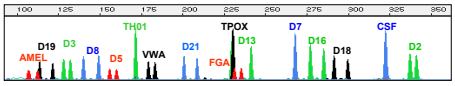


# Validation

John Butler

DNA Academy
University of Albany
June 13, 2005

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

## Current Areas of NIST Research Effort

- **Standard Information Resources** (STRBase information, training materials/review articles, validation standardization, calibration datasets)
- **Interlaboratory Studies** (Real-time PCR, mixture interpretation)
- **Resources for "Challenging Samples"** (miniSTRs for degraded DNA)
- **Information on New Loci** (Y-Chromosome, new STRs, SNPs)

## *Analytical Chemistry* Application Review

June 15, 2005 issue of *Analytical Chemistry*

**Forensic Science**

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*National Institute of Standards and Technology, Gaithersburg, Maryland 20899-8311*

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*Box 1334, Mount Laurel, New Jersey 08054*

Review Contents
- Forensic DNA Analysis
  - Collection, Characterization, Preservation, Extraction, and Quantitation of Biological Material
  - Short Tandem Repeats
  - Single-Nucleotide Polymorphisms
  - Y-STR Typing, Gender Identification, and X-Chromosome Analysis
  - Mitochondrial DNA Typing
  - Nonhuman DNA Typing Systems and Microbial Forensics
  - DNA Databases
  - Interpretation and Statistical Weight of DNA Typing Results
  - General Reviews

**250 articles referenced** covering forensic DNA analysis during 2003-2004

## Validation Project Purpose

- Review validation practices currently in use and available standards and guidelines (revised SWGDAM guidelines are too general)
- Help the community gain a better understanding of the validation process and how others have implemented validation in their labs so that validation in one's own lab may be performed more quickly
- Attempt to define a minimum number of samples that could be recommended for various validation scenarios
- Help with establishing uniformity throughout the field to aid auditors in their inspections

## 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**

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Scientific Working Group on DNA Analysis Methods (SWGDAM)

**3. Internal Validation**
…a total of **at least 50 samples**
(some studies may not be necessary…)

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.**

## 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

## 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…**

## DAB Quality Assurance Standards for Forensic DNA Testing Laboratories

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

## 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

## Pathway to Improved DNA Validation

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## Contacting the Community

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

## Representative Labs Interviewed

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

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

**Validation Standardization Questionnaire**
Please return to John Butler (NIST): john.butler@nist.gov or 301-975-8505 (fax)

*Purpose of questionnaire: We are embarking on an effort to define the minimum number of samples needed to reliably validate DNA typing procedures. As part of this effort, we are conducting a survey of standard practices currently used by practitioners in forensic DNA laboratories. Your honest responses to the following questions will help the entire community as we compile this information. Results will be summarized at the Promega meeting in October 2004 and made available on the NIST STRBase web site.*

General Questions

What does the term validation mean to you? *(define in a single sentence if possible)*

How do you know when you are finished validating a kit, instrument, software, or procedure?

What steps are needed in internal validation and how many samples should be run at a minimum?
- ☐ Precision studies ___(indicate types of samples –i.e., ladders), # samples/run ____; # runs ___
- ☐ Sensitivity studies_____, what range? _____
- ☐ Mixture studies _____ what mixture ratios are needed? _____
- ☐ Non-human DNA studies _____
- ☐ Non-probative cases _____

How many total samples do you think it takes to internally "validate" a new forensic kit?
- ☐ 10
- ☐ 50
- ☐ 500
- ☐ Other: _____

Validation Standardization Questionnaire (conducted June-August 2004)

## Review of Survey Questions

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

**Used to help define specific examples …**

## How I felt after taking on this project…

Me

**Literature, Validation Data, Survey Responses**

Validation Standardization Questionnaire (conducted June-August 2004)

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

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

Validation Standardization Questionnaire (conducted June-August 2004)

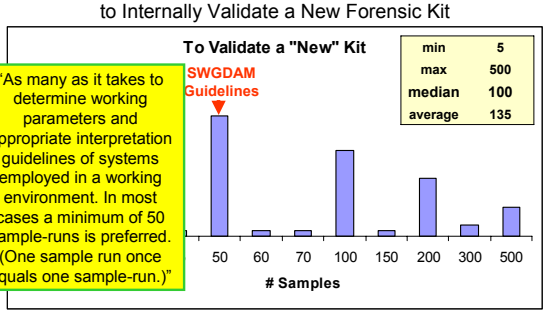
## How do you know when you are finished with a validation study? (2)

