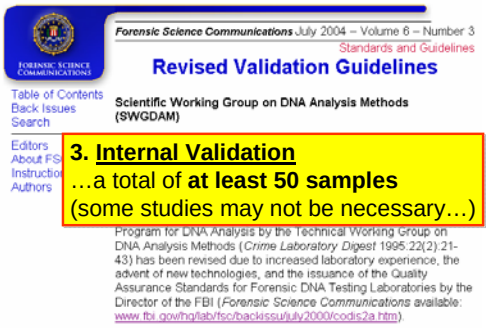
- **Review literature and learn the technique**
- Obtain equipment/reagents, if necessary
- Determine necessary validation studies (there can be overlap and you only need to run a total of 50 samples)
- Collect/obtain samples, if necessary
- **Perform validation studies maintaining all documentation**
- Summarize the studies and submit for approval to Technical Leader
- Write-up the analytical procedure(s). Include quality assurance (controls, standards, critical reagents and equipment) and data interpretation, as applicable
- Determine required training and design training module(s)
- Design qualifying or competency test

From Robyn Ragsdale (FDLE), Validation Workshop (Aug 24-26, 2005 at NFSTC)
http://www.cstl.nist.gov/biotech/strbase/validation/validationworkshop.htm

Revised SWGDAM Validation Guidelines

(July 2004)

http://www.fbi.gov/hq/lab/fsc/backissu/july2004/standards/2004_03_standards02.htm



The document provides validation guidelines and definitions approved by SWGDAM July 10, 2003.

Overview of Internal Validation Studies

3. Internal Validation: The internal validation process should include the studies detailed below encompassing **a total of at least 50 samples**. Some studies may not be necessary due to the method itself.

3.1 Known and nonprobative evidence samples
3.2 Reproducibility and precision
3.3 Match criteria
3.4 Sensitivity and stochastic studies
3.5 Mixture studies
3.6 Contamination
3.7 Qualifying test

SWGDAM Revised Validation Guidelines
http://www.fbi.gov/hq/lab/fsc/backissu/july2004/standards/2004_03_standards02.htm

3.1 Known and non-probative evidence samples:

- **Profiler Plus validation (JFS 2001) :** Analyzed nineteen non-probative cases that included blood standards for comparison to semen stains or bloodstains. Nine of these were previously analyzed in PM and D1S80.
- **PowerPlex 2.1 validation (JFS 2002):** Analyzed eleven proficiency tests as well as thirty samples for which previous PowerPlex 1.1 data was available as well as thirty-two cases for which previous RFLP, CTT or PowerPlex 1.1 data was available.
- **Identifiler Validation (Internal 2004):** Analyzed ten known samples of lab employees on 310 and 3100 genetic analyzers and compared results. Also analyzed nine cases and compared to the original case conclusions.

From Robyn Ragsdale (FDLE), Validation Workshop (Aug 24-26, 2005 at NFSTC)
http://www.cstl.nist.gov/biotech/strbase/validation/validationworkshop.htm

3.2 ***Reproducibility*** and precision:

- **Profiler Plus validation (JFS 2001) :** Interlaboratory reproducibility was assessed by analyzing fifty samples at two different sites; compared ten samples separated by gel electrophoresis versus capillary electrophoresis; evaluated results from twenty samples extracted organically and non-organically.
- **PowerPlex 2.1 validation (JFS 2002):** Concordance studies with 100 convicted offender samples and analyzed at four different sites *(one site only analyzed 25 samples)* . Also compared results of 25 of the samples with results obtained with Profiler Plus and Cofiler at a fifth site.
- **Identifiler Validation (Internal 2004):** Twenty samples of control 9974A were separately amplified at 1 ng target DNA and analyzed on 3 separate days.

From Robyn Ragsdale (FDLE), Validation Workshop (Aug 24-26, 2005 at NFSTC)
http://www.cstl.nist.gov/biotech/strbase/validation/validationworkshop.htm

3.2 Reproducibility and ***Precision***:

- **Profiler Plus validation (JFS 2001) :**
  - **Precision of allele determination:** Five known samples were injected twenty times and the base pair size and genotype data collected for one allele at each locus. Sizing data was also collected for the first allele of the allelic ladder for D3, amelogenin and D5 from 100 allelic ladder runs.
  - **Precision of relative peak height:** Used samples from reproducibility, stutter and above precision studies were used to determine the average heterozygote peak height ratio.
- **Identifiler Validation (Internal 2004):** Twenty samples of control 9974A were separately amplified at 1 ng target DNA and analyzed on 3 separate days. Each of the samples was re-injected throughout the three runs and base pair size determinations conducted.

