Advanced Topics in Forensic DNA Analysis

Validation Essentials

New Jersey State Police
Training Workshop

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Outline for This Section

- Urban legends of validation
- Validation definitions and resources
- Examples
- STRBase validation website

Timeline Regarding Forensic Validation Information

- 1989 *Casto* 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

Validation Information

- President's DNA Initiative: **Validation Workshop (Aug 2005) with Robyn Ragsdale** – slides on STRBase; NFSTC working on DVD
- **ABI Roadshow/HID University: Validation Workshop (May 2006)** – slides available on STRBase
- STRBase validation website: we need **your internal validation information** (e.g., Y-STRs)

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?

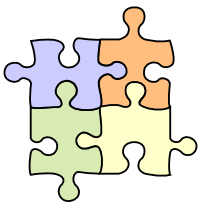
- **C**oncordance – are the same typing results obtained with the new technique as with an older one?
- **C**onstant Monitoring – check multiple allelic ladders in a batch against one another to confirm precision and consistent lab temperature
- **C**ommon Sense – are replicate tests repeatable?

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…

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**



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.

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.

Tools of Method Validation

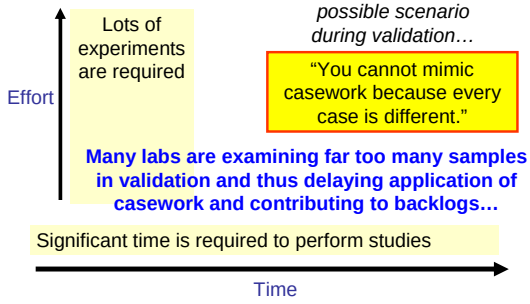
- Standard samples
  - positive controls
  - NIST SRMs
- Blanks
- Reference materials prepared in-house and spikes
- Existing samples
- Statistics
- **Common sense**

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

Common Perceptions of Validation

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

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



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

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

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

COURSE CONTENTS

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

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

Day #3
- Practical Exercises (Robyn)

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

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

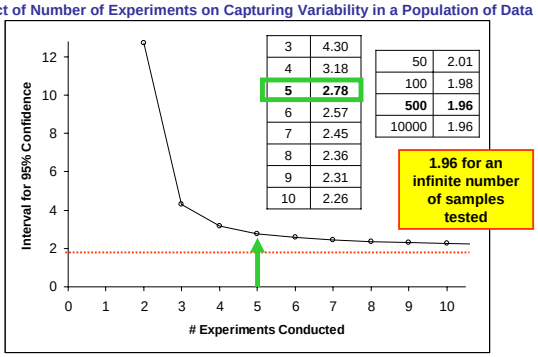
"Student" (real name: W. S. Gossett [1876-1937]) developed statistical methods to solve problems stemming from his employment in a brewery.

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

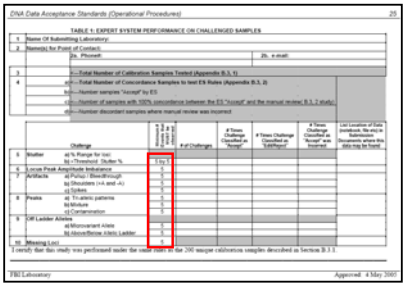
50	2.01
100	1.98
500	**1.96**
10000	1.96

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	Butler *et al.* (2003) *JFS* 48(4):908-911 Caucasian *N= 302*	Einum *et al.* (2004) *JFS* 49(6): 1381-1385 Caucasian ***N= 7,636***
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

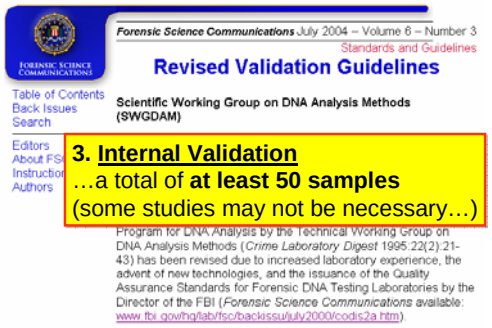
Minimum Allele Frequency = **5**/2N

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

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.

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

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
- …at new primer sets get the same results as original …ets – 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

Discussion

Developmental validation must precede the introduction of a novel method… A novel methodology may include an existing technology or testing… a specific technology (e.g., medical testing, genetic analysis… analysis. Citations in peer-reviewed scientific journal… novel methodology should be available.

8.1.2 Ha…

…ies and

…, stability, and mixture

…have access to a population database …ented and available for use in population …ics?

Where appropriate, has the database been tested for independence expectations?

8.1.2.3.1(FO-b) Does the database information include allele and frequency distributions for the locus or loci obtained from relevant populations?

