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

August 6, 2014

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

- Examined:
  - Peak Height Equivalency: \( \frac{PH_{\text{max}_{\text{small}}}}{PH_{\text{max}_{\text{large}}}} \)
  - Peak Height Ratio (PHR): \( \frac{PH_{\text{max}_{\text{small}}}}{PH_{\text{max}_{\text{large}}}} \)
  - ‘PHR Equivalency’ within a locus (PHRE):
  - Stutter Percentage per locus: \( \frac{PH_{\text{max}_{\text{small}}}}{PH_{\text{max}_{\text{large}}}} \times 100\% \)
  - Rate of Detection per profile: \( \frac{\text{Observed Alleles}}{\text{Expected Alleles}} \)
Dr. Catherine Grgicak

NIST DNA Analyst Webinar Series:
Validation Concepts and Resources- Part 1

08/06/2014

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-

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean</th>
<th>Standard Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Validation 1 (Capillaries)</td>
<td>0.891</td>
<td>0.10</td>
</tr>
<tr>
<td>Validation 2 (Injections)</td>
<td>0.969</td>
<td>0.027</td>
</tr>
<tr>
<td>Validation 3 (Amps)</td>
<td>0.716</td>
<td>0.22</td>
</tr>
<tr>
<td>Validation 4 (Kits)</td>
<td>0.710</td>
<td>0.23</td>
</tr>
</tbody>
</table>
'Peak Height Ratio Equivalency'

Parameter | Mean | Standard Deviation
--- | --- | ---
Validation #1 (Capillaries) | 0.975 | 0.025
Validation #2 (Injections) | 0.975 | 0.025
Validation #3 (Amps) | 0.950 | 0.035
Validation #4 (Kits) | 0.970 | 0.031

Rate of Detection

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Pr(DO)</th>
<th>Pr(DO)</th>
<th>Pr(DO)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Validation #2</td>
<td>0.6</td>
<td>0.4</td>
<td>0.2</td>
</tr>
<tr>
<td>Validation #1 (Capillaries)</td>
<td>0.6</td>
<td>0.4</td>
<td>0.2</td>
</tr>
<tr>
<td>Validation #3 (Amps)</td>
<td>0.6</td>
<td>0.4</td>
<td>0.2</td>
</tr>
<tr>
<td>Validation #4 (Kits)</td>
<td>0.6</td>
<td>0.4</td>
<td>0.2</td>
</tr>
</tbody>
</table>

Rate of Detection

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

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Pr(DO)</th>
<th>Pr(DO)</th>
<th>Pr(DO)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Validation #3 (Amps)</td>
<td>0.4</td>
<td>0.1</td>
<td>0.02</td>
</tr>
<tr>
<td>Validation #4 (Kits)</td>
<td>0.5</td>
<td>0.3</td>
<td>0.1</td>
</tr>
</tbody>
</table>
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-K550 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.