Validation of Forensic DNA Technologies:

Comparing Four Validation Designs: Impacts of Normal Laboratory Alterations on DNA Results

August 6, 2014

BOSTON UNIVERSITY NIST

Validation of Forensic DNA Analysis Methods

- To "determine the efficacy and reliability for forensic casework and/or database analysis."
- Assess:
  - Sensitivity
  - Stochastic effects
  - Repeatability
  - Reproducibility
  - Limit of detection

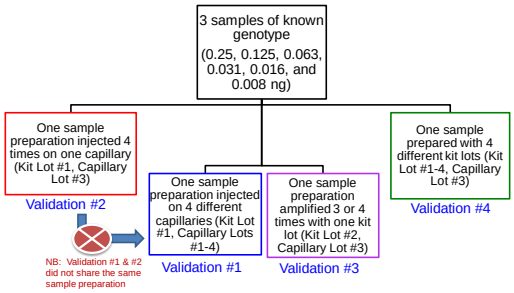
Aims of the Research

- Aim:
  - Characterize the effect of daily laboratory alterations on peak heights, peak height ratios, stutter and allele drop-out during forensic DNA analysis
  - Improve validation practices within our laboratory
  - Determine if differing validation practices would impact results

Purpose

- Compare Validation schemes:
  - Injecting samples, amplified once with one kit lot, multiple times on various capillaries (i.e., different capillary lots) (Validation #1)
  - Injecting samples, amplified once with one kit lot, multiple times on one capillary (Validation #2)
  - Amplifying samples multiple times with one kit lot and injecting once on one capillary (Validation #3)
  - Amplifying samples multiple times with multiple kit lots and injecting once on one capillary (Validation #4)
- Compared Validation #1 v. #2, and Validation #3 v. #4.

Methods



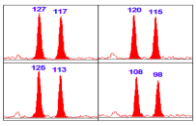
- Sorted data by target, experimental design, sample, and locus

Methods

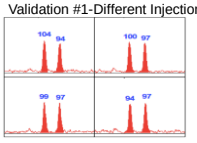
- Examined:
  - Peak Height Equivalency: $\frac{PH_{allele}}{PH_{allele,max}}$
  - Peak Height Ratio (PHR): $\frac{PH_{locus,smaller}}{PH_{locus,larger}}$
  - 'PHR Equivalency' within a locus (PHRE): $\frac{PHR_{locus}}{PHR_{locus,max}}$
  - Stutter Percentage per locus: $\frac{PH_{locus,stutter}}{PH_{locus,allele}} \times 100\%$
  - Rate of Detection per profile: $\frac{\#\,Observed\ Alleles}{\#\,Expected\ Alleles}$

Peak Heights

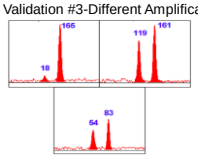
Validation #2-Different Capillary Lots



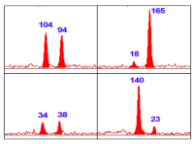
Validation #1-Different Injections



Validation #3-Different Amplifications

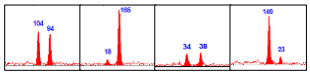


Validation #4-Different Kit Lots



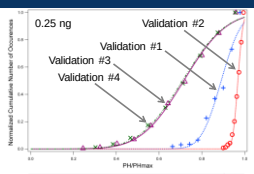
Peak Heights

- Examined:
  - 'PH Equivalency' within an allele (PHE): $\frac{PH}{PH\,max}$
    - ex. Multiple amplifications with different kit lots using 0.031 ng DNA

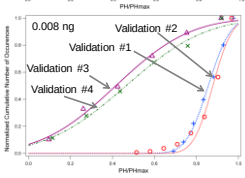


- Allele 1: 104, 18, 34, and 140 RFU
  - PHE= 104/140, 18/140 and 34/140 = 0.74, 0.13, and 0.24
- Allele 2: 94, 165, 38, 23
  - PHE = 94/165, 38/165 and 23/165 = 0.57, 0.23 and 0.14

'Peak Height Equivalency'