FBI DNA Quality Assurance Audit Document
Issue Date 07/04 (Rev. #6) 28

Validation Experiments Are Sometimes Driven by Fear of Auditors Rather than Good Science

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

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

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

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

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

Example with Identifiler STR Kit

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

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

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

ABI Kit Validation Papers

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

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

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

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

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

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

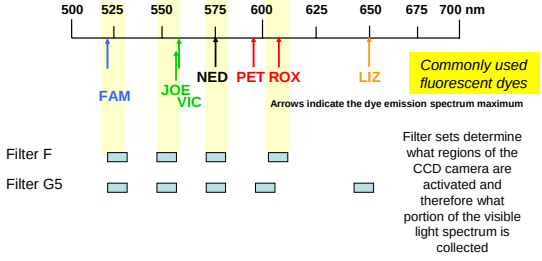
AmpFℓSTR® Identifiler™
PCR Amplification Kit
User's Manual

Population Studies with D2S1338 and D19S433

- These STR loci are part of the widely used SGM Plus kit
- Included in profile frequency calculator using 24 European populations and 5,700 individuals: http://www.str-base.org/calc.php
- Budowle, B. (2001) Genotype profiles for five population groups at the short tandem repeat loci D2S1338 and D19S433. *Forensic Sci. Comm.* 3(3); available at http://www.fbi.gov/hq/lab/fsc/backissu/july2001/budowle1.htm
- Budowle, B., *et al.* (2001) Population data on the STR loci D2S1338 and D19S433. *Forensic Sci. Comm.* 3(3); available at http://www.fbi.gov/hq/lab/fsc/backissu/july2001/budowle2.htm
- Butler, J.M., *et al.* (2003) Allele frequencies for 15 autosomal STR loci on U.S. Caucasian, African American, and Hispanic populations. *J. Forensic Sci.* 48(4):908-911; genotypes available at http://www.cstl.nist.gov/biotech/strbase/NISTpop.htm

Different Fluorescent Dyes

Visible spectrum range seen in CCD camera



	Blue	Green	Yellow	Red	Orange	Used with These Kits
Filter F	5FAM	JOE	NED	ROX		Profiler Plus
Filter G5	6FAM	VIC	NED	PET	LIZ	Identifiler

Mobility Shift with Non-Nucleotide Linker "Mobility Modifiers"

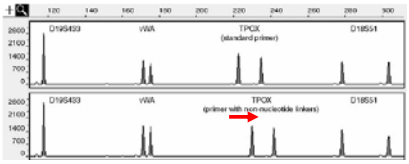


FIG. 1—*NED dye labeled loci from two amplifications of a single sample using TPOX primers both with and without non-nucleotide linkers. The X-axis indicates base pair size and the Y-axes RFU. The top panel depicts the amplification without non-nucleotide linkers. Sizes for the TPOX alleles for this panel were 222.93 and 234.81 bp. Sizes for the TPOX alleles in the amplification using the modified primer, depicted in the bottom panel, were 229.85 and 241.71 bp, indicating an average shift of 6.91 bp. Peaks heights, intralocus balance, and intracolor balance were similar in both amplifications.*

Collins PJ, Hennessy LK, Leibelt CS, Roby RK, Reeder DJ, Foxall PA. Developmental validation of a single-tube amplification of the 13 CODIS STR loci, D2S1338, D19S433, and amelogenin: the AmpFlSTR Identifiler PCR amplification kit. *J. Forensic Sci.* 2004; 49(6): 1265-1277.

Mobility Modifiers

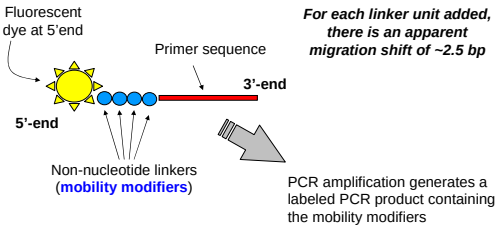


Figure 5.7, J.M. Butler (2005) *Forensic DNA Typing*, 2nd Edition © 2005 Elsevier Academic Press

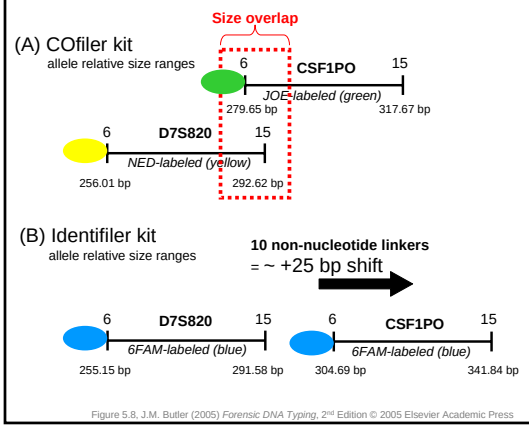


Figure 5.8, J.M. Butler (2005) *Forensic DNA Typing*, 2nd Edition © 2005 Elsevier Academic Press