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

Validation Standardization Questionnaire (conducted June-August 2004)

## Survey Summary for Recommended **Total Number of Samples**
to Internally Validate a New Forensic Kit

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




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

Validation Standardization Questionnaire (conducted June-August 2004)

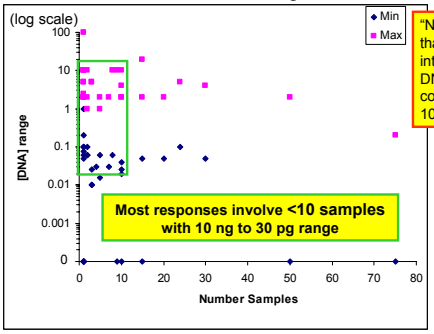
## Survey Summary for Recommended **Precision Studies**

A few of the responses:

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

Validation Standardization Questionnaire (conducted June-August 2004)

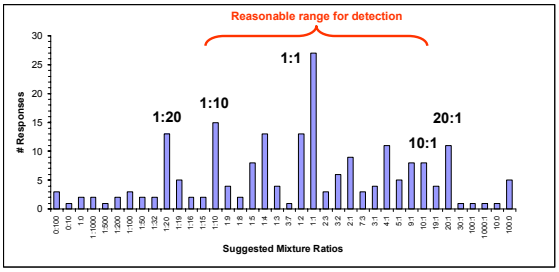
## Survey Summary for Recommended **Sensitivity Studies**




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

Validation Standardization Questionnaire (conducted June-August 2004)

## Survey Summary for Recommended **Mixture Studies**




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

Validation Standardization Questionnaire (conducted June-August 2004)

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

A few of the responses:

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

Validation Standardization Questionnaire (conducted June-August 2004)

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

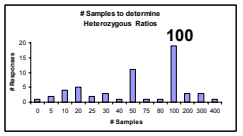
A few of the responses:

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

Validation Standardization Questionnaire (conducted June-August 2004)

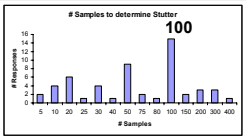
## Survey Summary for Recommended **Numbers of Samples**

**to Determine Heterozygote Peak Height Ratios and Stutter Values**




| | |
|---|---|
| min | 0 |
| max | 400 |
| median | 50 |
| average | 85 |

**Heterozygote Peak Height Ratios**



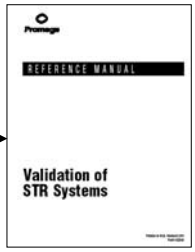

| | |
|---|---|
| min | 5 |
| max | 400 |
| median | 63 |
| average | 88 |

**Stutter Values**

Validation Standardization Questionnaire (conducted June-August 2004)

## Where do you look for guidance currently in validation?

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




Published in March 2001

Validation 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

## SWGDAM Revised Validation Guidelines

**Section 1.1** Validation is the process by which the scientific community acquires the necessary information to

(a) Assess the ability of a procedure to obtain reliable results.

(b) Determine the conditions under which such results can be obtained.

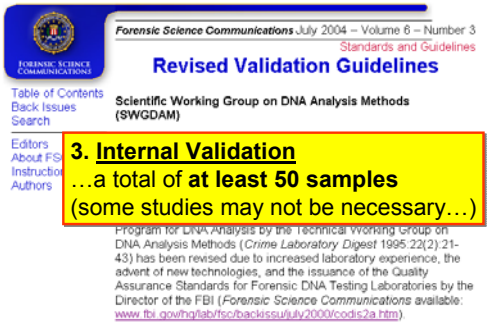
(c) Define the limitations of the procedure.

The validation process identifies aspects of a procedure that are critical and must be carefully controlled and monitored.

