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

Validation Workshop

Validation Overview

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Presentation Outline

Introductions: Presenters and Participants

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)

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 those of the author 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.

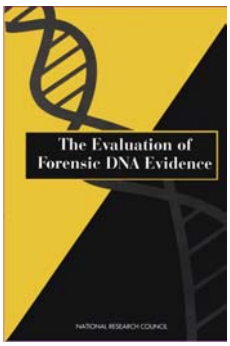
Overview of This Section

- 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?
- Define accuracy, precision, sensitivity, stability, reproducibility, and robustness as applied to general 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…
- 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…

NRC II Recommendation 3.1



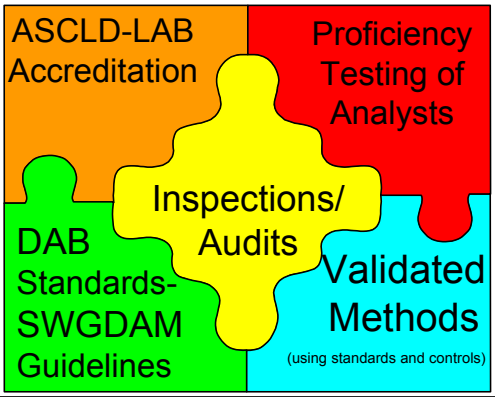
- Laboratories should adhere to high quality standards (such as those defined by TWGDAM and the DNA Advisory Board) and make every effort to be accredited for DNA work (by such organizations as ASCLD-LAB).

Some Desirable QC and QA Guidelines

Noted in NRC I pp. 104-105

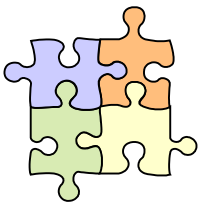
- Reagents and equipment are properly maintained and monitored.
- **Procedures used are generally accepted in the field and supported by published, reviewed data that were gathered and recorded in a scientific manner**.
- Appropriate controls are specified in procedures and are used.
- **New technical procedures are thoroughly tested to demonstrate their efficacy and reliability** for examining evidence material before being implemented in casework.

Ensuring Accurate Forensic DNA Results



Elements for Guaranteeing Quality Results in Forensic DNA Testing

- Accepted Standards and Guidelines for Operation
- Laboratory Accreditation
- Proficiency Testing of Analysts
- Standard Operating Procedures
- **Validated Methods**
- Calibrated Instrumentation
- Documented Results
- Laboratory Audits
- **Trustworthy Individuals**



Costs/Benefits of 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.

Organizations Involved in International Quality Assurance Issues

- International Standards Organization (ISO) **ISO 17025**
  - http://www.iso.ch
- AOAC International (Association of Official Analytical Chemists)
  - http://www.aoac.org
- Eurachem
  - http://www.eurachem.ul.pt
- VAM (Valid Analytical Measurement)
  - http://www.vam.org.uk
- CCQM (Comité Consultatif pour la Quantité de Matière; Consultative Committee for Amount of Substance – Metrology in Chemistry)
  - http://www.bipm.org/en/committees/cc/ccqm/
- CITAC (Co-operation on International Traceability in Analytical Chemistry)
  - http://www.citac.cc

Organizations Involved in International Quality Assurance Issues

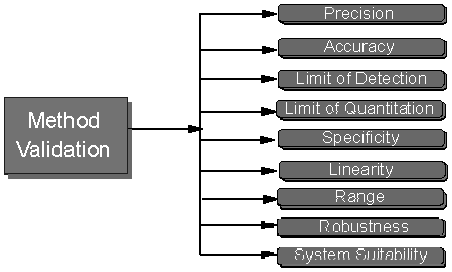
- ASTM International (American Society for Testing and Materials)
  - http://www.astm.org
- CLSI (Clinical and Laboratory Standards Institute)
  - http://www.clsi.org
- ANSI (American National Standards Institute)
  - http://www.ansi.org
- ILAC (International Laboratory Accreditation Cooperation)
  - http://www.ilac.org
- FDA (U.S. Food and Drug Administration)
  - http://www.fda.gov

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."