From Robyn Ragsdale (FDLE), Validation Workshop (Aug 24-26, 2005 at NFSTC)
http://www.cstl.nist.gov/biotech/strbase/validation/validationworkshop.htm

3.3 Match criteria:

- **Profiler Plus validation (JFS 2001) :** Data is addressed in the precision study
- **PowerPlex 2.1 validation (JFS 2002):** Not addressed
- **Identifiler Validation (Internal 2004):** Data is addressed in the precision study
- **DNA extraction with DNA IQ (Internal 2003):** Not addressed
- **3100 Validation (Internal 2003):** Data is addressed in the precision study
- **Quantifiler Validation (Internal 2004):** Not applicable

From Robyn Ragsdale (FDLE), Validation Workshop (Aug 24-26, 2005 at NFSTC)
http://www.cstl.nist.gov/biotech/strbase/validation/validationworkshop.htm

3.4 Sensitivity and stochastic studies:

- **Profiler Plus validation (JFS 2001) :** Prepared dilutions from 10 ng to 36 pg, amplified the samples and ran on 3 separate 310s. Also examined injection times ranging from five to twenty seconds on samples containing 0.6 ng to 36 pg of input DNA.
- **PowerPlex 2.1 validation (JFS 2002):** Prepared dilutions ranging from 25 ng down to 0.03125 ng, amplified samples and analyzed using gel electrophoresis.
- **Identifiler Validation (Internal 2004):** Nine samples of 9947A were amplified in duplicate by 2 separate analysts in concentrations ranging from 0.0125 to 1 ng and analyzed at 50 to 150 rfus.

From Robyn Ragsdale (FDLE), Validation Workshop (Aug 24-26, 2005 at NFSTC)
http://www.cstl.nist.gov/biotech/strbase/validation/validationworkshop.htm

3.5 Mixture studies:

- **Profiler Plus validation (JFS 2001) :** Two samples were mixed together at known proportions (1:200, 1:100, 1:20, 1:10, 1:2, and 1:1) to determine the ratio at which the major and minor components of a mixture could be resolved. Amplified 2 ng of target DNA
- **PowerPlex 2.1 validation (JFS 2002):** Preparations of a series of DNA:DNA ratios from already quantified samples were utilized as well as mixtures of body fluids in known volumes prior to DNA extraction and quantification. Amplified 1 ng of target DNA.

From Robyn Ragsdale (FDLE), Validation Workshop (Aug 24-26, 2005 at NFSTC)
http://www.cstl.nist.gov/biotech/strbase/validation/validationworkshop.htm

3.6 Contamination:

- **Profiler Plus validation (JFS 2001) :** Not discussed
- **PowerPlex 2.1 validation (JFS 2002):** Not discussed
- **Identifiler Validation (Internal 2003):** Although more instrument related that kit related, the lab put 9 sets of sample tubes in the sample tray for the 310 in a set pattern with some containing excessive size standard and injected in a specific order.
- **Automated extraction with DNA IQ (JFS 2004):** Use of appropriate controls (blanks) through out the validation study demonstrated no instances of contamination.

From Robyn Ragsdale (FDLE), Validation Workshop (Aug 24-26, 2005 at NFSTC)
http://www.cstl.nist.gov/biotech/strbase/validation/validationworkshop.htm

3.7 Qualifying test:

- **Profiler Plus validation (JFS 2001) :** Not discussed
- **PowerPlex 2.1 validation (JFS 2002):** Not discussed
- **Identifiler Validation (Internal 2004):** Analyzed a previously characterized external DNA proficiency test as well as NIST SRM 2391b.
- **DNA extraction with DNA IQ (Internal Validation 2003):** not discussed
- **3100 Validation (Internal 2003):** Analysts were required to run a set of previously characterized samples. Written examination also required.