**Reliability, Reproducibility, Robustness, Range**

## 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.**

Validation Standardization Questionnaire (conducted June-August 2004)

## Can Validation be Standardized?

Statements from survey responders…

Over 86% (45/52) said yes

Those who responded "no" said

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

**Our Conclusion…**

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

## A Thoughtful Comment from One Interviewee

Before a set of validation experiments is performed…

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

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

## 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

## How an Assay Evolves

NIJ-funded project or company efforts

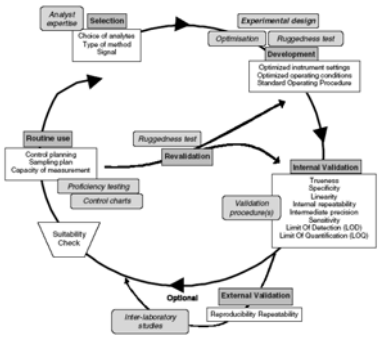
Performed by manufacturer

- Research
- Development
- Optimization

**Performed by forensic lab**

- Pre-Validation — Learning what questions to ask
- Validation — Performance Check (Kit QC or Following Instrument Repair)
- Re-Validation
- Implementation — Writing SOP, Training Others and Going "On-Line"

## The lifecycle of a method of analysis




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

## Steps Surrounding "Validation" in a Forensic Lab

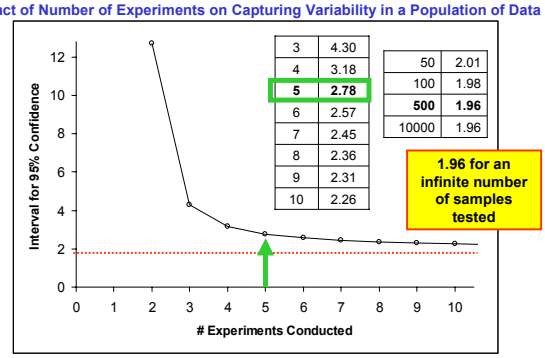
**Effort to Bring a Procedure "On-Line"**

**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

## 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**

## From ***The HitchHiker's Guide to the Galaxy***

http://www.bbc.co.uk/dna/h2g2/

**The Answer to the Ultimate Question Of Life, The Universe, And Everything**

**(and the Minimum Number of Samples for Internal Validation?)**

➢**42** +8 = **50** (SWGDAM Revised Validation Guidelines)

Validation Standardization Questionnaire (conducted June-August 2004)

## Survey Summary of Planned Near-term "Validation"

Commercial Kits

*Extraction*
- DNA IQ
- Qiagen
- Biomek 2000

*DNA Quant*
- **Quantifiler**

*STR Amp Kits*
- Identifiler
- PowerPlex Y
- Yfiler
- PowerPlex 16
- ProPlus/COfiler reduced volume

Software
- **GeneMapper*ID***
- GeneScan/ Genotyper NT
- TrueAllele
- SQL*LIMS and Forensic Solution

Analysis Instruments
- **ABI 3100 Avant**
- ABI 3100
- FMBIO III+
- MegaBACE

*For RT-PCR*
- **ABI 7000**
- Stratagene RT-PCR

Internal Validation

## 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**

Internal Validation

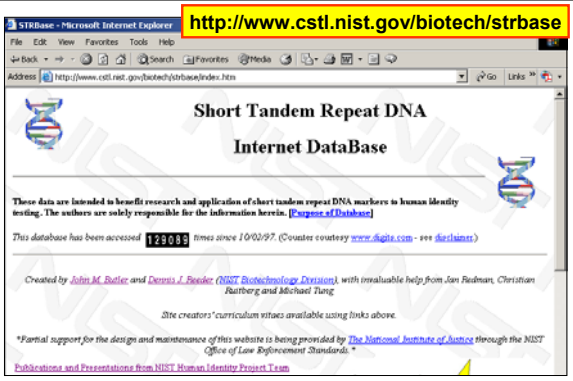
## Example: ABI 3100*Avant*

- Evaluation of a new ABI 3100*Avant* 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)**

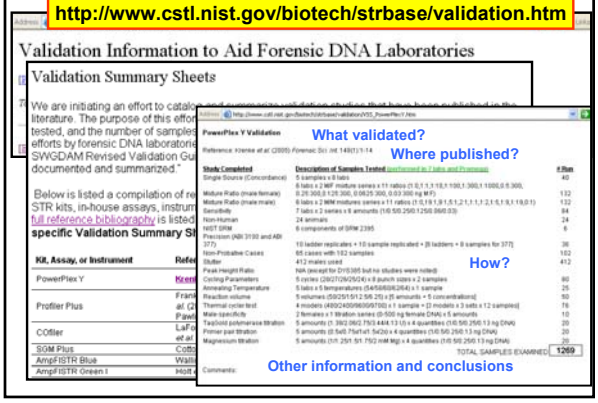
http://www.cstl.nist.gov/biotech/strbase




***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

## Summary of Literature Examined

Reported Developmental Validation Efforts

Numbers of Samples Run in Developmental Validation St...

