

Is it time to change our approach to validation?



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There are many laboratory activities to validate...



- New STR kits
- CE instruments
- Quantification kits or assays
- Genotyping software
- Rapid DNA instrument
- DNA extraction robotic process
- Probabilistic genotyping software

From JMB-Validation Overview, 8-6-2014

Planning validations is crucial!

- You need a team- Users, QA, people with special knowledge or skills etc.
- You need a team leader
- Develop a detailed plan with specific questions
- Get input
 - What information should be developed first?
 - What questions can be researched at the same time?
- Review the plan & the questions.
- Ask: **Will the information developed inform the use of the procedure in the lab and provide structure of the SOP?**
 - If yes-move ahead
 - If no-adjust the plan
- If you are the Tech Leader and you are doing this by yourself- you **are** missing something.

Validation Guidance for Forensic DNA- these documents are very helpful

November 2010 ENFSI DNA Working Group Guidelines
http://www.enfsi.eu/sites/default/files/documents/minimum_validation_guidelines_in_dna_profiling_-_v2010_0.pdf

Recommended Minimum Criteria for the Validation of Various Aspects of the DNA Profiling Process

DOCUMENT TYPE :	REF. CODE:	ISSUE NO:	ISSUE DATE:
POLICY	ENFSI DNA WORKING GROUP	001	November 2010

December 2012 SWGDAM Guidelines

http://swgdam.org/SWGDAM_Validation_Guidelines_APPROVED_Dec_2012.pdf

Scientific Working Group on
DNA Analysis Methods
Validation Guidelines for
DNA Analysis Methods



From JMB-Validation
Overview, 8-6-2014

The Plan

- What type of validation is needed
 - Developmental
 - Internal
- What standards/guidelines apply?
- **What are “the questions”**
- **What are not “the questions”**
- What samples will be used?
- What quantities of DNA?
- How many individuals?
- Mixtures-yes/no?
- Is additional equipment or resources needed?
- The timeline
 - other people need to make decisions based on the validation and implementation.



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 - May contain validation summaries and/or provide references
 - Read those references

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 - Especially when procedure or instrument was originally used for other applications
- Read and track what has been done vs requirements.
 - If application was not originally forensic, there may be gaps to fill.
 - If originally a forensic application, are all requirements met?
 - For either situation are there important questions, that are not QAS requirements and need additional validation?

Equipment/software/supplies for validation (not used in casework)-

- **Spending money may save time**
- Nanodrop or other UV-Vis spectrophotometer
- Plate tracker if loading any plates by hand
- Hemocytometer for cell counting
- Stats software other than Excel-
 - JMP, SAS
 - Macros etc. from NIST
- Purchased DNA samples or body fluids
 - American Type Culture Collection
 - Coriell Cell Repositories

Efficiency in the order of experiments:

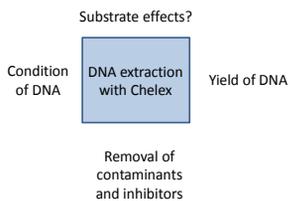
- New kits and instrument (or upgrade) happening simultaneously?
 - Make a single validation sample set or use NIST samples or both.
 - Amplify the samples with old kit (*SuperQuant*[®]) and new kit (*MegaSuperQuant*[®])
 - Run amplified products, *SuperQuant*[®] on pre-upgrade instrument.
 - Run amplified products, *SuperQuant*[®] on post-upgrade instrument.
 - Run amplified products, *MegaSuperQuant*[®] on post-upgrade instrument
- Then-

Efficiency in the order of experiments:

- Compare data from well known *SuperQuant*[®] using pre/post instruments.
- Compare data from well known *SuperQuant*[®] and newcomer *MegaSuperQuant*[®] using new/upgraded instrument (and software).
- Once you have compared the two system capabilities, do any needed replicate amps, plates, or multiple operators using new system that are needed. Look at means, SD etc.
- Use this data to design SOP including ideal template amount for profile kits, possible “stop testing” decisions, use of Ys or mini-STRs etc.