From Robyn Ragsdale (FDLE), Validation Workshop (Aug 24-26, 2005 at NFSTC)
http://www.cstl.nist.gov/biotech/strbase/validation/validationworkshop.htm

Design of Experiments Conducted for Validation Studies

- Before performing a set of experiments for validation, ask yourself:
  - **What is the purpose of the study?**
  - **Do we already know the answer?**
  - **Can we write down how we know the answer?**
- Think before you blindly perform a study which may have no relevance (e.g., extensive precision studies)
- **Too often we do not differentiate learning, validation, and training**

Points for Consideration

- Remove as many variables as possible in testing an aspect of a procedure
  - e.g., create bulk materials and then aliquot to multiple tubes rather than pipeting separate tubes individually during reproducibility studies
- Who can do (or should do) validation…
  - Outside contractor?
  - Summer intern?
  - Trainee?
  - Qualified DNA analyst

From a validation standpoint, having an outside group perform the validation studies on your instruments is legitimate, **but valuable experience and knowledge are lost…**

Steps Surrounding "Validation" in a Forensic Lab

Effort to Bring a Procedure "On-Line"

This is what takes the time…

- **Installation** – purchase of equipment, ordering supplies, setting up in lab
- **Learning** – efforts made to understand technique and gain experience troubleshooting; can take place through direct experience in the lab or vicariously through the literature or hearing talks at meetings
- **Validation of Analytical Procedure** – tests conducted in one's lab to verify range of reliability and reproducibility for procedure
- **SOP Development** – creating interpretation guidelines based on lab experience
- **QC of Materials** – performance check of newly received reagents
- **Training** – passing information on to others in the lab
- **Qualifying Test** – demonstrating knowledge of procedure enabling start of casework
- **Proficiency Testing** – verifying that trained analysts are performing procedure properly over time

Practical Examples

Practical Examples

- Profiler Plus/COfiler kit switch to Identifiler
- ABI 3100 upgrade to ABI 3130xl
- GeneScan/Genotyper to GeneMapperID
- New allelic ladder provided by company
- Bringing Quantifiler "on-line" (from Quantiblot)
- DNA IQ
- Corbett robot
- FSS-i3 expert system software
- Reduced volume reactions

Discuss each example - participants to provide what they would do…

Suggestions for an Internal Validation of an STR Kit

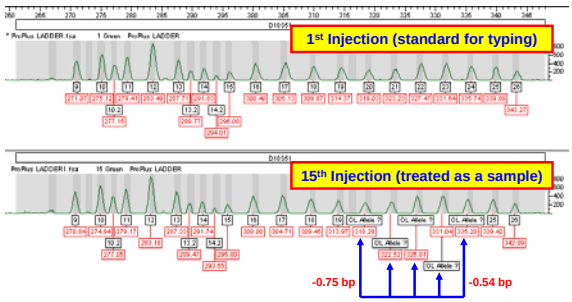
- Standard samples (3.1) **Between 1 and ~20 samples**
  - Verify correct type with positive control or NIST SRM samples
  - Concordance study with 5-10 (non-probative casework) samples previously typed with other kit(s)
- Precision samples (3.2) **5-10 samples**
  - Run at least 5-10 samples (allelic ladder or positive control)
- Sensitivity samples (3.4) **14 samples**
  - Run at least 2 sets of samples covering the dynamic range
  - 5 ng down to 50 pg—e.g., 5, 2, 1, 0.5, 0.2, 0.1, 0.05 ng
- Mixture samples (3.5) **10 samples**
  - Run at least 2 sets of samples
  - Examine 5 different ratios—e.g., 10:1, 3:1, 1:1, 1:3, 1:10

>50 samples

Additional Suggestions for Meeting the SWGDAM Revised Validation Guidelines

- Match Criteria (3.3)
  - As part of running a batch of samples (e.g., 10 or 96), run one allelic ladder at the beginning and one at the end
  - If all alleles are typed correctly in the second allelic ladder, then the match criteria (i.e., precision window of +/-0.5 bp) has likely been met across the entire size range and duration of the run
- Contamination Check (3.6)
  - Run negative controls (samples containing water instead of DNA) with each batch of PCR products
- Qualifying Test (3.7)
  - Run proficiency test samples

