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

Validation Workshop

Internal Validation

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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)

Overview of This Section

- Revisit each standard from the DAB standards
- Discuss the Revised Validation Guidelines and what they really mean
- Present examples of internal validation studies performed for each standard
- Discuss how to appropriately document internal validation studies
- Discuss what to do if upon implementation of the newly validated procedure, issues arise

DNA Advisory Board Standards (*Forensic Sci. Comm.* July 2000)

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.

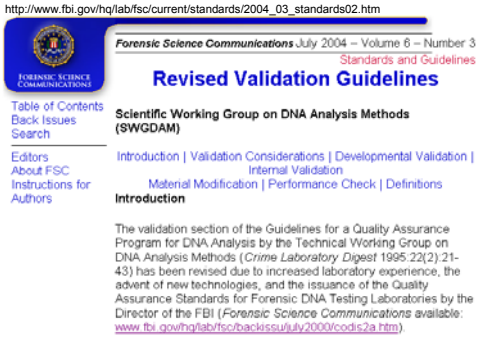
8.1.3.4 Material modifications made to analytical procedures shall be documented and subject to validation testing.

Quality Assurance Audit For Forensic DNA and Convicted Offender DNA Databasing Laboratories (*Forensic Sci. Comm.* July 2004)

		Yes	No	N/A
8.1.3.1(a)	Has the procedure been tested using known and non-probative evidence samples?	___	___	___
8.1.3.1 (CO-a)	Has the procedure been tested using known samples?	___	___	___
8.1.3.1(b)	Has the reproducibility and precision of the procedure been monitored and documented using human DNA control(s)?	___	___	___
8.1.3.2(FO)	Based on empirical data, have match criteria been established and documented?	___	___	___
8.1.3.3	Has the analyst or examination team successfully completed a qualifying test using the DNA analysis procedure prior to its incorporation into casework or database applications? (CO8.1.3.2)	___	___	___
8.1.3.4	Have material modifications to analytical procedures been documented and subjected to validation testing?	___	___	___
8.1.4(FO)	If methods are not specified, does the laboratory, wherever possible, select methods that have been published by reputable technical organizations or in relevant scientific texts or journals or that have been appropriately evaluated for a specific or unique application?	___	___	___

Revised SWGDAM Validation Guidelines
(July 2004)

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



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

Validation per Revised Validation Guidelines

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.

Revised Validation Guidelines

1.2.1 **Developmental** validation is the demonstration of the ***accuracy, precision,*** and ***reproducibility*** of a procedure by the manufacturer, technical organization, academic institution, government laboratory, or other party. Developmental validation must precede the the use of a novel methodology for forensic DNA analysis.

Revised Validation Guidelines

1.2.2 **Internal** validation is conducted by each forensic DNA testing laboratory and is the in-house demonstration of the ***reliability*** and ***limitations*** of the procedure. Prior to using a procedure for forensic applications, a laboratory must conduct internal validation studies.

Who should perform such studies?

How do you go about determining what studies are necessary?

Who approves the final product?

Revised Validation Guidelines

1.2.2.1 Internal validation studies must be sufficiently documented and summarized.

How are these studies documented?

What format should be used for the summary?

What documentation needs to be retained?

Revised Validation Guidelines

1.2.2.2 Internal validation should lead to the establishment of documented quality assurance parameters and interpretation guidelines.

Example: In the validation of Quantifiler and ABI 7000, the expected value for the CT for the IPC should be determined. The allows for assessment as to the performance of a sample relative to amplification.

Example: In determining guidelines for mixture interpretations, mixtures of known samples are diluted at known concentrations to determine the thresholds at which major and minor contributors may be determined.

Revised Validation Guidelines

1.2.2.3 Satellite laboratories must perform an internal validation independent of the main laboratory. Performance-based tests must be completed and documented for each laboratory location, whereas basic validation data may be shared by all locations in a laboratory system.

For implementation of a new robotic extraction platform, which studies would need to be performed by the "main lab" and which should be performed by the "satellite lab"?

Revised Validation Guidelines

1.2.2.4 A complete change of detection platform or commercial kit requires an internal validation.

What is a complete change of detection platform?
- *Gel-based to capillary based?*

What is a complete change in commercial kit?
- *Profiler Plus and CoFiler to PowerPlex 16?*

Revised Validation Guidelines Additions

3.0 The internal validation process includes the studies detailed below *(following slides)* encompassing a total of at least **50** samples. Some studies may not be necessary due to the method itself.