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

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

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

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

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.

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

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

How do you validate a method?

- Decide on analytical requirements
- 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.

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.

PubMed Literature Search

http://www.ncbi.nlm.nih.gov/PubMed

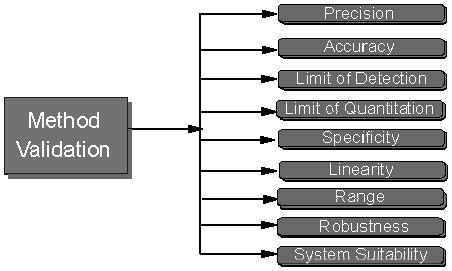
Search Results with term "validation" (8/15/05)
- *J. Forensic Sci.* - 78 references
- *Int. J. Legal Med.* - 24 references
- *Forensic Sci. Int.* - 62 references

- All of PubMed – **32,191 references**
- "validation" AND "forensic DNA" - 116

Review of Promega conference proceedings:
133 with "validation" in title of talk or poster

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

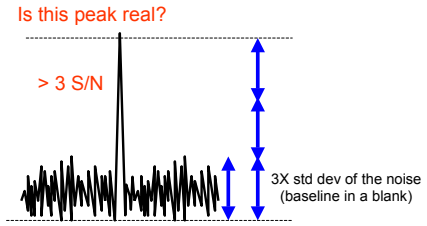
Sensitivity

- 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?

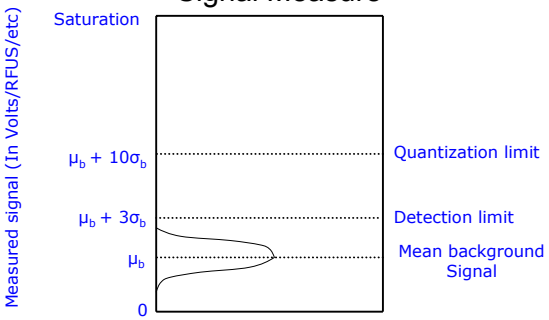
EURACHEM Guide (1998) *The Fitness for Purpose of Analytical Methods: A Laboratory Guide to Method Validation and Related Topics*, p. 43; available at http://www.eurachem.ul.pt/guides/valid.pdf

Limit of Detection (LOD)

- Typically 3 times the signal-to-noise (based on standard deviation of the noise)



Signal Measure



Travis Doom, "Background Noise in STR Testing," Presentation at *The Science of DNA Profiling: A National Expert Forum*; Held at Wright State (Dayton, OH), August 12, 2005; available at http://www.bioforensics.com/conference05/Doom_BackgroundNoise.ppt

Objective threshold determination

- The limit of detection is an extrapolated value.
- While easy to use, carte blanche thresholds make assumptions that may not be valid for a particular experiment/run.
- FBS study (currently unpublished)
  - Study characterizes noise signal in 42 runs taken from 7 cases analyzed by the FBI.
  - Each run contains a reagent blank, a positive control, and a negative control.
  - Output signal data was collected only from regions of the electropherogram free of analyte signal (positive control peaks, ROX peaks, +/-4 stutter) in all channels.
    - In-line reagent blanks/controls

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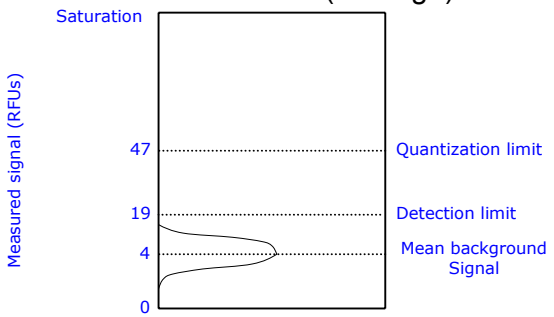
Study Results

Noise Characterization and Thresholds of Detection/Quantization (RFUs)