Sizing Precision with Non-Nucleotide Linkers

TABLE 1—*Sizing shift and sizing precision data for loci incorporating non-nucleotide linkers on the ABI PRISM 310 Genetic Analyzer.*

Locus	Increase in Detected Size (bp)	Range of Standard Deviation of Alleles for Identifiler Kit (bp)	Range of Standard Deviation of Alleles Previous Kit (bp)
CSF1PO	26	0.08–0.13*	0.03–0.10[†]
D2S1338	16	0.05–0.12*	0.02–0.15[‡]
D13S317	12	0.05–0.09*	0.02–0.09[§]
D16S539	23	0.06–0.09*	0.01–0.08[‡]
TPOX	7	0.03–0.08*	0.02–0.07[‡]

No apparent significant decrease in precision with mobility modifiers…

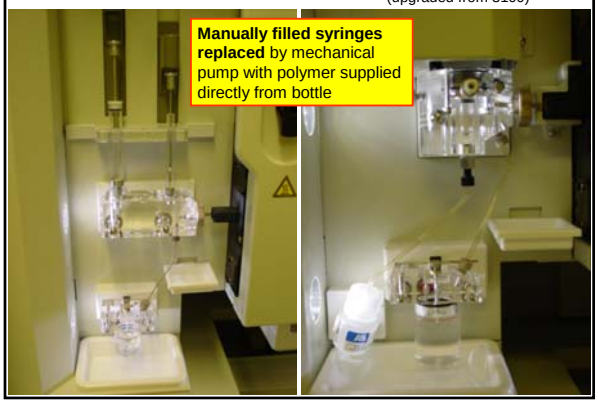
Collins PJ, Hennessy LK, Leibelt CS, Roby RK, Reeder DJ, Foxall PA. Developmental validation of a single-tube amplification of the 13 CODIS STR loci, D2S1338, D19S433, and amelogenin: the AmpFlSTR Identifiler PCR amplification kit. *J. Forensic Sci.* 2004; 49(6): 1265-1277.

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)



Manually filled syringes replaced by mechanical pump with polymer supplied directly from bottle

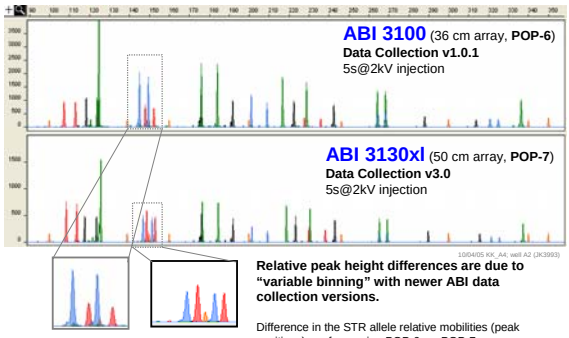
ABI 3130xl vs ABI 3100

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

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.

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)**

Validation Section of the DNA Advisory Board Standards

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

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

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

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

FORENSIC SCIENCE COMMUNICATIONS JULY 2000 VOLUME 2 NUMBER 3

Why is Documentation of Validation Important?

9. Documentation of Validated Methods

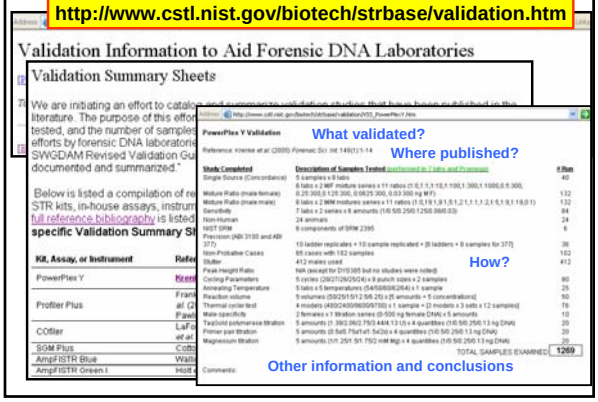
9.1 Once the validation process is complete it is important to document the procedures so that the method can be clearly and unambiguously implemented. There are a number of reasons for this. **The various assessments of the method made during the validation process assume that,in use, the method will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