Can the same samples be used to cover different studies in the same validation? What about other validations?

3.1 Known and non-probative evidence samples: ***The method must be evaluated and tested using known samples and, when possible, authentic case samples; otherwise, simulated case samples should be used. DNA profiles obtained from questioned items should be compared to those from reference samples. When previous typing results are available, consistency as to the inclusion or exclusion of suspects or victims within the limits of the respective assays should be assessed.***

- Known samples
- Authentic case samples *or*
- *Simulated case samples*
- Use previous data

Why do we do this? *To show that the technique works in our hands*

3.1 Known and non-probative evidence samples:

- **Profiler Plus validation (JFS 2001) :** Analyzed nineteen non-probative cases that included blood standards for comparison to semen stains or bloodstains. Nine of these were previously analyzed in PM and D1280.
- **PowerPlex 2.1 validation (JFS 2002):** Analyzed eleven proficiency tests as well as thirty samples for which previous PowerPlex 1.1 data was available as well as thirty-two cases for which previous RFLP, CTT or PowerPlex 1.1 data was available.
- **Identifiler Validation (Internal 2004):** Analyzed ten known samples of lab employees on 310 and 3100 genetic analyzers and compared results. Also analyzed nine cases and compared to the original case conclusions.

3.1 Known and non-probative evidence samples:

- **DNA extraction with DNA IQ (Internal 2003):** Twenty-four sets of body fluids (blood, semen, saliva, and vaginal fluid) as well as hair (n=12) from known individuals were extracted. All gave the expected results following DNA analysis demonstrating that the technique worked on the commonly seen samples in DNA. Mixed samples (post-coital) as well as samples applied to a variety of substrates were also extracted and demonstrated the expected results following DNA analysis.
- **3100 Validation (Internal 2003):** Thirty-four known samples were analyzed and compared to the previous platform.

3.1 Known and non-probative evidence samples:

- **Quantifiler Validation (Internal 2004):** Eleven samples were quantitated and compared with previous QF results. Also participated in the NIST Quantitation study (8 additional samples). All samples were amplified with Identifiler and analyzed on a 310.
- **Quantifiler Validation (Internal 2004):** Fifty two samples quantitated in Quantifiler, Quantiblot and AluQuant, amplified in PP/CF and analyzed on a 310 or 3100.

3.1 Known and non-probative evidence samples:

- **GMID Validation (Internal 2005):** One thousand twenty-six samples were analyzed and compared to GS/GT results.

Why such a large number when only 50 required?

3.2 Reproducibility and precision: *The laboratory must document the reproducibility and precision of the procedure using an appropriate control(s).*

What are these?

Reproducibility is being able to obtain the same results under the same conditions
- the IPC in QF or the allelic ladder used in STR analysis

Precision is the "tightness" or closeness of the results
- the range of the CT for the IPC of the base pair size of the alleles in the allelic ladder

You need a method that will give you the same result consistently with the same level of "tightness"

3.2 ***Reproducibility*** and precision:

- **Profiler Plus validation (JFS 2001) :** Interlaboratory reproducibility was assessed by analyzing fifty samples at two different sites; compared ten samples separated by gel electrophoresis versus capillary electrophoresis; evaluated results from twenty samples extracted organically and non-organically.
- **PowerPlex 2.1 validation (JFS 2002):** Concordance studies with 100 convicted offender samples and analyzed at four different sites *(one site only analyzed 25 samples)* . Also compared results of 25 of the samples with results obtained with Profiler Plus and Cofiler at a fifth site.
- **Identifiler Validation (Internal 2004):** Twenty samples of control 9974A were separately amplified at 1 ng target DNA and analyzed on 3 separate days.

3.2 ***Reproducibility*** and precision:

- **DNA extraction with DNA IQ (Internal 2003):** Same sample set as the known samples. Also, neat blood samples extracted under the same parameters yielded equivalent quantitation results.
- **3100 Validation (Internal 2003):** Same single source samples utilized for ***3.1 Known and non-probative evidence samples.*** Each of thirty-four samples was injected independently on each of the 16 capillaries.