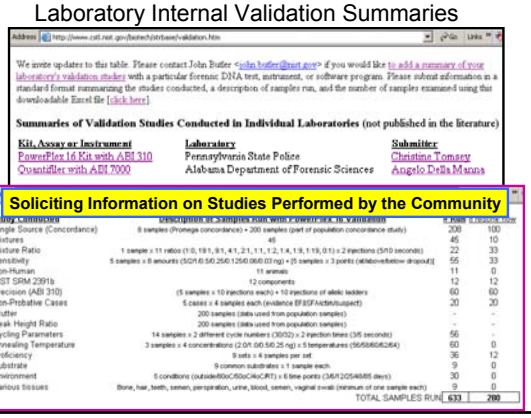
| Kit | Reference | Sensitivity | Precision | Stutter | Mixture | ases |
|---|---|---|---|---|---|---|
| PP16 | | | | | | |
| Profiler Plus | | | | | | |
| Cofiler | | | | | | |
| Identifiler | | | | | | |
| SGM Plus | | | | | | |
| PP1.1 | | | | | | |
| PP2.1 | | | | | | |
| PP 16 BIO | | | | | | |
| PP... | | | | | | |
| Sef... | | | | | | |
| Powe... | | | | | | |
| Y-PLEX... | | | | | | |
| Y-PLEX 5 | | | | | | |
| Y-PLEX 12 | | | | | | |
| Yfiler | | | | | | |

Information will be posted on new STRBase Validation Homepage http://www.cstl.nist.gov/biotech/strbase/validation.htm

**A total of 64 papers examined**

Full list of forensic DNA literature reviewed is available on STRBase

## Laboratory Internal Validation Summaries

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- Robin Cotton (Orchid Cellmark)
- Tim McMahon (AFDIL)

- **Many members of forensic DNA typing community for their valuable input on our validation questionnaire**

# Interlaboratory Studies

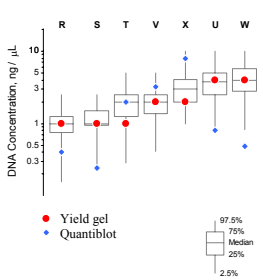
DNA Quantitation (2004),
Mixture Interpretation (2005)

## NIST Initiated Interlaboratory Studies

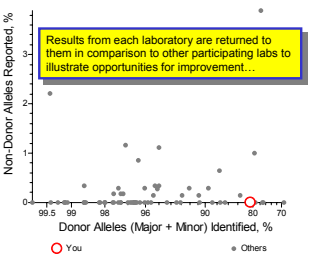
| Studies involving STRs | # Labs | Publications |
|---|---|---|
| Evaluation of CSF1PO, TPOX, and TH01 | 34 | Kline MC, Duewer DL, Newall P, Redman JW, Reeder DJ, Richard M. (1997) Interlaboratory evaluation of STR triplex CTT. *J. Forensic Sci.* 42: 897-906 |
| Mixed Stain Studies #1 and #2 (Apr–Nov 1997 and Jan–May 1999) | 45 | Duewer DL, Kline MC, Redman JW, Newall PJ, Reeder DJ. (2001) NIST Mixed Stain Studies #1 and #2: interlaboratory comparison of DNA quantification practice and short tandem repeat multiplex performance with multiple-source samples. *J. Forensic Sci.* 46: 1199-1210 |
| MSS3 Mixed Stain Study #3 (Oct 2000-May 2001) | 74 | Kline, M.C., Duewer, D.L., Redman, J.W., Butler, J.M. (2003) NIST mixed stain study 3: DNA quantitation accuracy and its influence on short tandem repeat multiplex signal intensity. *Anal. Chem.* 75: 2463-2469.<br>Duewer, D.L., Kline, M.C., Redman, J.W., Butler, J.M. (2004) NIST Mixed Stain Study #3: signal intensity balance in commercial short tandem repeat multiplexes, *Anal. Chem.* 76: 6928-6934. |
| DNA Quantitation Study (Jan-Mar 2004) QS04 | 80 | Kline, M.C., Duewer, D.L., Redman, J.W., Butler, J.M. (2005) Results from the NIST 2004 DNA Quantitation Study, *J. Forensic Sci.* 50(3):571-578 |
| MIX05 Mixture Interpretation Study (Jan-Mar 2005) | **64** | ***Data analysis currently on-going ...*** Will be presented at NIJ Grantees and SWGDAM (June 2005) and ISFG (Sept 2005) |