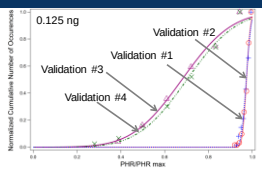


Parameter	Mean	Standard Deviation
Validation 1 (Capillaries)	0.891	0.10
Validation 2 (Injections)	0.969	0.027
Validation 3 (Amps)	0.716	0.22
Validation 4 (Kits)	0.710	0.23

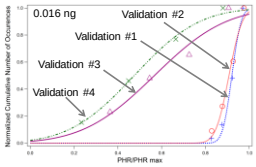


Parameter	Mean	Standard Deviation
Validation 1 (Capillaries)	0.857	0.12
Validation 2 (Injections)	0.878	0.10
Validation 3 (Amps)	0.42	0.38
Validation 4 (Kits)	0.46	0.42

'Peak Height Ratio Equivalency'

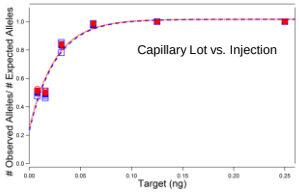


Parameter	Mean	Standard Deviation
Validation 1 (Capillaries)	0.973	0.024
Validation 2 (Injections)	0.974	0.020
Validation 3 (Amps)	0.68	0.25
Validation 4 (Kits)	0.70	0.25



Parameter	Mean	Standard Deviation
Validation 1 (Capillaries)	0.921	0.05
Validation 2 (Injections)	0.907	0.07
Validation 3 (Amps)	0.57	0.37
Validation 4 (Kits)	0.47	0.33

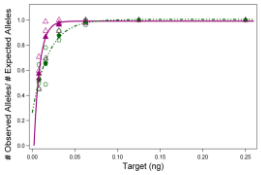
Rate of Detection

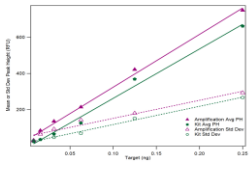


Parameter	$Pr(DO)_{0.008}$	$Pr(DO)_{0.016}$	$Pr(DO)_{0.031}$
Validation #1 (Capillaries) (□)	0.6	0.4	0.2
Validation #2 (Injections) (O)	0.6	0.4	0.2

Rate of Detection

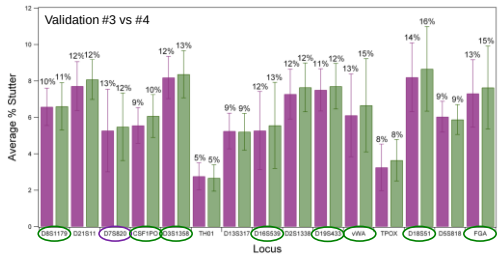
Amplification (Validation #3) vs. Kit Lot (Validation #4)





Parameter	$Pr(DO)_{0.008}$	$Pr(DO)_{0.016}$	$Pr(DO)_{0.031}$
Validation #3 (Amp) (△)	0.4	0.1	0.02
Validation #4 (Kit Lots) (O)	0.5	0.3	0.1

Stutter Percentages



Threshold = Average % Stutter + 3SD

Conclusions and Recommendations

- Variation in peak height from multiple capillaries > variation in peak height from multiple injections
- Amplification kit lots resulted in different stutter thresholds and rates of detection (Validation #3 resulted in higher peak heights, and lower drop out rates than Validation #4).
  - Amplifications with one kit lot may not be enough to characterize the peak heights, and drop-out rates expected over time
- Measurements of intermediate precision are required. Multiple kit lots over multiple capillary lots over an intermediate period of time.

Acknowledgements

- Kayleigh Rowan & Genevieve Wellner
- National Institute of Justice
  - This project was partially supported by Award No. NIJ2011-DN-BX-K558 and NIJ2012-DN-BX-K050 awarded by the National Institute of Justice, Office of Justice Programs, U.S. Department of Justice. The opinions, findings, and conclusions or recommendations expressed in this publication/program/exhibition are those of the author(s) and do not reflect those of the Department of Justice.

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