Reagent Blank	**Run Type**	**μ**	**σ**	**μ + 3σ**	**μ + 10σ**
	Maximum (Noisy)	15.4	6.65	35.4	81.9
	Average (n=43)	6.51	4.62	20.4	52.7
	Minimum	5.17	3.52	15.7	40.3
Negative Control	**Run Type**	**μ**	**σ**	**μ + 3σ**	**μ + 10σ**
	Maximum (Noisy)	16.3	24.5	89.9	262
	Average (n=43)	6.61	5.39	22.8	60.5
	Minimum	5.16	3.47	15.6	39.9
Positive Control	**Run Type**	**μ**	**σ**	**μ + 3σ**	**μ + 10σ**
	Maximum (Noisy)	15.4	6.00	33.4	75.4
	Average (n=43)	6.22	4.09	18.5	47.1
	Minimum	4.85	3.46	15.2	39.4

Travis Doom, "Background Noise in STR Testing," Presentation at *The Science of DNA Profiling: A National Expert Forum*; Held at Wright State (Dayton, OH), August 12, 2005; available at http://www.bioforensics.com/conference05/Doom_BackgroundNoise.ppt

Positive Control (Average)



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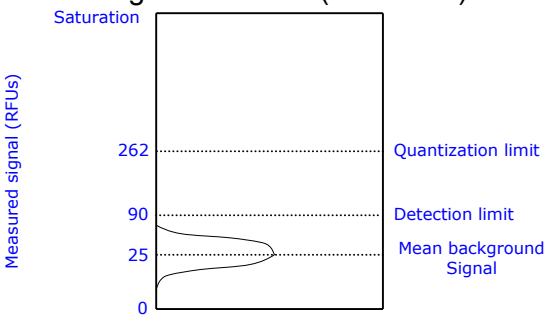
Negative Control (Maximum)



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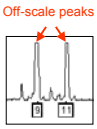
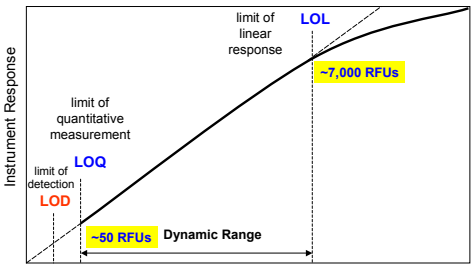
Limit of Linear Response (LOL)

- Point of saturation for an instrument detector so that higher amounts of analyte do not produce a linear response in signal
- In ABI 310 or ABI 3100 detectors, the CCD camera saturates leading to flat-topped peaks.



Useful Range of an Analytical Method



LOD = 3x SD of blank
LOQ = 10x SD of blank

Adapted from Figure 1-7 in Skoog, D.A., *et al.* (1998) *Principles of Instrumental Analysis* (5th Edition). Thomson Learning, Inc.

Linearity and Range

- Linearity "defines the ability of the method to obtain test results proportional to the concentration of analyte."
- "The Linear Range is by inference the range of analyte concentrations over which the method gives test results proportional to the concentration of the analyte."
- Working range is a "set of values of measurands for which the error of a measuring instrument is intended to lie within specified limits."

EURACHEM Guide (1998) *The Fitness for Purpose of Analytical Methods: A Laboratory Guide to Method Validation and Related Topics*, pp. 43, 46; available at http://www.eurachem.ul.pt/guides/valid.pdf

Specificity

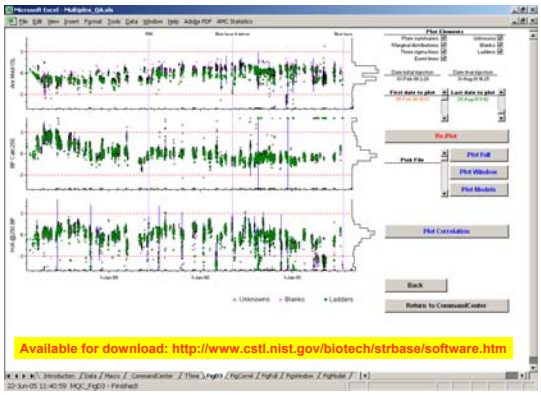
- "The ability of a method to measure only what it is intended to measure."
- "Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Typically these might include impurities, degradants, matrix, etc."
- The primers in PCR amplification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