Build a Box

- For what range of samples, DNA amounts or values does the method work?
- Where does this break down?



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Extraction Methods

- Evaluate yield and purity
 - Yield: $\frac{\text{ng DNA obtained}}{\text{starting ng DNA in starting material (based on cell count)}}$
 - Reproducibility of yield
 - Purity - two separate questions
 - Have non-nucleic acid components been removed
 - For differential extraction-amount of carryover in each fraction
 - Practical measure - Does the DNA amplify?
- Need some characterization of the starting sample; otherwise you are reduced to comparing results with existing methods for which you may not know the % yield.

EXAMPLE 1

Validation Needed	New Extraction Method
Known samples	Absolutely
Precision & Accuracy: Repeatability	Does method work every time & for each operator
Precision & Accuracy: Reproducibility	May be a good technique but does not do well with particular substrates
Sensitivity	Is there a difference in DNA recovery for different starting sample amounts or types
Stochastic studies	N/A
Mixture studies	N/A except for differential ext. methods
Contamination assessment	Did you observe any? Are there steps that would be especially vulnerable?
Non-probative/mock evidence	Yes, but this is always limited
Other questions	Robotic-Yes Manual-No

Example 2	
Validation Needed	New Quantitation Method
Known samples	NIST, dilution series-male, female, limited mixtures (only measuring total male and total human)
Precision & Accuracy: Repeatability	Does method work every time & for each operator
Precision & Accuracy: Reproducibility	High accuracy may be ideal. High precision would give good relative values.
Sensitivity	At the high end, where is dilution needed to be in linear range? At low end, does value truly coincide inability to obtain profile
Stochastic studies	Yes-Ask if relative amplification of human and male and/or other probes is constant at LT amounts
Mixture studies	At what ratio of male to female does detection of both fail.
Contamination assessment	? If reaction set up is similar to previous assay, would this have changed
Non-probative/mock evidence	Yes, but this is always limited
Other questions-if assay measures degradation-	There is no standard sample which will allow lab to lab comparison of results. Accept developmental validation?

Example 3	
Validation Needed	New STR Amplification kits
Known samples	Absolutely, single source and mixtures that mimic casework, NIST samples
Precision & Accuracy: Repeatability	Same sample, multiple amps
Precision & Accuracy: Reproducibility	NIST first, single source dilution series, mixtures Look at PH, PHR, contributor proportion
Sensitivity	Dilution series, NIST samples, knowns, mixture with varying contributor ratio
Stochastic studies	NIST first, single source dilution series, mixtures Look at PH, PHR, contributor proportion
Mixture studies	Absolutely
Contamination assessment	Will this be any different from previous STR amps
Non-probative/mock evidence	Yes, but this is always limited
Other questions	Robotic-Yes Manual-No

- And the list goes on!**
- Detection platforms-what features need careful evaluation
 - Precision (demonstrate and stop)
 - Injection parameters-may be tied to AT
 - Analytical threshold-may differ based on color, ng amplified, injection parameters
 - Robots-buy one and keep forever?
 - Software upgrades- do we need 400 labs to do this (it's a computer)
 - Probabilistic genotyping software-standard set of mixtures (it's a computer). However, how to implement may differ lab to lab.
 - Rapid DNA
 - *Next Gen Sequencing and SNPs*

Done, but still have....

- Documentation and summary
- Write data driven by validation SOP
- Staff review all data (i.e. study not read two page summary)
- Training
- Experience
- Modifications if needed



We need to move to a better box!

- Could NIST help with some additional standard samples?
 - Standard degraded DNAs
 - Standard mixtures
 - Standard cell pellets with known cell number
- Could reasonable minds agree on what data can be shared?
- Make a public repository for validation data using a common format for the information
 - Good science that is available is more defensible than unavailable science
- *Expand on the current well written SWGDAM Validation Guidelines and, considering the needs of NDIS, write for the future and make some reality driven changes to reduce burden of individual labs while enhancing user knowledge and practice.*