Validation Workshop

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

Pennsylvania State Police
DNA Laboratory Training Workshop
Greensburg, PA
June 5, 2007

Proposed Agenda for PSP Training

- Introductions – Presenter and Participants
- **Validation Workshop**

Lunch

- *Answer questions*
- Cover other topics (depending on time)
  - Mixture Interpretation
  - qPCR and Low-Copy Number (LCN) DNA Testing

My Goal is to Answer YOUR Questions – So Please Ask Them…

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.

Introductions

My Background

- **My mom grew up in Pittsburg and my parents met at Penn State so I have Pennsylvania roots…**
- PhD **(Analytical Chemistry)** from University of Virginia (Aug 1995)
- Research conducted at FBI Academy under Bruce McCord doing CE for STR typing
- NIST Postdoc – developed STRBase website
- GeneTrace Systems – private sector experience validating assays
- NIST Human Identity Project Leader since 1999
- Invited guest to SWGDAM since 2000
- **Member of SWGDAM Validation Subcommittee**
- Served on WTC KADAP and helped evaluate and validate new miniSTR, mtDNA, and SNP assays
- Author of *Forensic DNA Typing: Biology, Technology, and Genetics of STR Markers* (2nd Edition)
- Married with 6 children – I have "validated" that they are mine using STR typing…

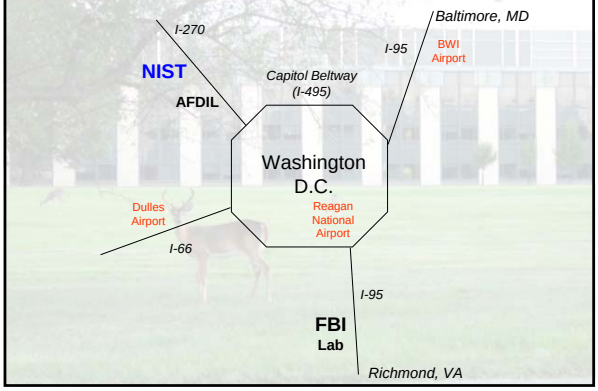
NIST History and Mission

- National Institute of Standards and Technology (NIST) was created in 1901 as the National Bureau of Standards (NBS). The name was changed to NIST in 1988.
- NIST is **part of the U.S. Department of Commerce** with a mission to develop and promote measurement, standards, and technology to enhance productivity, facilitate trade, and improve the quality of life.
- NIST supplies over 1,300 Standard Reference Materials (SRMs) for industry, academia, and government **use in calibration of measurements**.
- **NIST defines time for the U.S.**

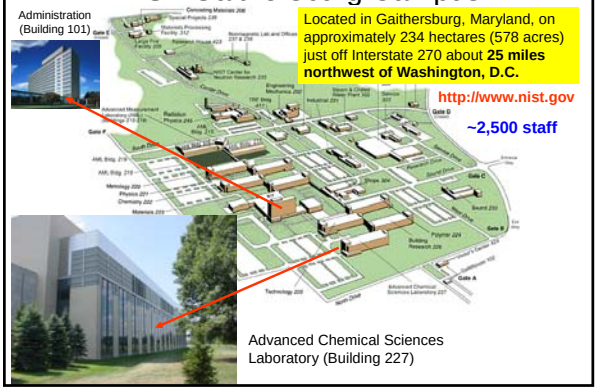
$573 for 3 jars


DNA typing standard

Location of NIST (near Washington, DC)



NIST Gaithersburg Campus



NIST Human Identity Project Team

All NIST 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

National Institute of Justice

The Research, Development, and Evaluation Agency of the U.S. Department of Justice

Current Areas of NIST Effort with Forensic DNA

- **Standards** http://www.cstl.nist.gov/biotech/strbase/
  - Standard Reference Materials
  - Standard Information Resources (STRBase website)
  - Interlaboratory Studies
- **Technology**
  - Research programs in SNPs, miniSTRs, Y-STRs, mtDNA, qPCR
  - Assay and software development
- **Training Materials**
  - Review articles and workshops on STRs, CE, validation
  - PowerPoint and pdf files available for download