Stability

- Will the method produce a result reliably over time?
- Control charts are an effective tool for monitoring stability and quality assurance over time
  - Dave Duewer at NIST has developed a software program called **Multiplex_QA** that permits a view of sensitivity and resolution of STR data in order to monitor instrument performance over time.
  - The program is available for download on the NIST STRBase website: http://www.cstl.nist.gov/biotech/strbase/software.htm

NIST Multiplex_QA Program for Monitoring Performance Over Time



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.

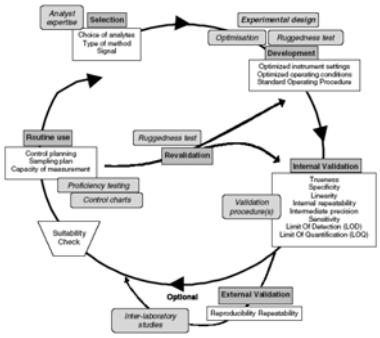
EURACHEM Guide (1998) *The Fitness for Purpose of Analytical Methods: A Laboratory Guide to Method Validation and Related Topics*, p. 49; available at http://www.eurachem.ul.pt/guides/valid.pdf

System Suitability

- Fitness for purpose is the "degree to which data produced by a measurement process enables a user to make technically and administratively correct decisions for a stated purpose."

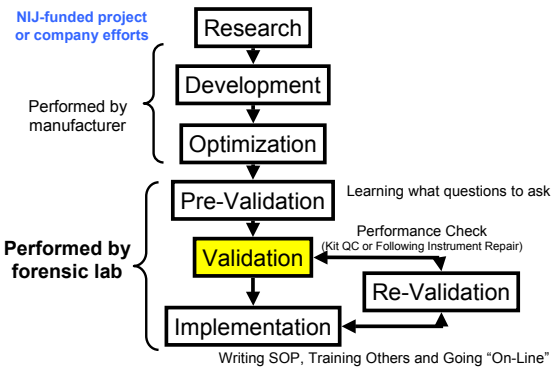
EURACHEM Guide (1998) *The Fitness for Purpose of Analytical Methods: A Laboratory Guide to Method Validation and Related Topics*, p. 42; available at http://www.eurachem.ul.pt/guides/valid.pdf

The lifecycle of a method of analysis



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

How an Assay Evolves



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

If *N*=5, 95% of the time the actual mean would be in the range: $X_{avg} \pm 2.78\ \sigma/N^{1/2}$

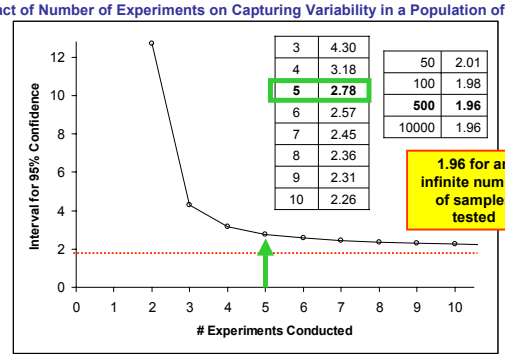
Student's *t*-Tests

"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**). For example, it it likely that the true mean size of maple leaves is "close" to the mean calculated from a sample of *N* randomly collected leaves. If *N*=5, 95% of the time the actual mean would be in the range: $X_{avg} \pm 2.776\ \sigma/N^{1/2}$; if *N*=10: $X_{avg} \pm 2.262\ \sigma/N^{1/2}$; if *N*=20: $X_{avg} \pm 2.093\ \sigma/N^{1/2}$; if *N*=40; $X_{avg} \pm 2.023\ \sigma/N^{1/2}$; and for "large" *N*: $X_{avg} \pm 1.960\ \sigma/N^{1/2}$. (These "small-sample" corrections are included in the descriptive statics report of the 95% confidence interval.)

