DNA Mixture Interpretation Principles: Observations from a NIST Scientific Foundation Review

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Writing Limitations into your SOPs

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Why set limits?

- Why not? Limits protect you!
- Highways have maximum speed limits and it differs from one locale to the next. Some even have minimum speed limits.
- Every elevator has a weight limit.
- Conference rooms have capacity limits.
- Planes have load limits.
- If you have kids, you set limits for them:
  - Play time limits
  - Snack limits (type of snack and amount)
  - Television limits (time and content)

Limits

- Google it!
  - *noun*. a point or level beyond which something does not, or may not, extend or pass. “the *limits* of presidential power”
- Never be afraid to set limits for DNA Interpretation.
- Never be “peer-pressured” to push these limits.
- Never be forced by your customers to push these limits.
- Trust in the wisdom of your laboratory management.
- Trust in the wisdom of your DNA Technical Leader!

When to set limits

- Limits should be well-defined after validation
  - Analytical thresholds and stochastic thresholds
  - Stutter filters and global filters
  - Minimum amplification amount and Optimal amplification amount
  - Whether or not controls need to be “perfect” to be able to deem data “passing”. (Does a run fail if there is signal in the negative control? Or does it mean that we need to interpret the data carefully?)
  - Quality of data – degradation, stochastic effects, mixture ratios, etc.
  - Quantity of data – minimum number of alleles, minimum number of loci, number of contributors, etc.
  - Source attribution?
- Boils down to “what types of profiles are suitable for comparison or interpretation?”

When to set limits (continued)

- Limits should be continuously explored after implementation
  - Do we want to be as aggressive to determine the genotype at a particular locus? Would we rather INC it or “Q” it? (17, Q instead of 17, 18)
  - If you are using probabilistic genotyping and currently do not have a minimum number of loci to interpret, should you implement one?
  - Is it time to increase the global filter for known single-source samples (like exemplars) to minimize editing?
  - Is it time to revisit the validation and implement dye-specific analytical thresholds?
  - Is it time to revisit the validation and implement allele-specific stutter filters?
  - Is it time to decrease the optimal amplification amount to prevent saturation?
  - If specific poor-quality profiles produce poor comparisons, is it time to deem them “not suitable for comparison”?

Why put limits into your procedures?

- Because our standards require us to have SOPs to enable consistency.
- FBI Quality Assurance Standards Audit for Forensic DNA Testing Laboratories (9/1/11)
  - 9.1 Does the laboratory have and follow written analytical procedures…?
  - 9.1.1 Does the laboratory have a documented standard operating procedure for each analytical method used?
- ISO/IEC 17025:2017
  - 7.2.1.1 The laboratory shall use appropriate methods and procedures for all laboratory activities…

Why put limits into your procedures?

- Because guideline-documents recommend us to do so.
- SWGDAM Interpretation Guidelines for Autosomal STR Typing by Forensic DNA Testing Laboratories – APPROVED 01/12/2017:
  - (Background) *"…laboratories…are required by the Quality Assurance Standards…to establish and follow documented procedures for the interpretation of DNA typing results and reporting."*
  - (Background) *"…the laboratory should utilize written procedures for interpretation of analytical results with the understanding that specificity in the standard operating protocols will enable greater consistency…"*
  - (Background) *"It is recommended that standard operating procedures for the interpretation of DNA typing results be sufficiently detailed…"*

How to write limits into your SOPs

- BE VERY CLEAR!
  - Benefits your people
  - Benefits your customers
- Know your audience
  - Some people learn verbally
  - Some people learn visually
  - Some people learn logically
- Don’t hesitate to be repetitive!
- **Don’t hesitate to be repetitive!**

Fusion Sample Input Amount
Optimal – 525pg*
Minimum – 37.5pg

*The option for amplification with a greater input amount is available if determined appropriate for the sample by the analyst.

6.2.1 The optimal DNA input amount for amplification was determined to be **525pg** and the minimum DNA input amount is **37.5pg**.

6.2.2 The AT was determined to be 50 relative fluorescent units (RFU).

6.2.3 The ST was determined to be 300 RFU.

Is there a “right way” to push your limits?

- For a change in SOP, it all depends on how much validation data you have to support your procedure.
  - Laboratory Management and (especially) the DNA Technical Leader must be comfortable with the procedure.
  - For example, if your minimum amplification threshold is currently at 100pg and would like to “push it down” to 75pg, you should have data at and below 75pg to determine if you can comfortably reset the limit.
- For a one-time deviation, it all depends on whether or not you can scientifically justify it.
  - For example, if resulting evidence data shows contamination from an investigator and your laboratory’s policy is to “INC” the sample, can you deviate and interpret it (carefully!) upon request? Maybe. Probabilistic Genotyping software may be able to help you do it.

Deviations to Procedures

- In my opinion, any proposed deviation to written standard operating procedures is “pushing the limit”.
- Must be accompanied with scientific justification when standard courses of actions will not be followed.
  - ISO 17025 7.2.1.7: “Deviations from methods…shall occur only if…documented, technically justified, (and) authorized…”
  - As the DNA Technical Leader for my lab, my analysts need to convince me. Otherwise, the data cannot be used. “Tell me why it is still acceptable to use the resulting data.”
  - “We have no more sample to retest” is never a good reason. There must be scientific justification!
- Deviations should be a rarity and an approval in one instance should not mean automatic approvals in future instances. Otherwise, it must be written into the procedure.

Limits – Mixture Resource Committee Discussions

- All of us agreed that limitations exist for DNA Interpretation
- All of us agreed that pushing the limits could be dangerous
- Not all of us agreed that a specific limit for one lab is a limit for another lab.
  - Reason could be that some labs are using Probabilistic Genotyping Software and some labs are manually deconvoluting.
- Overall tip was to use validation data to establish your standard procedures (and limitations) and decide what profiles are suitable for comparison.
  - Very subjective. Again, could be based on a laboratory's deconvolution methodology.
- A suggestion was to use validation data from other labs to help guide a lab to establish procedures. But this would require publishing validation or a "central repository" to publicly share validation data.