Seminars and Training Workshops to Individual Forensic DNA Laboratories

MYRIAD — Feb 3, 2005
FBI Laboratory — May 19, 2005
June 8, 2005
June 13-14, 2005
Apr 27-28, 2006
June 6, 2006
BCA — Aug 7, 2006
Nov 15, 2006
State Police WV — Dec 5-6, 2006
March 7, 2007
March 14, 2007
Apr 3-4, 2007
Apr 5, 2007
June 5, 2007

Training Workshops Planned

- ISFG Meeting (August 2007, Copenhagen, Denmark)
  - **CE Fundaments and Troubleshooting**
  - **Validation**
- SAFS Meeting (September 2007, Atlanta, GA)
  - **Mixture Interpretation**
- Int. Symposium on Human Identification (Promega) Meeting (October 2007, Hollywood, CA)
  - **Validation**

Background of Participants…

Name
Experience (years) with DNA typing
Something memorable about yourself
What you hope to learn from this workshop

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

NIJ National Forensic Science Technology Center
President's DNA Initiative - Workshops

Validation Workshop

Robyn Ragsdale, PhD
Florida Department of Law Enforcement (FDLE)

John M. Butler, PhD
National Institute of Standards and Technology (NIST)

FDLE NIST

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…

Validation Workshop Outline

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

BREAK

Practical
- Developmental Validation
- Internal Validation and Examples
- Documentation

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

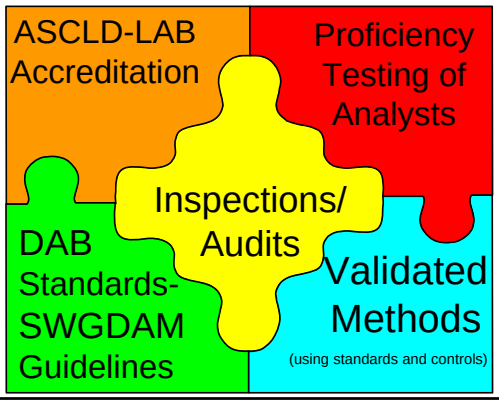
- **Quality assurance** (QA) – planned or systematic actions necessary to provide adequate confidence that a product or service will satisfy given requirements for quality
- **Quality control** (QC) – day-to-day operational techniques and activities used to fulfill requirements of quality
- **Validation** – 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

Ensuring Accurate Forensic DNA Results



Checks and Controls on DNA Results

Community	FBI DNA Advisory Board's Quality Assurance Standards *(also interlaboratory studies)*
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

History of Forensic DNA Validation Guidelines

Timeline Regarding Forensic Validation Information

- 1989 *Castro* case – concern over quality in forensic DNA cases
- 1989 TWGDAM – focus on RFLP
- 1991 TWGDAM – updated with PCR info
- 1995 TWGDAM – updated with more PCR info
- **1998/99 DNA Advisory Board Standards**
- 2004 Revised SWGDAM Validation Guidelines

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.

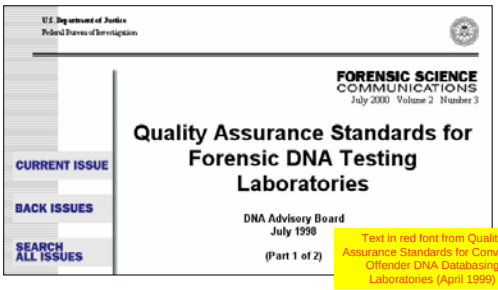
DNA Advisory Board (DAB)

DNA Advisory Board (DAB) Members

- **Joshua Lederberg** (Rockefeller University) – chair 1995-1998
- **Arthur Eisenberg** (University of North Texas Health Science Center) – chair 1998-2000
- **John Hicks** (Alabama Department of Forensic Sciences)
- **Shirley Abrahamson** (Wisconsin State Supreme Court)
- **Ranajit Chakraborty** (University of Texas Health Science Center)
- **Bruce Budowle** (FBI Laboratory)
- **Larry Presley** (FBI Laboratory)
- **Jack Ballantyne** (Suffolk County Crime Lab)
- **Jay Miller** (FBI Laboratory)
- **Dennis Reeder** (National Institute of Standards and Technology)
- **Margaret Kuo** (Orange County Sheriff's Office)
- **Bernard Devlin** (Carnegie Mellon University)
- **Marcia Eisenberg** (Laboratory Corporation of America)
- **Paul Ferrara** (Virginia Division of Forensic Science)
- **Terry Laber** (Minnesota State DNA Lab)
- Dwight Adams, Randall Murch, Barry Brown (FBI Laboratory)
- David Coffman (Florida Department of Law Enforcement)
- Fred Bieber (Harvard Medical School)
- Mary Gibbons (Oakland Police Department)
- Eric Juengst (Case Western Reserve University)
- Susan Narveson (Phoenix Police Department)
- Mohammad Tahir (Indianapolis-Marion County Crime Lab)
- Dawn Herkenham (FBI Laboratory)