## Individual Performance in an Interlaboratory Study

DNA Quantitation




Accuracy in STR Typing




Results from each laboratory are returned to them in comparison to other participating labs to illustrate opportunities for improvement…

See Kline, M.C., *et al.* (2003) *Anal. Chem.* 75: 2463-2469

## NIST Quantitation Study 2004 (QS04)

Consisted of:
- 8 DNA extracts labeled A – H
- Shipped Dec 2003 –Jan 2004 to 84 laboratories for quantification; data received back by April 2004
- Labs were requested to use multiple methods / multiple analysts

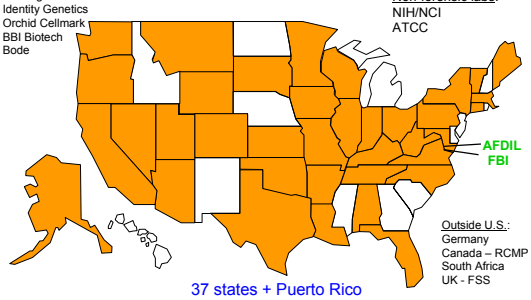
We received data from 80 Labs (95%)
**Total of 287 sets of data**
Participants used 19 different quantification methods (primarily variations on Quantiblot and Real-time PCR)

Information from this interlab study is being used to help construct SRM 2372 (Human DNA Quantitation Standard)

## Participation in NIST Interlaboratory Study on DNA Quantitation (QS04)

Companies:
Applied Biosystems
Promega
Identity Genetics
Orchid Cellmark
BBI Biotech
Bode

Non-forensic labs:
NIH/NCI
ATCC




Outside U.S.:
Germany
Canada – RCMP & CFS
South Africa
UK - FSS

37 states + Puerto Rico

84 laboratories were sent samples (80 returned results)

## 8 DNA Samples in This NIST Study


*Laboratories are only being asked to provide their quant values (no typing results expected)*

Mixed source DNA

Single source DNA

Teflon tube

Volume of each DNA sample provided = 100 µL

Table 2. The percent success rate reported for a sample.

| | | % Quantitative Results[a] | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Target [DNA] ng/µL | | 1.5 | 0.5 | 0.5 | 0.16 | 0.16 | 0.05 | 0.05 | 0.05 |
| Method | $N_{eval}$ | A | B | E | C | F | D | G | H |
| Quantifiler | 37 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| Other RT-PCR | 23 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| "ACES" | 14 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| AluQuant | 13 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| PicoGreen | 12 | 100 | 100 | 92 | 100 | 100 | 92 | 83 | 83 |
| ECL | 75 | 100 | 99 | 99 | 93 | 95 | 84 | 77 | 87 |
| TMB | 98 | 100 | 100 | 99 | 93 | 94 | 59 | 62 | 63 |
| Yield gel | 14 | 57 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 286 | | | | | | | | |

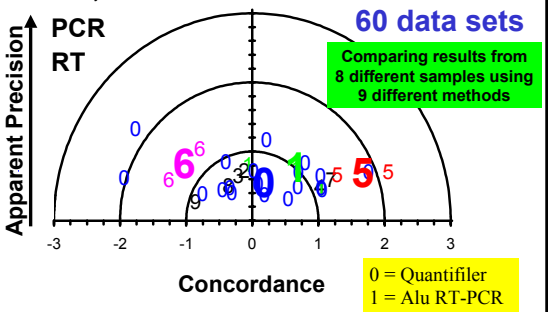
a Quantitative results are those that were reported as values between contiguous calibration standards, values reported standard if smaller than the target [DNA], or values repo calibration standard if larger than the target[DNA].

