Excellence in Forensic Leadership
Policy and Practice in the 21st Century
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ASCLD
AMERICAN SOCIETY OF CRIME LABORATORY DIRECTORS

Method to DNA Validation in the 21st Century

Validation Organization
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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
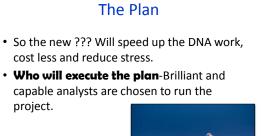- Probabilistic genotyping software

From JMB-Validation Overview, 8-6-2014

Validation Guidance for Forensic DNA: Useful Documents

November 2010 ENFSI DNA Working Group Guidelines
ENFSI 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

Planning 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 scientific questions
- Get input
  – What information should be developed first?
  – What questions can be researched at the same time?

- 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 the Tech Leader is doing this alone – he/she **is** missing something.

The Plan

- So the new ??? Will speed up the DNA work, cost less and reduce stress.
- **Who will execute the plan**-Brilliant and capable analysts are chosen to run the project.

This is NOT your plan!

Consider time frame:
Short-term or Long-term implementation of changes?

- Validate- **What**
  - Quant- giving information for human, male & degradation level
  - Large STR (L STR) kits- required January 2017
  - 3500 CE- Required for some L-STR kits
    - 3130's going away, but not before January 2017
    - 3130 fitted for six dyes
- Validate- **When**
  - All at once, one at a time, two at a time????
    (I personally am averse to timelines but this has not served me well. They are needed by others so they have to be made.)

One-thing-at-time approach

- What is most urgent? Implementation of L- STR kit
- Pros
  - Fewer staff removed from casework
  - Financial outlay spread out over longer time frame
  - More manageable for making SOP and QA changes
  - Gradual learning curve for analysts, other lab staff and customers
- Cons
  - Long period of constant change as each subsequent change is completed

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Every-thing-at-one-time approach

- What can be combined? New quant, amp and CE validated in concert.
- Pros
  - Works well if lab can create or has a dedicated validation group
  - Single, one-time, change over for staff
  - New technical systems validated as a "unit"
  - Single SOP and QA switch over
- Cons
  - All costs up front,
  - Switch over more likely to encounter problems
  - Steep learning curve for analysts

Decision Made

- Mike discussed what the larger goals are such as better speed, cost etc.
- **How** - Define the scientific goals
  - Those that are part of requirements
    - QAS
    - Accreditation
  - Those that are needed **in addition** to the requirements e.g.
    - Those studies which define type of samples to which the technique is applied
    - Defining how much or what type of samples can be used
    - Characterizing DNA extraction yield or removal or mitigation of impurities
    - What specific benefits does an enhancement procedure provide

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Make a list of all scientific questions and needs:

- Needs will be-
  - Scientific questions
  - Data analysis
  - Standard operating procedures
  - Associated QA procedures
  - Changes/Additions to LIMS
  - Changes to training

Scientific Questions

- Break down the scientific questions e.g.
  - **New and improved** DNA quantification kit- comparison to current kit
    - Sensitivity for all probes (human, male, other i.e. degradation)
    - Use of standard curve
    - Reproducibility
    - Precision and accuracy
    - Prediction of successful STR amplification

Scientific Questions

- Break down the scientific questions e.g.
  - **New and improved** STR kit-comparison to current kit
    - What range of amounts of single source DNA can be amplified
      - Least amount of DNA that can be detected
      - Most amount of DNA that can be used without signal or baseline artifacts
    - Comparison to current kit
      - Are there any surprises, new artifacts,
      - Compare profile parameters such as stutter, peak height and peak height ratio to profiles from current instrument using same amplified product.

Scientific Questions

- Break down the scientific questions e.g.
  - **New and improved** CE model-comparison to current instrument
    - What is the analytical threshold
    - What range of amounts of single source DNA can be used
      - Least that can be detected
      - Most that can be used without signal or baseline artifacts or loss of linearity
    - Comparison to current instrument model
      - Are there any surprises, new artifacts,
      - Compare profile parameters such as stutter, peak height and peak height ratio to profiles from current instrument using same amplified product.

Review existing literature:

- Equipment and amplification kit manuals
  - May contain validation summaries and should provide references
  - Read those references

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- Journal articles – from forensic and other journals
  - Search back for a reasonable number of years
  - Was procedure or instrument originally used for other applications

Review existing literature:

- Equipment and kit manuals
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- Journal articles – from forensic and other journals
  - Search back for a reasonable number of years
  - Was procedure or instrument originally used for other applications
- What has been done and published vs validation requirements?
  - If application was not originally forensic, there may be gaps to fill.
  - If originally a forensic application, have all requirements met?
  - In either situation are there important questions, that are not requirements and still need additional validation?

After READing

- Redesign plan as needed

- You are allowed to use existing data
  - May eliminate need for some studies
  - May not eliminate but may reduce the extent of the study (may verify previous data, limit number of samples or range of conditions you may have considered testing)

Contact nearest graduate level forensic science program

- Maybe they can help with access to journals
- They have students
  - You need hands
  - Use student hands where a mistake will not sink the ship. (**Caution**-not all students are equal)

Samples/Equipment/Software/Supplies for validation (not casework)

- **Spending money may save time and money!**
  - Hemocytometer for cell counting
  - Nanodrop or other UV-Vis spectrophotometer
  - Best known samples-NIST standard reference samples (for quant and amp sensitivity)
  - Purchased DNA samples or body fluids
    - American Type Culture Collection
    - Coriell Cell Repositories
    - BioreclamationIVT-ask for single source samples-sperm and others
  - Prepare DNA extracts in sufficient quantity, use now and store for future validations and comparisons