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

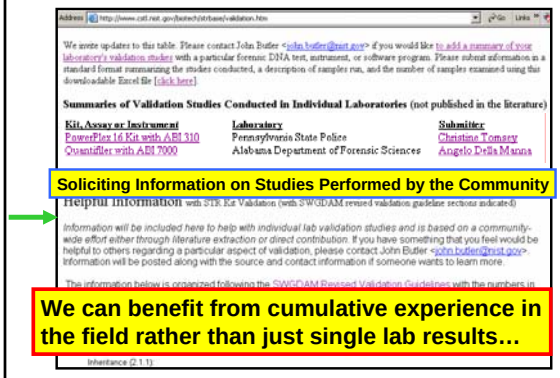
EURACHEM Guide (1998) *The Fitness for Purpose of Analytical Methods: A Laboratory Guide to Method Validation and Related Topics*, p. 37; available at http://www.eurachem.ul.pt/guides/valid.pdf

Validation Homepage on STRBase

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



Laboratory Internal Validation Summaries



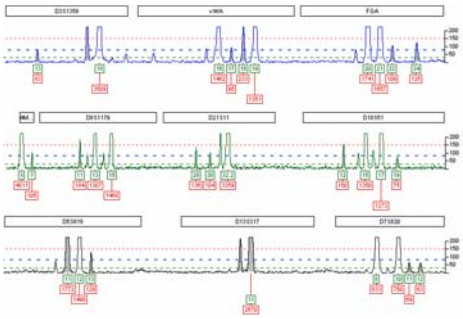
Setting thresholds for the ABI 310/3100

- Where do current ideas on instrument thresholds for the ABI 310/3100 come from?
- How do I set these values in my laboratory?
- Why might they vary from one instrument to the next?
- How do these thresholds affect data interpretation?

Future defense attacks will likely focus on detection thresholds – can you defend your current threshold (e.g., 50 RFU or 150 RFU)?

Lines in the sand: a two-person mix?

Slide from Dan Krane, MAFS Workshop (Oct 2006)

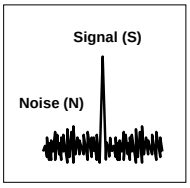


Two reference samples in a 1:10 ratio (male:female). Three different thresholds are shown: 150 RFU (red); LOQ at 77 RFU (blue); and LOD at 29 RFU (green). Gilder et al., January 2007 *JFS*.

What is a true peak (allele)?

GeneScan function

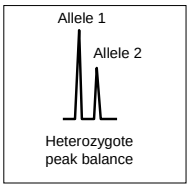
Peak detection threshold



Signal > 3x sd of noise

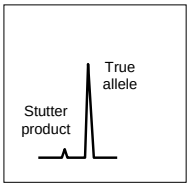
Genotyper function

Peak height ratio (PHR)



PHR consistent with single source Typically above 60%

Stutter percentage



Stutter location above 15%

TWGDAM validation of AmpFlSTR Blue

Wallin *et al.* (1998) *J. Forensic Sci.* 43(4): 854-870

- Minimum cycle # (27-30 cycles examined)
- Amplification adjusted to 28 cycles so that quantities of DNA below 35pg gave very low peaks or no peaks (below the analytical threshold!)
- 35 pg is approx 5 cells
- (but is 35pg the analytical threshold?) Determining this value might be a useful goal of a validation study

TWGDAM validation of AmpFlSTR Blue

Wallin *et al.* (1998) *J. Forensic Sci.* 43(4): 854-870

Determination of Minimum Sample

- Goal: avoid situations where peak imbalance results in only one detectable allele from a heterozygous pair.
- Perform serial dilution (1ng- 8pg) of 2 control samples which were heterozygous at all 3 loci
  - **Samples above 125pg had peak height RFUs above 150**
  - **Below 125pg peak heights were not significantly above background**
  - **At 31 pg peaks were very low or undetectable**
- **"Peaks below 150 RFU should be interpreted with caution"** **Why? Noise and stochastic fluctuation!**

Example of Validation Documentation

Alabama Department of Forensic Sciences
Birmingham DNA

ABI Prism® 7000 Validation

TABLE OF CONTENTS

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

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

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

Date

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

Documentation of Alabama Validation for ABI 7000 and Quantifiler Assay

What Section of QAS Validation Requirements

Experiments Performed

Summary of Results

Conclusions

Known and Non-Probative Samples

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

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

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

Results:

Non-Probative Samples				
Sample Name	Sample Type	Quantifiler Human Result	Quantiblot Result	Percent Difference 100-[(QF/QB)*100]
96MB84292-1D	vaginal swab	0.0914	0.12	23.83
00BH01157-1A	victim standard	6.03	2	-201.50
96MB84292-1A	victim standard	1.36	1	-36.00
98BH00578-3	blood from currency	0.0993	0.03	-231.00
98BH29999-1C	vaginal swab	0.303	0.24	-26.25
98BH29999-1A1	victim standard	0.684	1	31.6
98BH32136-2A	semen on comforter	0.103	0.12	14.17
98BH32136-3A	suspect standard	2.14	1	-114.00
98BH32136-1A	victim standard	4.14	2	-107.00

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

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

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

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.
- 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

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