Use of Second Allelic Ladder to Monitor Potential Match Criteria Problems



These alleles have drifted outside of their genotyping bins due to temperature shifting over the course of the sample batch

Example with Identifiler STR Kit

- **Your lab is currently running ProfilerPlus/COfiler and wants to switch to Identifiler**. What is needed for your internal validation?
- **What is different between Identifiler and ProfilerPlus/COfiler**?
  - **Two new STR loci: D19S433 and D2S1338**
  - **Different fluorescent dyes**
  - **Additional fluorescent dye (5-dye vs 4-dye)**
  - **Different dye on internal size standard**
  - **More loci being amplified in the multiplex**
  - **Mobility modifiers to move allele sizes**

Different
Loci (2 extra STRs)
Dyes
Mobility Modifiers
Software (5-dye)

- PCR primer sequences are the same so potential allele discordance due to primer binding site mutations should not be an issue
- **What has been reported in terms of developmental validation for Identifiler?**

ABI Kit Validation Papers

J. Forensic Sci. 2002; 47(1): 66-96

Cydne L. Holt,[1] Ph.D.; Martin Buoncristiani,[2] M.P.H.; Jeanette M. Wallin,[1] M.P.H.; Theresa Nguyen,[1] B.S.; Katherine D. Lazaruk,[1] Ph.D.; and P. S. Walsh,[1] M.P.H.

TWGDAM Validation of AmpFℓSTR™ PCR Amplification Kits for Forensic DNA Casework

J. Forensic Sci. 2004; 49(6): 1265-1277

Patrick J. Collins,[1] B.A.; Lori K. Hennessy,[1] Ph.D.; Craig S. Leibelt,[1] A.B.; Rhonda K. Roby,[1,1] M.P.H.; Dennis J. Reeder,[1] Ph.D.; and Paul A. Foxall,[1] Ph.D.

Developmental Validation of a Single-Tube Amplification of the 13 CODIS STR Loci, D2S1338, D19S433, and Amelogenin: The AmpFℓSTR® Identifiler® PCR Amplification Kit

AmpFℓSTR® Identifiler™
PCR Amplification Kit
User's Manual

Example: PowerPlex 16

- Switch from ProfilerPlus/COfiler kits to PowerPlex 16
- Retaining same instrument platform of ABI 310

Recommendations:

- Concordance study (somewhat, but better to review literature to see impact across a larger number of samples and which loci would be expected to exhibit allele dropout-e.g., D5S818)
- Stutter quantities, heterozygote peak height ratio
- Some sensitivity studies and mixture ratios
- **Do not need precision studies to evaluate instrument reproducibility**

Example: ABI 3130

- Evaluation of a new ABI 3130 when a laboratory already has experience with ABI 310
- STR kits used in lab will remain the same

Recommendations:

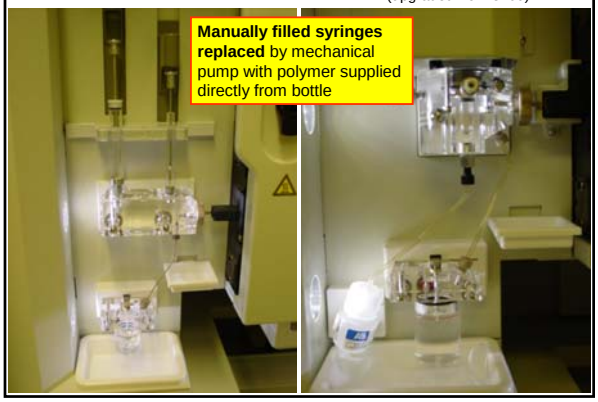
- Precision studies to evaluate instrument reproducibility
- Sensitivity studies
- **Do not need new stutter, mixture ratio, peak height ratio, etc. (these relate to dynamics of the the kit used)**

Instrument/Software Upgrades or Modifications

- What should be done to "validate" new upgrade?
  - ABI 7000 to ABI 7500
  - ABI 3100 to ABI 3130xl
  - GeneScan/Genotyper to GeneMapper*ID*
- Try to understand what is different with the new instrument or software program compared to the one you are currently using (e.g., ask other labs who may have made the switch)
- If possible, try to retain your current configuration for comparison purposes for the validation period