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

A Comment on Minimum Numbers of Samples for Validation Studies…

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

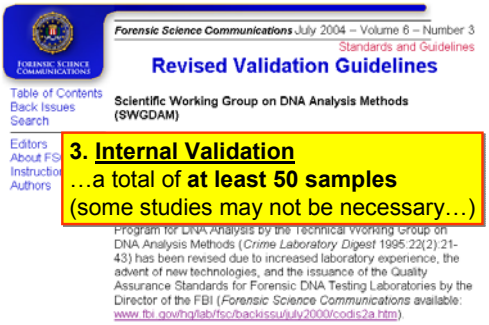
50	2.01
100	1.98
500	**1.96**
10000	1.96

1.96 for an infinite number of samples tested

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.

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 →

Historical Perspective on Implementing Forensic DNA Analysis

Crouse, C.A. (2001) Implementation of forensic DNA analysis on casework evidence at the Palm Beach County Sheriff's Office Crime Laboratory: historical perspective. Croatian Med. J. 42(3): 247-251.

- "A 2001 survey by the STR Megaplex Advanced Research and Training group (SMART) reported **the average time it now takes for a laboratory to completely validate a fluorescent STR system is one year**."
- "It is highly recommended that laboratories obtain SWGDAM validation guidelines and exchange validation information with other laboratories."

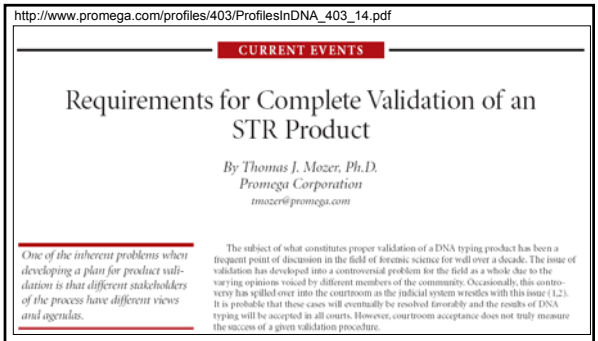
Design of Experiments Conducted for Validation Studies

- 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 individuals during reproducibility studies
- Who can do (or should do) validation…
  - Outside contractor?
  - Summer intern
  - Trainee
  - Qualified DNA analyst

http://www.promega.com/profiles/403/ProfilesInDNA_403_14.pdf



CURRENT EVENTS

Requirements for Complete Validation of an STR Product

By Thomas J. Mozer, Ph.D.
Promega Corporation
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One of the inherent problems when developing a plan for product validation is that different stakeholders of the process have different views and agendas.

The subject of what constitutes proper validation of a DNA typing product has been a frequent point of discussion in the field of forensic science for well over a decade. The issue of validation has developed into a controversial problem for the field as a whole due to the varying opinions voiced by different members of the community. Occasionally, this controversy has spilled over into the courtroom as the judicial system wrestles with this issue (1,2). It is probable that these cases will eventually be resolved favorably and the results of DNA typing will be accepted in all courts. However, courtroom acceptance does not truly measure the success of a given validation procedure.

Validation of STR Systems Reference Manual by Promega Corporation
http://www.promega.com/techserv/apps/hmnid/referenceinformation/powerplex/ValidationManual.pdf

Written from the perspective of only validating a STR kit…
(in this case PowerPlex 16)