3.2 ***Reproducibility*** and precision:

- **Quantifiler Validation (Internal 2004):** A sample of K562 was diluted from 2 ng/ul to 0.06 ng/ul and quantitated in replicates of 4 (or more) by two separate analysts on two separate days for at least 3 runs. Select samples from the reproducibility study were amplified and the average peak heights determined.
- **Quantifiler Validation (Internal 2004):** Twenty single source samples were quantified on three different days. Each of the twenty samples was also quantified in triplicate on a single run. Male: female mixtures were also prepared and quantitated in triplicate *(one time in duplicate)* over several days. (Same samples as precision samples)

3.2 ***Reproducibility*** and precision:

- **GMID Validation (Internal 2005):** Positive control samples from Profiler Plus and CoFiler demonstrated the expected results over numerous runs on numerous days from several different capillary electrophoresis platforms from 6 different labs.

3.2 Reproducibility and ***Precision***:

- **Profiler Plus validation (JFS 2001) :**
  - **Precision of allele determination:** Five known samples were injected twenty times and the base pair size and genotype data collected for one allele at each locus. Sizing data was also collected for the first allele of the allelic ladder for D3, amelogenin and D5 from 100 allelic ladder runs.
  - **Precision of relative peak height:** Used samples from reproducibility, stutter and above precision studies were used to determine the average heterozygote peak height ratio.

3.2 Reproducibility and ***Precision***:

- **PowerPlex 2.1 validation (JFS 2002):** Not discussed
- **Identifiler Validation (Internal 2004):** Twenty samples of control 9974A were separately amplified at 1 ng target DNA and analyzed on 3 separate days. Each of the samples was re-injected throughout the three runs and base pair size determinations conducted.

3.2 Reproducibility and ***Precision***:

- **DNA extraction with DNA IQ (JFS 2004):** Same as reproducibility samples
- **3100 Validation (Internal 2003):** Profiler Plus and Cofiler ladders were injected numerous times (Profiler Plus 944 injections and Cofiler 1600 injections) and the average base pair size for each allele determined and from that the mean for each locus as well as standard deviation determined. ***Note: The average base pair size from the previous samples utilized in the reproducibility study may also have been used.***

3.2 Reproducibility and ***Precision***:

- **Quantifiler Validation (Internal 2004):** A set of 8 standard dilutions of Quantifiler human DNA standards was made ranging in concentrations of 50 ng to 0.023 ng. These were run in 3 separate plates on 2 separate days. The CT values were complied, averages and SD determined. Also, the CT values for 330 IPCs were complied, averaged, and the SD determined.
- **Quantifiler Validation (Internal 2004):** Twenty single source samples were quantified on three different days. Each of the twenty samples was also quantified in triplicate on a single run. Male: female mixtures were also prepared and quantitated in triplicate *(one time in duplicate)* over several days. (Same samples as reproducibility samples)

3.2 Reproducibility and ***Precision***:

- **GMID Validation (Internal 2005):** Positive control samples from Profiler Plus and CoFiler demonstrated the expected results over numerous runs on numerous days from several different capillary electrophoresis platforms from 6 different labs. Also, the one thousand plus samples yielded concordant allelic calls when compared to results obtained with the previous analysis software. These samples were also run on numerous days from several different capillary electrophoresis platforms from 6 different labs.

What does this tell us relative to algorithms used to define a peak? About stutter filters? Allelic bins?

3.3 Match criteria: ***For procedures that entail separation of DNA molecules based on size, precision of sizing must be determined by repetitive analyses of appropriate samples to establish criteria for matching or allele designation.***

What does that mean?????

Concerns procedures that involve DNA separation

- need to determine the precision of that separation
- the reliability of the separation

Why?????

- so that the criteria used for matching alleles (to the allelic ladder) or determining an allelic designation are sound.

3.3 Match criteria:

- **Profiler Plus validation (JFS 2001) :** Data is addressed in the precision study
- **PowerPlex 2.1 validation (JFS 2002):** Not addressed
- **Identifiler Validation (Internal 2004):**Data is addressed in the precision study
- **DNA extraction with DNA IQ (Internal 2003):** Not addressed
- **3100 Validation (Internal 2003):**Data is addressed in the precision study
- **Quantifiler Validation (Internal 2004):** Not applicable
- **Quantifiler Validation (Internal 2004):** Not applicable
- **GMID Validation (Internal 2005):** Same 1000+ samples utilized.