Run the same plate of samples on the original instrument/software and the new one

ABI 3100 | ABI 3130xl (upgraded from 3100)



ABI 3130xl vs ABI 3100

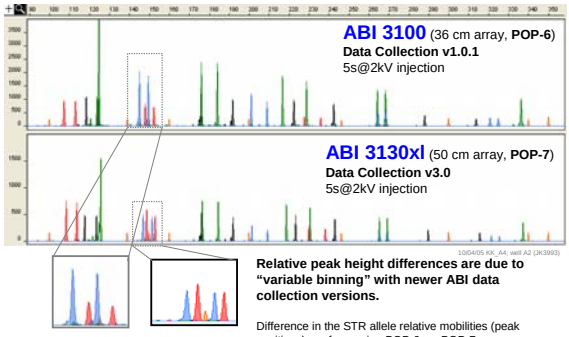
What NIST did to "validate" a 3130xl upgrade

- Ran plates of samples on both instruments with same injection and separation parameters and compared results
  - Data Collection version 1.0.1 (3100) vs 3.0 (3130xl)
  - POP-6 (3100) vs POP-7 (3130xl)
  - 36 cm array (3100) vs 50 or 80 cm array (3130xl)
- Ran several plates of Identifiler samples and compared allele calls (noticed a sensitivity difference with equal injections and relative peak height differences between dye colors) – **all obtained allele calls were concordant**
- Ran a plate of Profiler Plus samples and compared sizing precision – **precision was not significantly different**
- Also examined SNaPshot products and mtDNA sequencing data

Environmental conditions may change over time so original validation is no longer valid…

Comparison of ABI 3100 Data Collection Versions

Same DNA sample run with Identifiler STR kit (identical genotypes obtained) GeneScan display



Relative peak height differences are due to "variable binning" with newer ABI data collection versions.

Difference in the STR allele relative mobilities (peak positions) are from using POP-6 vs. POP-7.

Documentation

Documentation of Internal Validation Studies

What is the best way to do this? Standardized format?

Who needs to review?

Who needs to approve?

Should it be presented or published?

From Robyn Ragsdale (FDLE), Validation Workshop (Aug 24-26, 2005 at NFSTC)
http://www.cstl.nist.gov/biotech/strbase/validation/validationworkshop.htm

Appropriate Documentation…

- Publications in the Peer-Reviewed Literature
  - See provided reference list
  - http://www.cstl.nist.gov/biotech/strbase/validation.htm
- In terms of documentation, is the community doing too much? Too little?
  - **Benefit of STRBase Validation website**
- Should we be requesting more information from the manufacturers of commercial kits in terms of developmental validation studies?

ABI 7500 Quantifiler Validation Documentation

http://www.appliedbiosystems.com

Applied Biosystems

Experimental data supports that the 7500 system with v1.2.3 software provides consistent performance when compared to the ABI PRISM® 7000 Sequence Detection System previously validated for forensic applications. Therefore, the 7500 system can be sold to Human Identification customers at this time. Further guidance for specific operating conditions will follow.

Validation of the Applied Biosystems 7500 Real-Time PCR System with v1.2.3 Software

Applied Biosystems scientists have conducted experiments following the guidance provided by the DAB/SWGDAM to validate the Applied Biosystems 7500 Real-Time PCR System with v1.2.3 software ("7500 System") for use in forensic applications using the Quantifiler Human and Quantifiler Y kits. We are pleased to let you know that the Applied Biosystems 7500 Real-Time PCR System equipped with v1.2.3 software is now validated for use in forensic sample testing pursuant to these guidelines using the Quantifiler kits. We conducted experiments, reviewed data, and determined that the 7500 Real-Time PCR System provides results that are robust, reliable, reproducible and provide accurate results when used in conjunction with the Quantifiler™ Human and Quantifiler™ Y kits for the analysis of genomic DNA samples.

