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

Validation Workshop

Introduction to the DNA Advisory Board (DAB) Standards

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

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

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)

Brief Historical Overview

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

CURRENT EVENTS

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

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

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

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DNA Identification Act (1994)
Public Law 103-322

42 § 14131. Quality assurance and proficiency testing standards
(a) Publication of quality assurance and proficiency testing standards

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

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

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

DNA Advisory Board (DAB)

DNA Advisory Board (DAB) Members

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

DAB Standards

U.S. Department of Justice
Federal Bureau of Investigation

FORENSIC SCIENCE COMMUNICATIONS
July 2000 Volume 2 Number 3

Quality Assurance Standards for Forensic DNA Testing Laboratories

DNA Advisory Board
July 1998

(Part 1 of 2)

CURRENT ISSUE
BACK ISSUES
SEARCH ALL ISSUES

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

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

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

Outline of DAB Standards

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

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

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

8.1.1 Developmental validation that is conducted shall be appropriately documented.

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

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

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

8.1.2.3 Population distribution data are documented and available.

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

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

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

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

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

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

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

FORENSIC SCIENCE COMMUNICATIONS   JULY 2000   VOLUME 2   NUMBER 3

Developmental Validation Overview

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Locus Definition and Characterization

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

Developmental Validation Studies

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

Population Data and Independence Testing

8.1.2.3 Population distribution data are documented and available.

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Reproducibility and Precision

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

Match Criteria

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

Qualifying Test

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

Material Modifications

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

9. ANALYTICAL PROCEDURES

STANDARD 9.1 The laboratory shall have and follow written analytical procedures approved by the laboratory management/technical manager.

9.1.1 The laboratory shall have a standard operating protocol for each analytical technique used.

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

9.1.3 The laboratory shall have a procedure for differential extraction of stains that potentially contain semen.

FORENSIC SCIENCE COMMUNICATIONS   JULY 2000   VOLUME 2   NUMBER 3

Suitable Reagents

STANDARD 9.2 The laboratory shall use reagents that are suitable for the methods employed.

9.2.1 The laboratory shall have written procedures for documenting commercial supplies and for the formulation of reagents.

9.2.2 Reagents shall be labeled with the identity of the reagent, the date of preparation or expiration, and the identity of the individual preparing the reagent.

9.2.3 **The laboratory shall identify critical reagents** *(if any)* and evaluate them prior to use in casework. These critical reagents include but are not limited to: *(THIS LAST PORTION NOT IN CONVICTED OFFENDER DATABASING STANDARDS)*
- (a) Restriction enzyme
- (b) Commercial kits for performing genetic typing
- (c) Agarose for analytical RFLP gels
- (d) Membranes for Southern blotting
- (e) K562 DNA or other human DNA controls
- (f) Molecular weight markers used as RFLP sizing standards
- (g) Primer sets
- (h) Thermostable DNA polymerase

Human DNA Quantitation

STANDARD 9.3 The laboratory shall have and follow a procedure for **evaluating the quantity of the human DNA in the sample where possible.** *(NOT IN CONVICTED OFFENDER DATABASING STANDARDS)*

9.3.1 For casework RFLP samples, the presence of high molecular weight DNA should be determined.

Appropriate Controls and Standards

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

9.4.1 The following controls shall be used in RFLP casework analysis:

9.4.1.1 Quantitation standards for estimating the amount of DNA recovered by extraction. *(When required by the analytical procedure, standards for estimating the amount of DNA recovered by extraction shall be used.)*

9.4.1.2 K562 as a human DNA control. (In monitoring sizing data, a statistical quality control method for K562 cell line shall be maintained.)