Existed from 1995-2000

DAB Standards


U.S. Department of Justice
Federal Bureau of Investigation

FORENSIC SCIENCE COMMUNICATIONS
July 2000 Volume 2 Number 3

CURRENT ISSUE
BACK ISSUES
SEARCH ALL ISSUES

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

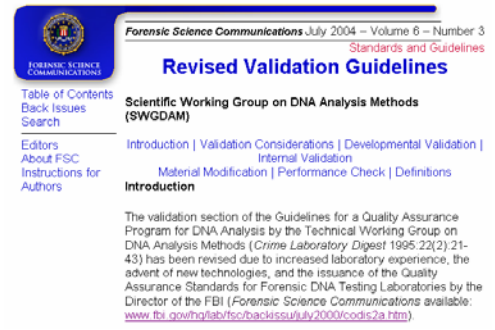
Quality Assurance Standards (QAS)

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

Revised SWGDAM Validation Guidelines

(July 2004)

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


Forensic Science Communications July 2004 – Volume 6 – Number 3
Standards and Guidelines

Revised Validation Guidelines

Table of Contents
Back Issues
Search

Editors
About FSC
Instructions for Authors

Scientific Working Group on DNA Analysis Methods (SWGDAM)

Introduction | Validation Considerations | Developmental Validation | Internal Validation
Material Modification | Performance Check | Definitions

Introduction

The validation section of the Guidelines for a Quality Assurance Program for DNA Analysis by the Technical Working Group on DNA Analysis Methods (*Crime Laboratory Digest* 1995;22(2):21-43) has been revised due to increased laboratory experience, the advent of new technologies, and the issuance of the Quality Assurance Standards for Forensic DNA Testing Laboratories by the Director of the FBI (*Forensic Science Communications* available: www.fbi.gov/hq/lab/fsc/backissu/july2000/codis2a.htm).

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

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

Community Needs 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.

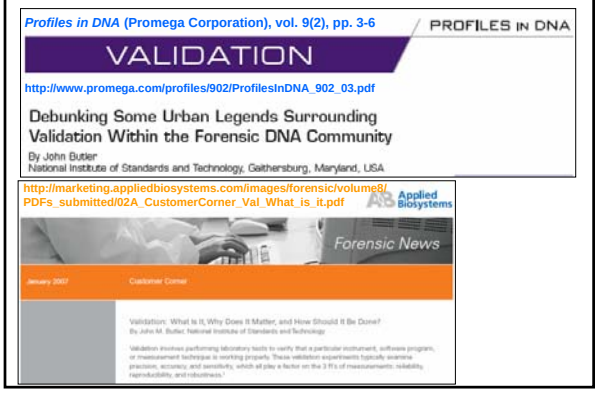
Roper, P., *et al.* (2001) *Applications of Reference Materials in Analytical Chemistry*. Royal Society of Chemistry, Cambridge, UK, pp. 110-111.

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

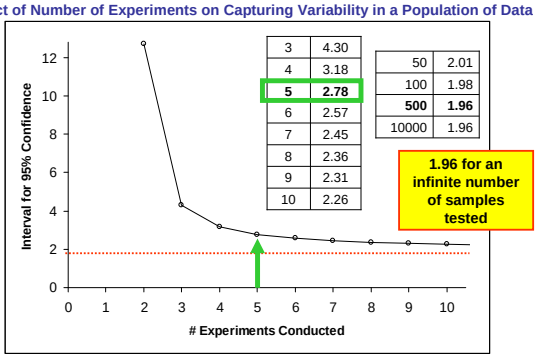
"Student" (real name: W. S. Gossett [1876-1937]) developed statistical methods to solve problems stemming from his employment in a brewery.