At least one lab used poor performance of their Quantiblot with low level samples to justify purchase of qPCR instrumentation and conversion to Quantifiler kit DNA quantitation

Kline, *et al., J. Forensic Sci.* 50(3): 571-578

## Interlaboratory Comparisons

Laboratory Performances with Real-Time PCR Methods




Kline, *et al.* (2005) *J. Forensic Sci.* 50(3):571-578

## Real-time qPCR Work at NIST

- Careful examination of published assays on the same set of DNA samples
- Lot-to-lot variability with Quantifiler "standard"
  - *qPCR is a relative measurement that depends on the quality of the material used to generate the standard curve*

## Variability of Quantifiler DNA Standards

Two lots of ABI "standards" using Quantifiler Human assay

| Sample (n = 4) | Standard Lot 1 (ng/mL) | Standard Lot 2 (ng/mL) |
|---|---|---|
| 1 | 4* | 2.91 ± 0.04 |
| 2 | 7.26 ± 0.79 | 4* |
| 3 | 2.93 ± 0.27 | 1.88 ± 0.09 |
| 4 | 3.46 ± 0.30 | 2.22 ± 0.08 |
| 5 | 2.99 ± 0.28 | 1.91 ± 0.08 |
| 6 | 2.62 ± 0.22 | 1.70 ± 0.03 |

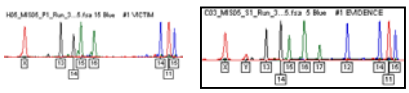
* - indicates "standard" value based on starting material provided by the manufacturer

Samples 1-3 = commercially available kit standards

Samples 4-6 = in-house standards based on UV absorbance

## Mixture Interpretation Interlab Study (MIX05)

- **Only involves interpretation of data**
- **91 labs enrolled** for participation (20 from overseas)
- **64 labs have returned results**
- Four mock cases supplied with "victim" and "evidence" electropherograms (GeneScan .fsa files – that can be converted for Mac or GeneMapper; gel files made available to FMBIO labs)
- Data available with Profiler Plus, COfiler, SGM Plus, PowerPlex 16, Identifiler, PowerPlex 16 BIO (FMBIO) kits
- Summary of results will involve training materials to illustrate various approaches to solving mixtures



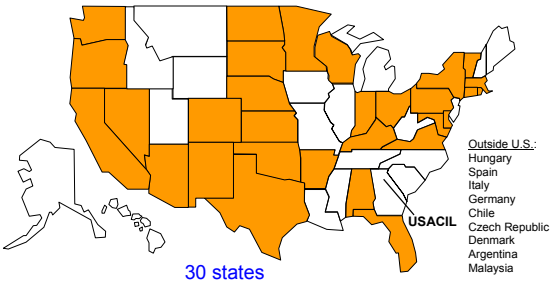
Perpetrator Profile(s) ??

Along with reasons for making calls and any stats that would be reported

## Participation in NIST Interlaboratory Study on Mixture Interpretation (MIX05)

Companies:
Myriad Genetics

**20 labs outside of U.S.** signed up

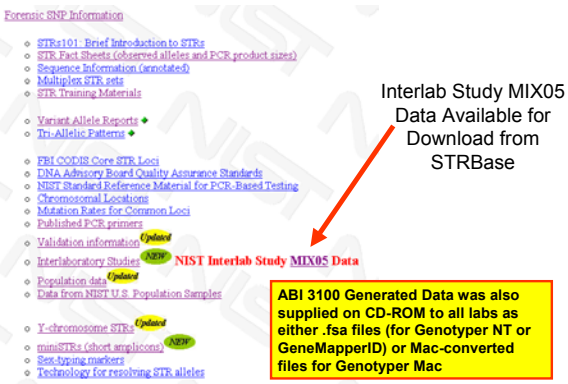



30 states

Outside U.S.:
Hungary
Spain
Italy
Germany
Chile
Czech Republic
Denmark
Argentina
Malaysia

91 laboratories signed up for study (64 returned results so far)

## http://www.cstl.nist.gov/biotech/strbase/interlab/MIX05.htm




Interlab Study MIX05 Data Available for Download from STRBase

**ABI 3100 Generated Data was also supplied on CD-ROM to all labs as either .fsa files (for Genotyper NT or GeneMapperID) or Mac-converted files for Genotyper Mac**

## MIX05 Results on Multiple Kits

**Case 1 evidence (mixture)**