Promega Material Modification Reported for PP16 Primer Mix Storage

http://www.promega.com/applications/hmnid/11072-AN-GI-final.pdf

promega Application Notes

Amplifications Using the PowerPlex® 16 System and a 10X Primer Pair Mix Stored in TE⁻⁴ Buffer or in Water Yield Comparable Results

Abstract

Promega is changing the solution used to prepare the 10X Primer Pair Mix in the PowerPlex® 16 System from water to TE⁻⁴ buffer. We show that the amplification results with the 10X Primer Pair Mix prepared in TE⁻⁴ buffer are comparable to those with the 10X Primer Pair Mix prepared in water, even when we varied reaction parameters (the amount of DNA template, volume of TE⁻⁴ buffer, the thermal cycler used, amount of AmpliTaq Gold® DNA polymerase used and primer concentrations). Because the performance with both primer pair formulations is comparable, users of the PowerPlex® 16 System should see no adverse effects on amplification results.

Introduction

DNA is traditionally stored in water or a buffered solution, such as TE⁻⁴ (10mM Tris-HCl, 0.1mM EDTA [pH 8.0]). DNA stored in TE⁻⁴ buffer is more stable due to the buffering capacity of Tris and the presence of EDTA (1). For this reason, the 10X Primer Pair Mix for the

DNA polymerase and 1.5mM $MgCl_2$, unless otherwise indicated. DNA templates were diluted in 19.2µl of TE⁻⁴ buffer, unless otherwise indicated. Amplifications were carried out using the Perkin-Elmer GeneAmp® PCR System 9600, unless otherwise indicated, for 32 cycles (10/22 cycling). Amplification products were detected using the ABI PRISM® 310 or 3100 Genetic Analyzer and the GeneScan® analysis software. Sample files were imported into the Genotyper® program and analyzed using the PowerTyper™ 16 Macro.

Variations in Amplification Reaction Conditions

Primers stored in TE⁻⁴ buffer are more stable than primers stored in water (data not shown), but we wanted to be sure that the use of TE⁻⁴ buffer to resuspend the primers did not affect amplification results. We varied the amplification conditions and compared the results obtained with the two primer pair formulations to determine if there were any effects due to the new formulation.

Validation Section of the DNA Advisory Board Standards

issued July 1998 (and April 1999); published in *Forensic Sci. Comm.* July 2000

STANDARD 8.1 The laboratory shall use validated methods and procedures for forensic casework analyses *(DNA analyses)*.

8.1.1 Developmental validation that is conducted **shall be appropriately documented**.

8.1.3 Internal validation **shall be performed and documented by the laboratory**.

FORENSIC SCIENCE COMMUNICATIONS JULY 2000 VOLUME 2 NUMBER 3

Why is Documentation of Validation Important?

9. Documentation of Validated Methods

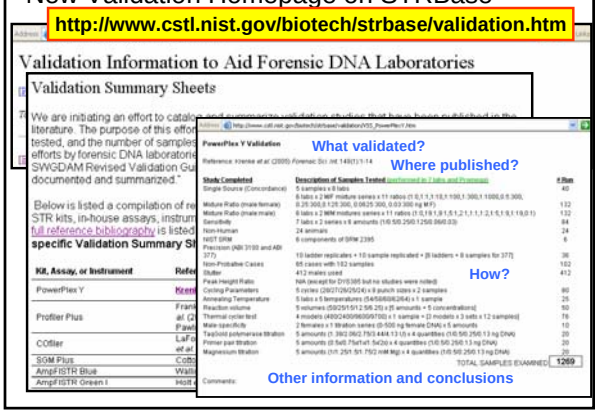
9.1 Once the validation process is complete it is important to document the procedures so that the method can be clearly and unambiguously implemented. There are a number of reasons for this. **The various assessments of the method made during the validation process assume that,in use, the method will be used in the same way each time**. If it is not, then the actual performance of the method will not correspond to the performance predicted by the validation data. Thus the **documentation must limit the scope for introducing accidental variation to the method**. In addition, proper documentation is necessary for auditing and evaluation purposes and may also be required for contractual or regulatory purposes.