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

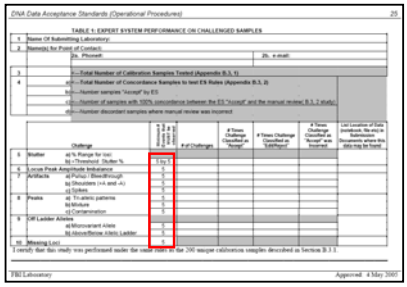
50	2.01
100	1.98
500	**1.96**
10000	1.96

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)



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

Allele	Caucasian *N= 302* (Butler *et al.* (2003) *JFS* 48(4):908-911)	Caucasian ***N= 7,636*** (Einum *et al.* (2004) *JFS* 49(6): 1381-1385)
11	0.0017*	0.0009
12	0.0017*	0.0007
13	--	0.0031
14	0.1027	0.1240
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*

Most common allele

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

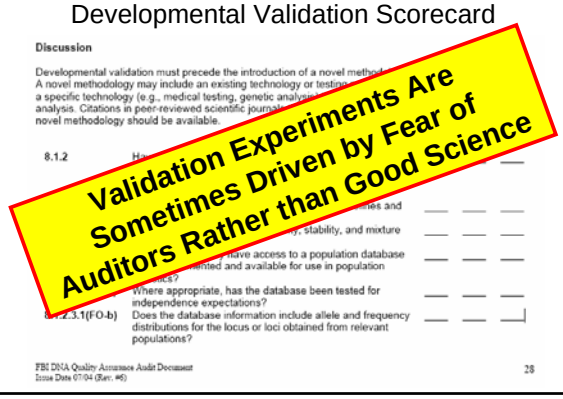
Want to sample at least 5 chromosomes to provide a somewhat reliable estimate of an allele's frequency in a population

What are the goals of validation studies involving a new STR typing kit?

- Stutter product amounts
  Why?: aids in mixture interpretation guidelines (how often does laboratory call peaks below 15% of an adjacent allele?)
- Precision studies
  Why?: aids in defining allele bin wind… change the ±0.5 bp from th…
- Sensitivity studies
  Why?: aid…
- M…
  …s of detecting the minor component
- …hat new primer sets get the same results as original …sets – potential of polymorphism causing allele dropout…
- …eak height ratio studies
  Why?: aids in mixture interpretation guidelines (how often does your laboratory call peaks below a 60% heterozygote peak height ratio?)

Too often validation experiments are performed but observations are not considered for implementation purposes

FBI DNA Quality Assurance Audit Developmental Validation Scorecard



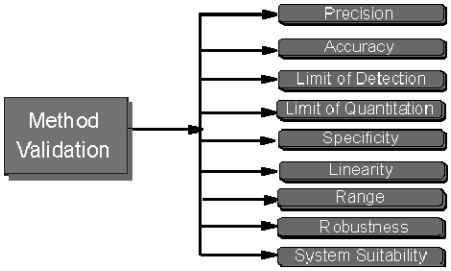
Validation in Other Fields
(Besides Forensic DNA Testing)

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."

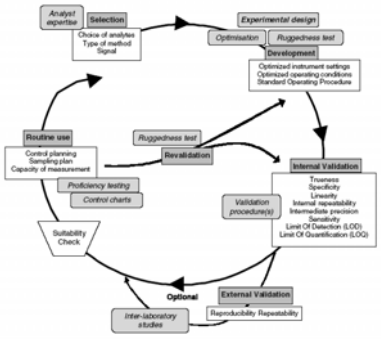
EURACHEM Guide (1998) *The Fitness for Purpose of Analytical Methods: A Laboratory Guide to Method Validation and Related Topics*, pp. 47-48; available at http://www.eurachem.ul.pt/guides/valid.pdf

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 Papers 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

Developmental Validation

DNA Advisory Board Quality Assurance Standards

Section 2. Definitions

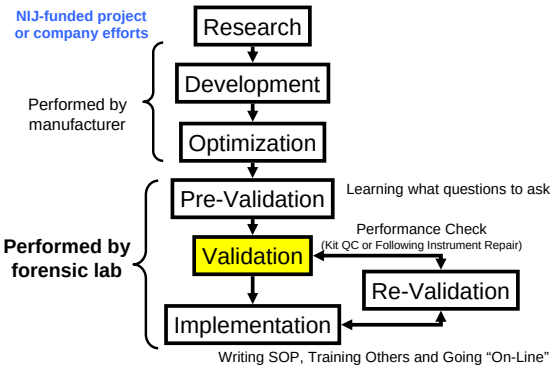
- (ff) Validation is a process by which a procedure is evaluated to determine its **efficacy and reliability** for forensic casework analysis *(DNA analysis)* and includes:

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

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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

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.