Community Needs Training

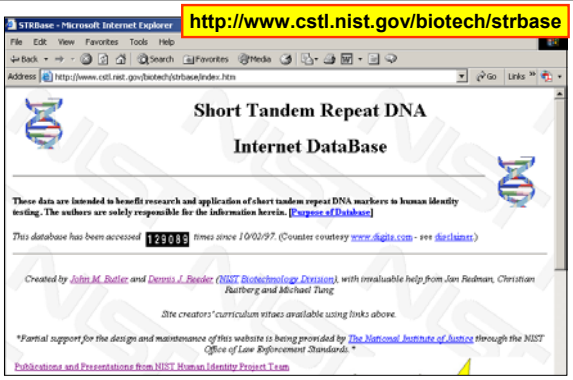
- To better understand what validation entails and how it should be performed (why a particular data set is sufficient)
- Many labs already treat DNA as a "black box" and therefore simply want a "recipe" to follow
- People are currently driven by fear of auditors and courts rather than scientific reasoning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

Pathway to Improved DNA Validation

- Collection of Current Philosophy on Validation
  - Community survey
  - Interviews
  - Literature summary
- Training
  - Auditors must be consistent in treatment of labs
- Providing Tools to Enable Improved Validation
  - Sample set(s)
  - Workbook – provide specific examples
  - Standard report form – documentation standardization
- Collection of Validation Data from Labs
  - NIJ-funded labs to submit data to STRBase validation website

VALIDATION WORKSHOP

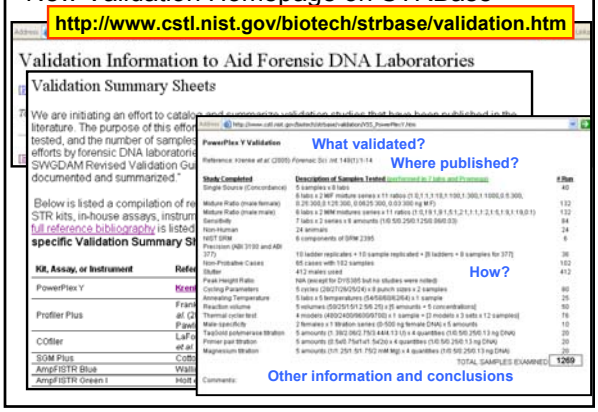
http://www.cstl.nist.gov/biotech/strbase



A Human Identity Testing Community Resource…

New Validation Homepage on STRBase

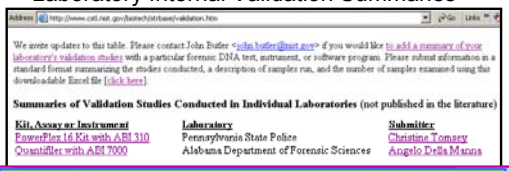
http://www.cstl.nist.gov/biotech/strbase/validation.htm



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



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

Resources to Aid Future Validation Studies

- STRBase Validation Website
  - **http://www.cstl.nist.gov/biotech/strbase/validation.htm**
  - Validation summary sheets
  - *Helpful information on aspects of validation studies*
- Multiplex_QA Program (Dave Duewer, NIST)
  - Software to monitor STR electropherogram performance (resolution, sensitivity) over time – **can aid performance checks**
  - Available for download: http://www.cstl.nist.gov/biotech/strbase/software.htm
- NIST Calibration Data Set (MIX05 data set is a prototype)
  - **We may construct a set of ~200 sample data files** that can be used to evaluate common STR typing "artifacts" such as stutter, non-template addition, spikes, peak imbalance, tri-allelic patterns, variant alleles, single base resolution

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.

Helpful Resource Books on Validation

- P. Roper, *et al.* (2001) *Applications of Reference Materials in Analytical Chemistry*. Royal Society of Chemistry, Cambridge, UK
- J.K. Taylor (1987) *Quality Assurance of Chemical Measurements*. Lewis Publishers: Chelsea, MI
- H. Gunzler, ed. (1996) *Accreditation and Quality Assurance in Analytical Chemistry*. Springer: New York
- J.K. Taylor (1990) *Statistical Techniques for Data Analysis*. Lewis Publishers: Chelsea, MI
- H.Y. Aboul-Enein, *et al.* (2001) *Quality and Reliability in Analytical Chemistry*. CRC Press: Washington, DC
- G.D. Christian (2004) *Analytical Chemistry* (6th Ed.). John Wiley & Sons, Inc.: Hoboken, NJ