3.4 Sensitivity and stochastic studies: *The laboratory must conduct studies that ensure the reliability and integrity of results. For PCR-based assays, studies must address stochastic effects and sensitivity levels.*

- Must determine the sensitivity of the method being validated to ensure reliability and integrity of the results -
- If the method is a PCR-based assay, you must determine how (if) stochastic effects and sensitivity levels have an affect on your data.

Why?????

so that you know the limits of the method being validated

Only related to low level samples? What happens in STR amplification if a sample is seriously overloaded? Does this correlate to RT PCR? What about extraction methods like magnetic bead technology?

3.4 Sensitivity and stochastic studies:

- **Profiler Plus validation (JFS 2001) :** Prepared dilutions from 10 ng to 36 pg, amplified the samples and ran on 3 separate 310s. Also examined injection times ranging from five to twenty seconds on samples containing 0.6 ng to 36 pg of input DNA.
- **PowerPlex 2.1 validation (JFS 2002):** Prepared dilutions ranging from 25 ng down to 0.03125 ng, amplified samples and analyzed using gel electrophoresis.
- **Identifiler Validation (Internal 2004):** Nine samples of 9947A were amplified in duplicate by 2 separate analysts in concentrations ranging from 0.0125 to 1 ng and analyzed at 50 to 150 rfus.

3.4 Sensitivity and stochastic studies:

- **DNA extraction with DNA IQ (Internal 2003):** Extracted blood dilutions from neat to $1x10^{-4}$ in triplicate to determine the sensitivity of the extraction method. Also varied the elution volume. Also extracted timed mock sexual kits to determine the limits of detecting sperm in a mixed sample.
- **3100 Validation (Internal 2003):** Samples from known sources (volunteers or positive controls) were quantitated and amplified in PP and/or CF targeting 0.06 to 2 ng of input DNA.

3.4 Sensitivity and stochastic studies:

- **Quantifiler Validation (Internal 2004):** Not addressed
- **Quantifiler Validation (Internal 2004):** Profiler Plus positive control was diluted from neat to 1:200. Also quantitated dilutions of DNA extracted from saliva, bloodstains and semen with various extraction methods. Also tested approximately 85 reagent blanks from previous training and proficiency tests as well as low level and high level samples and inhibited samples
- **GMID Validation (Internal 2005):** Not addressed

3.5 Mixture studies: *When appropriate, forensic casework laboratories must define and mimic the range of detectable mixture ratios, including detection of major and minor components. Studies should be conducted using samples that mimic those typically encountered in casework (e.g., post-coital vaginal swabs).*

Labs need to look at how mixtures affect results and need to design mixture interpretation guidelines based on these studies. These guidelines need to be utilized in casework.

What would be some good samples to use to help define your mixture guidelines?

3.5 Mixture studies:

- **Profiler Plus validation (JFS 2001) :** Two samples were mixed together at known proportions (1:200, 1:100, 1:20, 1:10, 1:2, and 1:1) to determine the ratio at which the major and minor components of a mixture could be resolved. Amplified 2 ng of target DNA
- **PowerPlex 2.1 validation (JFS 2002):** Preparations of a series of DNA:DNA ratios from already quantified samples were utilized as well as mixtures of body fluids in known volumes prior to DNA extraction and quantification. Amplified 1 ng of target DNA.

3.5 Mixture studies:

- **Identifiler Validation (Internal 2004):**
  - Peak Height ratio study: Ten single source samples were amplified in duplicate and analyzed
  - Five second injection study: Two known DNA samples (male and female) were mixed in a variety of ratios and injected for 5 seconds
  - Nine second injection study: same as above
- **DNA extraction with DNA IQ (Internal 2003):** Extracted 4 timed mock sexual assault kits to determine when the male component of the mixture could no longer be determined.
- **3100 Validation (Internal 2003):** Prepared 2 sets of mixtures from 1:1 to 1:16 with male and female major components.

3.5 Mixture studies:

- **Quantifiler Validation (Internal 2004):** Not performed
- **Quantifiler Validation (Internal 2004):** Female to male mixtures were made utilizing various body fluids and quantitated in both total human and total Y to determine the lowest amount of male DNA that could still be amplified and detected in the presence of female DNA (total DNA)
- **GMID Validation (Internal 2005):** Looked at numerous mixtures and compared results to those obtained in previous analysis with GenoTyper.