Material Modification

- Decrease in reaction volume from manufacturer's specifications
- Centricon tube membrane change
- Minimum peak threshold
- Injection times for genetic analyzers
- Increased amplification cycle numbers
- Others?

Performance Check

- Relocation of lab to a new facility
- Change of laser or other critical component on a genetic analyzer
- Software changes
  - Mac-based GS/GT to NT-based GS/GT
  - Mac-based collection software to NT or Windows-based collection software
- Additional instrumentation (i.e., 2nd 3130)

How would you evaluate each of these?

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

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.

Internal Validation

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

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"

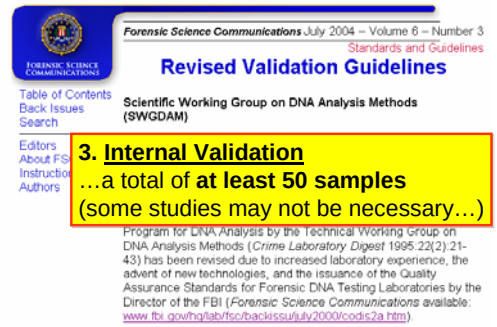
This is what takes the time…

- **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

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

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

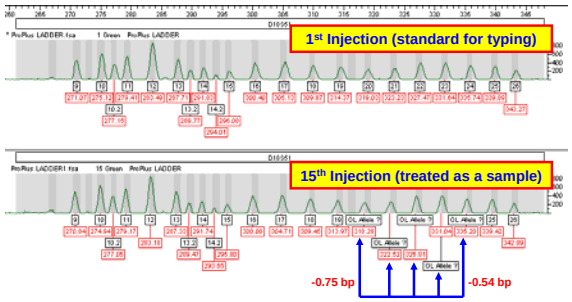
- Standard samples (3.1) **Between 1 and ~20 samples**
  - Verify correct type with positive control or NIST SRM samples
  - Concordance study with 5-10 (non-probative casework) samples previously typed with other kit(s)
- Precision samples (3.2) **5-10 samples**
  - Run at least 5-10 samples (allelic 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] 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:

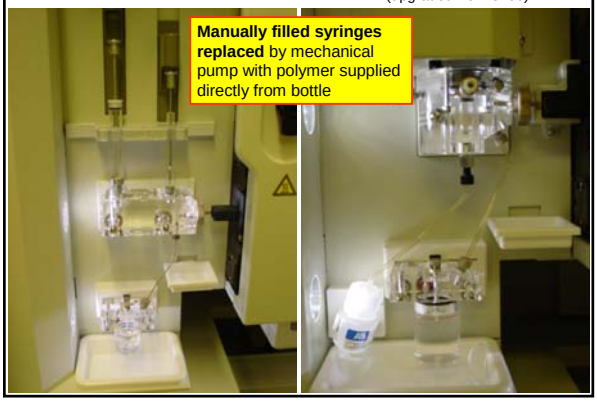
- Precision studies to evaluate instrument reproducibility
- Sensitivity studies
- **Do not need new stutter, mixture ratio, peak height ratio, etc. (these relate to dynamics of the the kit used)**

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

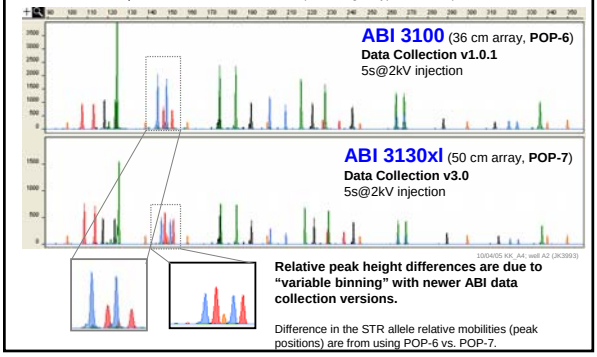
What NIST did to "validate" a 3130xl upgrade

- 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
an Applera Corporation business

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 1× 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**.