Samples/Equipment/Software/Supplies

- Consider purchase and QC of reagents as a batch to remove sources of variation prior to beginning validation
- Plate tracker if loading any plates by hand
- Repeating pipetter for master mix and plate loading
- Small extraction robot (standardized methods for DNA extracts with little variation in purity)
- Stats software other than Excel-
  - JMP, SAS
  - Macros etc. from NIST for stutter and other calculations

Efficiency in the order of experiments:

- New kits and instrument (or upgrade) happening simultaneously?
  - Make a single validation sample set using NIST and other samples
  - Quant-
  - Amplify the samples with old STR kit and new L-STR kit
  - Run both sets of amplified products, on pre-upgrade instrument.
    - Assess kit differences on the instrument lab is familiar with
    - Quantitate differences using loci common to both kits
    - Stutter per locus, average peak height per ng amplified
    - peak height across dye color,
    - Peak height ratio distribution with DNA mass, drop out
  - Run amplified products, on post-upgrade instrument.
    - Do same analysis as above
  - Then-

Efficiency in the order of experiments:

- Compare data from current STR kit using pre/post instruments
  - Are there changes, what are they, characterize changes

- Compare data from current and new L-STR kits using data from upgraded instrument.

- Once you have compared the two system capabilities, do any needed replicate amps, reruns, or additional data collection needed. Look at means, SD etc.

- Use this data to design SOP including ideal template amount for amplification kits, possible "stop testing" decisions
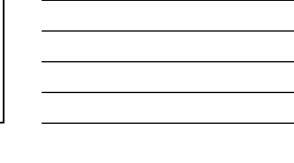
After the basic system is characterized-

- Test the limits
- Characterize the uses
- How are two systems different
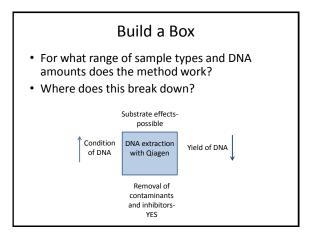- Build a box which defines the limits of the system
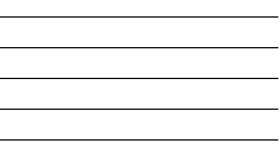
Using DNA extraction methods as an example:

- Evaluate yield and purity
  - Yield: $\dfrac{ng\ DNA\ obtained}{starting\ ng\ DNA\ in\ starting\ material\ (based\ on\ cell\ count)}$

  - 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.

Build a Box

- For what range of sample types and DNA amounts does the method work?
- Where does this break down?

Substrate effects?

Condition of DNA | DNA extraction with Chelex or Qiagen | Yield of DNA

Removal of contaminants and inhibitors

Build a Box

- For what range of sample types and DNA amounts does the method work?
- Where does this break down?

Substrate effects- possible

Condition of DNA | DNA extraction with Chelex | Yield of DNA

Removal of contaminants and inhibitors- NO

Build a Box

- For what range of sample types and DNA amounts does the method work?
- Where does this break down?

Substrate effects-
possible

Condition of DNA → DNA extraction with Qiagen ← Yield of DNA ↓

Removal of contaminants and inhibitors-
YES

Using initial list of the questions-

- Have you covered the required validation standards
- Do you have questions which are not in the standards? (probably will)
  – If you use the standards to guide the validation you will miss something.
  – If you simply ask the right questions you likely will cover the standards and answer some remaining important questions.

However beautiful the strategy;

You should occasionally look at the results.

The data is here!

Plan A

Plan B

"Not everything that counts can be counted, and not everything that can be counted counts."

Albert Einstein

Now there's data & lots of it!

DNA validation generates a great deal of data!

- A plan for data analysis is needed
- What specific profile parameters need to be extracted from the data
- What software?
- What statistics?
- Does the group need additional expertise from outside
- Use scientific literature for ideas and guidance

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 in CONTROLS or profile? Are there steps that would be especially vulnerable?
Non-probative/mock evidence	Yes, but this is always limited
Other questions	DNA yield? Are substrate inhibitors removed or mitigated? Check profile quality Can procedure by used on robotic equipment?

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, what DNA mass departs from 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 either 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?

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

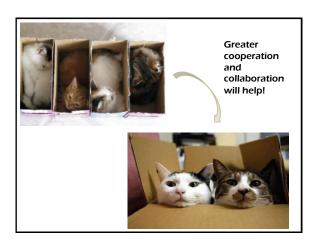- *Next Gen Sequencing and SNPs*

Done, but still have….

- Documentation and write up of results
- Include well labelled displays of data to show trends
- Staff review all data (i.e. STUDY-not read two page summary)
- Write procedures
- Develope QA procedures
- Make LIMS changes
- Train analysts
- Introduce slowly
  - With experienced analysts-first
  - On single source samples with sufficient DNA to repeat-first
- Make modifications when indicated after checking original validation data and filling in a gap if needed

How can we reduce burden of validation, produce less repetitive data and facilitate learning.

- 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 on individual labs while enhancing user knowledge and practice.*

Greater cooperation and collaboration will help!

Thanks

Two of the world's best collaborators;

Mike Coble and Charlotte Word

- I Keep six honest serving-men:
  (They taught me all I knew)
  Their names are What and Where and When
  And How and Why and Who.
  I send them over land and sea,
  I send them east and west;
  But after they have worked for me,
  I give them all a rest.

- I let them rest from nine till five.
  For I am busy then,
  As well as breakfast, lunch, and tea,
  For they are hungry men:
  But different folk have different views:
  I know a person small--
  She keeps ten million serving-men,
  Who get no rest at all!
  She sends 'em abroad on her own affairs,
  From the second she opens her eyes--
  One million Hows, two million Wheres,
  And seven million Whys!