9.2 Appropriate documentation of the method will help **to ensure that application of the method from one occasion to the next is consistent.**

EURACHEM Guide (1998) *The Fitness for Purpose of Analytical Methods: A Laboratory Guide to Method Validation and Related Topics*, p. 37; available at http://www.eurachem.ul.pt/guides/valid.pdf

New Validation Homepage on STRBase

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



Validation Summary Sheet for PowerPlex Y

Study Completed (17 studies done)	Description of Samples Tested (performed in 7 labs and Promega)	# Run
Single Source (Concordance)	5 samples x 8 labs	40
Mixture Ratio (male:female)	6 labs x 2 M/F mixture series x 11 ratios (1:0,1:1,1:10,1:100,1:300,1:1000,0.5:300, 0.25:300,0.125:300, 0.0625:300, 0.03:300 ng M:F)	132
Mixture Ratio (male:male)	6 labs x 2 M/M mixtures series x 11 ratios (1:0, 19:1, 9:1, 5:1, 2:1, 1:1, 1:2, 1:5, 1:9, 1:19, 0:1)	132
Sensitivity	7 labs x 2 series x 6 amounts (1/0.5/0.25/0.125/0.06/0.03)	84
Non-Human	24 animals	24
NIST SRM	6 components of SRM 2395	6
Precision (ABI 3100 and ABI 377)	10 ladder replicates + 10 sample replicated + [8 ladders + 8 samples for 377]	36
Non-Probative Cases	65 cases with 102 samples	102
Stutter	412 males used	412
Peak Height Ratio	N/A (except for DYS385 but no studies were noted)	
Cycling Parameters	5 cycles (28/27/26/25/24) x 8 punch sizes x 2 samples	80
Annealing Temperature	5 labs x 5 temperatures (54/58/60/62/64) x 1 sample	25
Reaction volume	5 volumes (50/25/15/12.5/6.25) x [5 amounts + 5 concentrations]	50
Thermal cycler test	4 models (480/2400/9600/9700) x 1 sample + [3 models x 3 sets x 12 samples]	76
Male-specificity	2 females x 1 titration series (0-500 ng female DNA) x 5 amounts each	10
TaqGold polymerase titration	5 amounts (1.38/2.06/2.75/3.44/4.13 U) x 4 quantities (1/0.5/0.25/0.13 ng DNA)	20
Primer pair titration	5 amounts (0.5x/0.75x/1x/1.5x/2x) x 4 quantities (1/0.5/0.25/0.13 ng DNA)	20
Magnesium titration	5 amounts (1/1.25/1.5/1.75/2 mM Mg) x 4 quantities (1/0.5/0.25/0.13 ng DNA)	20
Krenke *et al.* (2005) *Forensic Sci. Int.* 148:1-14	TOTAL SAMPLES EXAMINED	1269

Laboratory Internal Validation Summaries

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

We invite updates to this table. Please contact John Butler <john.butler@nist.gov> if you would like to add a summary of your laboratory's validation studies with a particular forensic DNA test, instrument, or software program. Please submit information in a standard format summarizing the studies conducted, a description of samples run, and the number of samples examined using this downloadable Excel file [click here].

Summaries of Validation Studies Conducted in Individual Laboratories (not published in the literature)

Kit, Assay or Instrument	Laboratory	Submitter
PowerPlex 16 Kit with ABI 310	Pennsylvania State Police	Christine Tomsey
Quantifiler with ABI 7000	Alabama Department of Forensic Sciences	Angelo Della Manna

Soliciting Information on Studies Performed by the Community

Study Conducted	Description of Samples Run with PowerPlex 16 Validation	# Run	# Reports Now
Single Source (Concordance)	8 samples (Promega concordance) + 200 samples (part of population concordance study)	208	100
Mixtures	45	45	10
Mixture Ratio	1 sample x 11 ratios (1:0, 19:1, 9:1, 4:1, 2:1, 1:1, 1:2, 1:4, 1:9, 1:19, 0:1) x 2 injections (5/10 seconds)	22	33
Sensitivity	5 samples x 8 amounts (5/2/1/0.5/0.25/0.125/0.06/0.03 ng) + [5 samples x 3 points (at/above/below dropout)]	55	33
Non-Human	11 animals	11	0
NIST SRM 2391b	12 components	12	12
Precision (ABI 310)	(5 samples x 10 injections each) + 10 injections of allelic ladders	60	60
Non-Probative Cases	5 cases x 4 samples each (evidence E/ESF/victim/suspect)	20	20
Various tissues	Bone, hair, teeth, semen, perspiration, urine, blood, semen, vaginal swab (minimum of one sample each)	9	0
	TOTAL SAMPLES RUN	633	288