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FORENSIC SCIENCE COMMUNICATIONS JULY 2000 VOLUME 2 NUMBER 3

Why is Documentation of Validation Important?

9. Documentation of Validated Methods

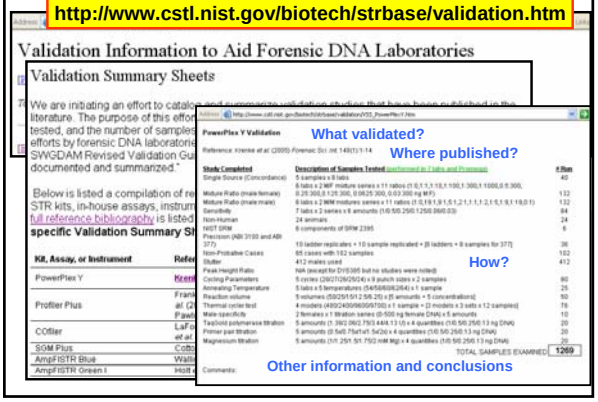
9.1 Once the validation process is complete it is important to document the procedures so that the method can be clearly and unambiguously implemented. There are a number of reasons for this. **The various assessments of the method made during the validation process assume that,in use, the method will 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

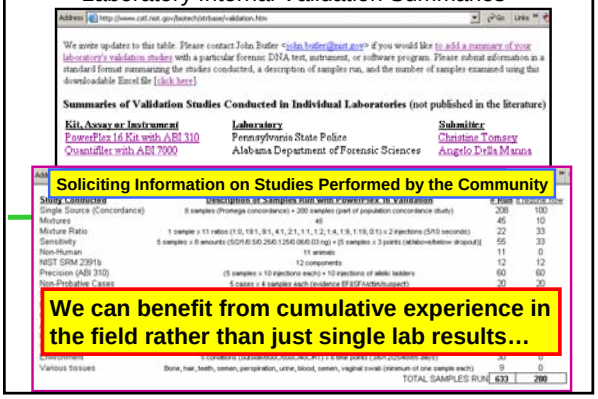


Validation Summary Sheet for PowerPlex Y

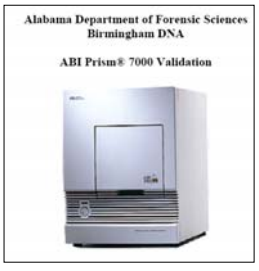
Study Completed (17 studies done)	Description of Samples Tested (performed in 7 labs and Promega)	# Run
Single Source (Concordance)	5 samples x 8 labs	40
Mixture Ratio (male:female)	6 labs x 2 M/F mixture series x 11 ratios (1:0,1:1,1:10,1:100,1:300,1:1000,0.5:300, 0.25:300,0.125:300, 0.0625:300, 0.03:300 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



Example of Validation Documentation



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

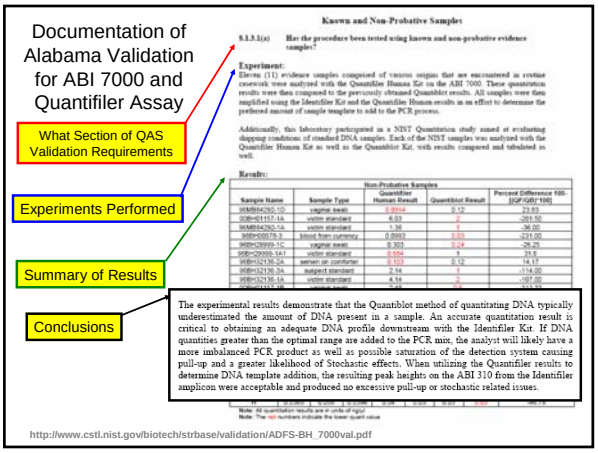
Documentation of Alabama Validation for ABI 7000 and Quantifiler Assay

What Section of QAS Validation Requirements

Experiments Performed

Summary of Results

Conclusions



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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