Beyond the Validation: Finding the balance of QA Requirements and Understanding the Data Prior to Implementation into Casework

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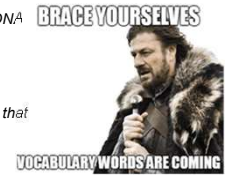
Definition of Validation

Developmental validation

Determination of conditions and limitations of a new or novel DNA methodology

Internal validation

Accumulation of test data within the laboratory to demonstrate that established methods and procedures perform as expected



Scientific Working Group on DNA analysis methods. Validation Guidelines for DNA analysis methods. (https://www.swgdam.org/publications)

What is the problem?

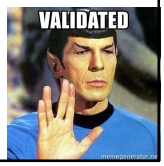
Resources

Standards for validation

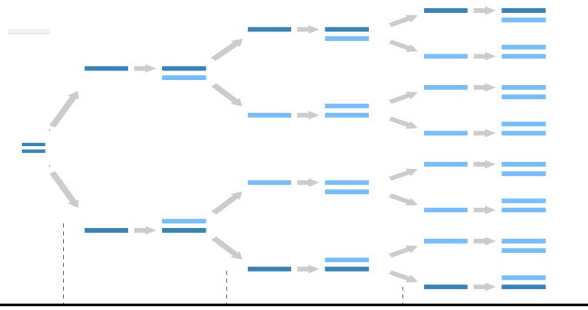
How to prevent an internal validation ballooning into a developmental validation?

- **Validation Guidelines for DNA Analysis Methods,** SWGDAM (Scientific working group on DNA analysis methods)
- **Debunking Some Urban Legends Surrounding Validation Within the Forensic DNA Community**, John Butler, National Institute of Standards and Technology

How to ensure that the validation reflects what will be encountered in casework and ensures limitations of any process are captured?



Validation by numbers



Rephrase the question

Validation:
When enough is not enough

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Lesson 1

Importance of testing a range of sample inputs and sample concentrations

Upgrade of quant system

Included experiments related to sensitivity, standard stability, male/female mixtures and concordance

Concordance

Different sample types that had been encountered in casework

Samples primarily processed using DNA IQ

~60 samples including different DNA templates used for concordance alone

~20 negative controls

However… our approach was flawed

Issue A: Different extraction methods

- Caused inhibition (low IPC)

Issue B: Negative control data

- Previously all clear during validation

Negative control data

POSTER # 26 : How meaningful is a quant result?

3:00-5:00pm this afternoon

Lesson 2 Reproducibility

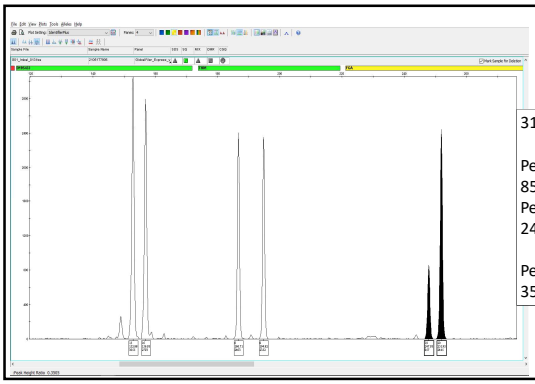
Importance of understanding peak height variance in new kit

Kits with more loci, more dyes – what is the consequence for data quality?

"..the larger the multiplex, the less efficient the amplification may become. This means that success rates could be compromised.

Clearly there is a balance to be struck between the size of the multiplex and its efficiency"

Gill P, Fereday. L, Morling N, Schneider P; The evolution of DNA Databases-Recommendations for new European STR loci, Forensic Science International 156 (2006)242-244



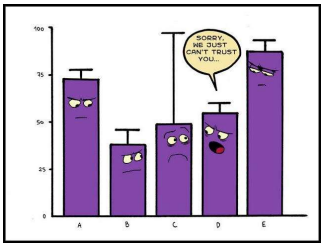
3130xl data

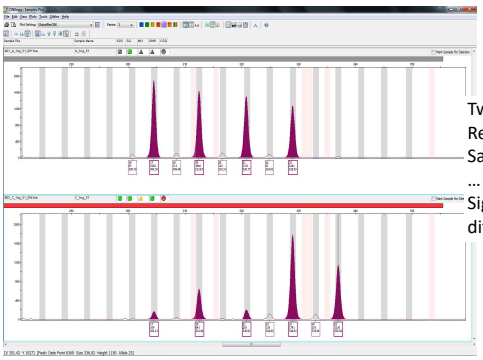
Peak height allele 1: 857 rfu
Peak height allele 2: 2445 rfu

Peak imbalance: 35%

The associated issues…

- Larger variability than previously encountered
- Analysts expectations had to change
- Increased rework of profiles for confidence
- Increased costs





Two person mix
Repeat amplifications
Same template DNA
…
Significant peak height differences

Lesson 3 Limitations of a kit

Importance of understanding developmental validation limitations

Developmental validation completed yet does experimental design reflect casework?

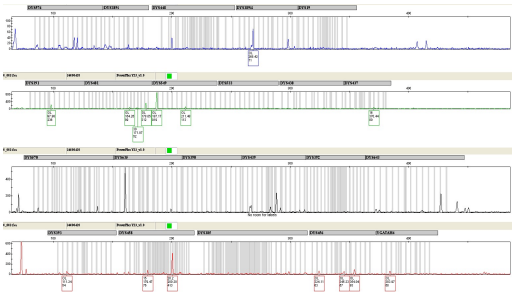
I don't care if we *are* out of coffee filters…This is a completely inappropriate use of the spin columns!

Our experiences with Y STR kits

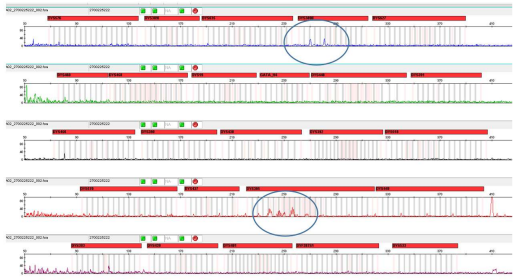
- We use Y STR kits predominantly on high concentration female samples
- Looking for trace male DNA
- Not uncommon to encounter **total DNA** values between 50-100ng/µL

Issue with additional peaks/artefacts due to excess specific female DNA

Y STR kit 1



Y STR kit 2



Internal validation

accumulation of test data within the laboratory to demonstrate that established methods and procedures perform as expected in the laboratory. Prior to using a procedure for forensic applications, a laboratory shall conduct internal validation studies.

Conclusions

- Understand limits of developmental validation
  - Are you intending to use the product outside recommendations?
  - Plan all inputs and outputs
- Manage expectations
  - Of analysts, management, stakeholders
- Dedicate resources
  - Projects that are managed with resources, plans and milestones are more likely to succeed
- Be flexible
  - Plans may change. Do sufficient work to ensure confidence in results

Thank you for your attention

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