3.6 Contamination: *The laboratory must demonstrate that its procedures minimize contamination that would compromise the integrity of the results. A laboratory should employ appropriate controls and implement quality practices to assess contamination and demonstrate that its procedure minimizes contamination.*

Demonstrate that procedures minimize this -

HOW?????

Use of accepted controls and established procedures.

The accepted controls must consistently yield the expected results.

3.6 Contamination:

- **Profiler Plus validation (JFS 2001) :** Not discussed
- **PowerPlex 2.1 validation (JFS 2002):** Not discussed
- **Identifiler Validation (Internal 2003):** Although more instrument related that kit related, the lab put 9 sets of sample tubes in the sample tray for the 310 in a set pattern with some containing excessive size standard and injected in a specific order.
- **Automated extraction with DNA IQ (JFS 2004):** Use of appropriate controls (blanks) through out the validation study demonstrated no instances of contamination.

3.6 Contamination:

- **3100 Validation (Internal 2003):**
  - Mechanical carryover (carryover from one injection to the next): wells of positive controls were injected followed immediately by injection of blanks
  - Optical carryover (signal from one capillary being detected and associated with the adjacent capillary by the detection cell):wells of positive control injected adjacent to wells of blanks
- **Quantifiler Validation (Internal 2004):** Not discussed
- **Quantifiler Validation (Internal 2004):** Not discussed
- **GMID Validation (Internal 2005):** Not discussed

3.7 Qualifying test: ***The method must be tested using a qualifying test. This may be accomplished through the use of proficiency test samples or types of samples that mimic those that the laboratory routinely analyzes. This qualifying test may be administered internally, externally, or collaboratively.***

Test method in a hands on format -

like an old proficiency test

Written format? Laboratory format?

The audit document states that this can be either.

3.7 Qualifying test:

- **Profiler Plus validation (JFS 2001) :** Not discussed
- **PowerPlex 2.1 validation (JFS 2002):** Not discussed
- **Identifiler Validation (Internal 2004):** Analyzed a previously characterized external DNA proficiency test as well as NIST SRM 2391b.
- **DNA extraction with DNA IQ (Internal Validation 2003):** not discussed
- **3100 Validation (Internal 2003):** Analysts were required to run a set of previously characterized samples. Written examination also required.

3.7 Qualifying test:

- **Quantifiler Validation (Internal 2004):** Not discussed
- **Quantifiler Validation (Internal 2004):** Previously characterized samples were re-run and analyzed. Written test also required.
- **GMID Validation (Internal 2005):** Previously collected data was provided for analysis.

Other DAB Standards to Consider

9.1.1 The laboratory shall have an ***standard protocol*** for each analytical technique used.

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

9.2.3 The laboratory shall identify ***critical reagents*** (if any) and evaluate them prior to use in casework……

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

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

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

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

Documentation of Internal Validation Studies

What is the best way to do this? Standardized format?

Who needs to review?

Who needs to approve?

Should it be presented or published?

Implementation of the Newly Validated Procedure

Ok, the validation studies are complete and approved, the procedure is written and approved and the lab is ready to implement the new procedure into casework.

So, what about training?

Who needs to be trained and what is the extent of the training? How is the training documented? What constitutes completion of training? Per individual or per lab?

What if……...

What if……...

You had validated RT PCR such that you were able to drop "negative samples" like extraction blanks and low level samples that were below your detection level for your DNA analysis…….

What if……...

You had validated RT PCR such that you were able to drop "negative samples" such as extraction blanks and low level samples that were below your detection level for your DNA analysis…….

and now your "negative samples" were all showing the presence of low level DNA?

What if……...

You have validated Identifiler and in that validation determined that your target amount of DNA was 1 ng to obtain a complete profile…….

What if……...

You have validated Identifiler and in that validation determined that your target amount of DNA was 1 ng to obtain a complete profile……...

And now you are seeing numerous instances of overloaded samples

What if……..

You have validated Identifiler and in that validation determined that your target amount of DNA was 1 ng to obtain a complete profile…….

or you are now unable to obtain a complete profile at 1 ng of target DNA

What if……..

You had validated the 3100 with a minimum peak threshold of 100 rfus……..

What if……..

You had validated the 3100 with a minimum peak threshold of 100 rfus……..

but now you were seeing numerous artifacts greater than 100 rfus