9.4.1.3 Molecular weight size markers to bracket known and evidence samples. *(Molecular weight size markers to bracket samples on an analytical gel: No more than five lanes shall exist between marker lanes.)*

9.4.1.4 A Procedure *shall be available* to monitor the completeness of restriction enzyme digestion. *(Interpretation of the autorad/lumigraph is the ultimate method of assessment but a test gel or other method may be used as necessary.)*

9.4.2 The following controls shall be used for PCR casework analysis *(database analysis)*:

9.4.2.1 **Quantitation standards**, which estimate the amount of human nuclear DNA recovered by extraction. *(When required by the analytical procedure, standards which estimate the amount of human nuclear DNA recovered by extraction shall be used.)*

9.4.2.2 **Positive and negative amplification controls**.

9.4.2.3 **Reagent blanks**. *(Contamination controls.)*
9.3.2.3.1 Samples extracted prior to the effective date of these standards without reagent blanks are acceptable as long as other samples analyzed in the batch do not demonstrate contamination.

9.4.2.4 **Allelic ladders and/or internal size makers** for variable number tandem repeat sequence PCR based systems.

Traceability to NIST Standard Reference Material

STANDARD 9.5 The laboratory shall check its DNA procedures annually or whenever substantial changes are made to the protocol(s) against an appropriate and available NIST standard reference material or standard traceable to a NIST standard.

Written Guidelines for Data Interpretation

STANDARD 9.6 The laboratory shall have and follow written general guidelines for the interpretation of data.

9.6.1 The laboratory shall verify that **all control results are within established tolerance limits**.

9.6.2 Where appropriate, visual matches shall be supported by a numerical match criterion. *(NOT IN CONVICTED OFFENDER DATABASING STANDARDS)*

9.6.3 For a given population(s) and/or hypothesis of relatedness, the **statistical interpretation** shall be made following the recommendations 4.1, 4.2 or 4.3 as deemed applicable of the National Research Council report entitled "The Evaluation of Forensic DNA Evidence" (1996) and/or court directed method. These calculations shall be derived **from a documented population database appropriate for the calculation**. *(NOT IN CONVICTED OFFENDER DATABASING STANDARDS)*

10. EQUIPMENT CALIBRATION AND MAINTENANCE

STANDARD 10.1 The laboratory shall use equipment suitable for the methods employed.

FORENSIC SCIENCE COMMUNICATIONS   JULY 2000   VOLUME 2   NUMBER 3

Instrument Calibration

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

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

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

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

Instrument Maintenance

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

10.3.1 New *(critical)* instruments and equipment, or *(critical)* instruments and equipment that have undergone repair or maintenance, shall be calibrated before being used in casework analysis.

10.3.2 **Written records or logs shall be maintained for maintenance service performed on instruments and equipment**. Such documentation shall be retained in accordance with applicable Federal or state law.

Revised SWGDAM Validation Guidelines
(July 2004)

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

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

FORENSIC SCIENCE COMMUNICATIONS

Table of Contents
Back Issues
Search

Editors
About FSC
Instructions for
Authors

Scientific Working Group on DNA Analysis Methods (SWGDAM)

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

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

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

Previous Guidelines Regarding Validation

Technical Working Group on DNA Analysis Methods (TWGDAM)

- TWGDAM (1995) – *Crime Lab Digest* 22(2):20-43
  – Budowle *et al.* "Guidelines for a quality assurance program for DNA analysis"

- TWGDAM (1991) – *Crime Lab Digest* 18(2):44-75
  – Kearney *et al.* "Guidelines for a quality assurance program for DNA analysis"

- TWGDAM (1989) – *Crime Lab Digest* 16(2):40-59
  – Kearney *et al.* "Guidelines for a quality assurance program for DNA restriction fragment length polymorphism analysis"

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

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

Differences between 1991 and 1995 TWGDAM Guidelines

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

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

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

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

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

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

Differences between 1991 and 1995 TWGDAM Guidelines

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

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

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

Comparison of DAB Standards and Previous Validation Guidelines

DNA Loci

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

PCR Considerations

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

Comparison of DAB Standards and Previous Validation Guidelines

Developmental Validation

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

Comparison of DAB Standards and Previous Validation Guidelines

Internal Validation

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