We can benefit from cumulative experience in the field rather than just single lab results…

Example of Validation Documentation

Alabama Department of Forensic Sciences Birmingham DNA

ABI Prism® 7000 Validation

TABLE OF CONTENTS

TAB	TITLE
8.1.1	Developmental Validation
8.1.3.1(a)	Known and Non-Probative Samples
8.1.3.1(b)	Reproducibility • Quantitation • Peak Height Experiment – ABI 310
8.1.3.1(b)	Precision

The validation studies referenced above have been reviewed and provide the necessary documentation required by the FBI Director's "Quality Assurance Standards for Forensic DNA Testing Laboratories" for a quantitation method to be used in the forensic casework section of the Alabama Department of Forensic Sciences Birmingham DNA laboratory.

Angelo Della Manna, MSFS, D-ABC
Forensic Biology Discipline Chief
Statewide Technical Leader

Date

Available on STRBase Validation Website:
http://www.cstl.nist.gov/biotech/strbase/validation/ADFS-BH_7000val.pdf

Documentation of Alabama Validation for ABI 7000 and Quantifiler Assay

What Section of QAS Validation Requirements

Experiments Performed

Summary of Results

Conclusions

Known and Non-Probative Samples

8.1.3.1(a) Has the procedure been tested using known and non-probative evidence samples?

Experiment:
Eleven (11) evidence samples comprised of various origins that are encountered in routine casework were analyzed with the Quantifiler Human Kit on the ABI 7000. These quantitation results were then compared to the previously obtained Quantiblot results. All samples were then amplified using the Identifiler Kit and the Quantifiler Human results in an effort to determine the preferred amount of sample template to add to the PCR process.

Additionally, this laboratory participated in a NIST Quantitation study aimed at evaluating shipping conditions of standard DNA samples. Each of the NIST samples was analyzed with the Quantifiler Human Kit as well as the Quantiblot Kit, with results compared and tabulated as well.

Results:

Non-Probative Samples

Sample Name	Sample Type	Quantifiler Human Result	Quantiblot Result	Percent Difference 100-[(QF/QB)*100]
[illegible]	vaginal swab	0.0914	0.12	23.83
[illegible]	victim standard	6.03	2	-201.50
[illegible]	victim standard	1.36	1	-36.00
[illegible]	blood from currency	0.0993	0.03	-231.00
[illegible]	vaginal swab	0.303	0.24	-26.25
[illegible]	victim standard	0.684	1	31.6
[illegible]	semen on comforter	0.103	0.12	14.17
[illegible]	suspect standard	2.14	1	-114.00
[illegible]	victim standard	4.14	2	-107.00

The experimental results demonstrate that the Quantiblot method of quantitating DNA typically underestimated the amount of DNA present in a sample. An accurate quantitation result is critical to obtaining an adequate DNA profile downstream with the Identifiler Kit. If DNA quantities greater than the optimal range are added to the PCR mix, the analyst will likely have a more imbalanced PCR product as well as possible saturation of the detection system causing pull-up and a greater likelihood of Stochastic effects. When utilizing the Quantifiler results to determine DNA template addition, the resulting peak heights on the ABI 310 from the Identifiler amplicon were acceptable and produced no excessive pull-up or stochastic related issues.

Note: All quantitation results are in units of ng/µl
Note: The red numbers indicate the lower quant value

http://www.cstl.nist.gov/biotech/strbase/validation/ADFS-BH_7000val.pdf

Implementation of the Newly Validated Procedure

Ok, the validation studies are complete and approved, the procedure is written and approved and the lab is ready to implement the new procedure into casework.

So, what about training?

Who needs to be trained and what is the extent of the training? How is the training documented? What constitutes completion of training? Per individual or per lab?

From Robyn Ragsdale (FDLE), Validation Workshop (Aug 24-26, 2005 at NFSTC)
http://www.cstl.nist.gov/biotech/strbase/validation/validationworkshop.htm

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