Inter and intra-variation observed from a NIST interlaboratory study on DNA mixture interpretation in the U.S. (MIX13)

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NIST Interlaboratory Studies on DNA Mixture Interpretation

NIST and NIJ Disclaimer

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Summary of DNA Mixture Interlaboratory Studies

<table>
<thead>
<tr>
<th>Study</th>
<th>Year</th>
<th># Labs</th>
<th># Samples</th>
<th>Mixture Types</th>
</tr>
</thead>
<tbody>
<tr>
<td>MSS 1</td>
<td>1997</td>
<td>22</td>
<td>11 stains</td>
<td>ss, 2p, 3p</td>
</tr>
<tr>
<td>MSS 2</td>
<td>1999</td>
<td>45</td>
<td>11 stains</td>
<td>ss, 2p, 3p</td>
</tr>
<tr>
<td>MSS 3</td>
<td>2000-01</td>
<td>74</td>
<td>7 extracts</td>
<td>ss, 2p, 3p</td>
</tr>
<tr>
<td>MIX05</td>
<td>2005</td>
<td>69</td>
<td>4 cases (.fsa)</td>
<td>only 2p</td>
</tr>
<tr>
<td>MIX13</td>
<td>2013</td>
<td>108</td>
<td>5 cases (.fsa)</td>
<td>2p, 3p, 4p</td>
</tr>
</tbody>
</table>

- Other recent studies
  - UK Regulator
  - DFSC (US Department of Defense Lab)

MIX13 Participants from **108 Laboratories**

| 46 states had at least one lab participate |

Due to the number of laboratories responding and the federal, state, and local coverage obtained, this MIX13 interlaboratory study can be assumed to provide a **reasonable representation of current U.S. forensic DNA lab procedures across the community**
Purpose of MIX13 Cases

<table>
<thead>
<tr>
<th>Case</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>~1:1 mixture (2-person)</td>
</tr>
<tr>
<td>2</td>
<td>Low template profile with potential dropout (3-person)</td>
</tr>
<tr>
<td>3</td>
<td>Potential relative involved (3-person)</td>
</tr>
<tr>
<td>4</td>
<td>Minor component (2-person)</td>
</tr>
<tr>
<td>5</td>
<td>Complex mixture (&gt;3-person) with # of contributors; inclusion/exclusion issues</td>
</tr>
</tbody>
</table>

According to German Stain Commission (2009) mixture types: 1 = A, 2 = C, 3 = ?, 4 = B, 5 = ?

MIX13 Study (Case 01)

- Summary – Mock sexual assault, 2 person 50:50 mixture, all alleles above a ST of 150 RFU.
- Purpose – How many labs would consider the victim’s profile and determine genotypes (deconvolution) for a mRMP statistic?

Case 01 – PP16HS

All alleles are above a ST of 150RFU “Indistinguishable”
Conclusions

- No false exclusions

- Wide range of variation in stats reported for labs that inferred genotypes (RMP or LR).

Case 02 – IDFiler

![Case 02 – IDFiler Graph](image)

NOTE: BU sample AT = 30; ST = 150

<table>
<thead>
<tr>
<th>Individual</th>
<th>Included?</th>
<th>Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Suspect A</td>
<td>Yes</td>
<td>6</td>
</tr>
<tr>
<td>Suspect B</td>
<td>Yes</td>
<td>1.5</td>
</tr>
<tr>
<td>Suspect C</td>
<td>Yes</td>
<td>1</td>
</tr>
<tr>
<td>Suspect D</td>
<td>No</td>
<td>--</td>
</tr>
</tbody>
</table>

Total Input DNA = 300 pg
Intra-Laboratory Results (n = 8)

<table>
<thead>
<tr>
<th>Analyst</th>
<th>Suspect A</th>
<th>Suspect B</th>
<th>Suspect C</th>
<th>Suspect D</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Inconclusive - A, B, C</td>
<td>Excluded</td>
<td>Excluded</td>
<td>Excluded</td>
</tr>
<tr>
<td>2</td>
<td>6.74 Quad</td>
<td>23.6</td>
<td>Excluded</td>
<td>Excluded</td>
</tr>
<tr>
<td>3</td>
<td>Inconclusive - A, B, C</td>
<td>Excluded</td>
<td>Excluded</td>
<td>Excluded</td>
</tr>
<tr>
<td>4</td>
<td>9.4 for A, B</td>
<td>C</td>
<td>Excluded</td>
<td>Excluded</td>
</tr>
<tr>
<td>5</td>
<td>4.1 Quint</td>
<td>37</td>
<td>Excluded</td>
<td>Excluded</td>
</tr>
<tr>
<td>6</td>
<td>230 for A, B</td>
<td>Inconclusive</td>
<td>Excluded</td>
<td>Excluded</td>
</tr>
<tr>
<td>7</td>
<td>9.4 for A, B</td>
<td>Excluded</td>
<td>Excluded</td>
<td>Excluded</td>
</tr>
<tr>
<td>8</td>
<td>37.3 for A, B</td>
<td>Excluded</td>
<td>Excluded</td>
<td>Excluded</td>
</tr>
</tbody>
</table>

Concerns with Case 02

- One lab included Suspect D (False Inclusion).
- A substantial number of labs falsely excluded the two low-level contributors.
- Major concern with labs using CPI for contributor #3…
CPI with Suspect C

Contributors
A = 15, 15
B = 14, 15
C = 12, 14

15 of 108 labs used CPI to include Suspect C (13.8%)
4 of these 15 (26.6%) used D19 as a locus for CPI

Case 03 – IDPlus

Case 03 – Two Suspects

<table>
<thead>
<tr>
<th>Individual</th>
<th>Inclusion?</th>
<th>Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Victim</td>
<td>Included</td>
<td>7</td>
</tr>
<tr>
<td>Boyfriend (CP)</td>
<td>Included</td>
<td>2</td>
</tr>
<tr>
<td>Suspect 3A (Brother)</td>
<td>Included</td>
<td>1</td>
</tr>
<tr>
<td>Suspect 3B (Friend)</td>
<td>Excluded</td>
<td>--</td>
</tr>
</tbody>
</table>
The Brothers

Markers  DBS1179  D21511  D7S820  CSF1PO  D3S1358  THO1
Victim 03A  12,15  31,2 31,2  10,10 10,11  14,14  9,3,9,3
Cons Partner  14,14  28,35  10,11  10,12 14,18  7,8
Suspect 03A  14,15  28,35  10,11  12,12 14,18  7,8

Markers  D135317  vWA  TPOX  D18S51  D5S818
Victim 03A  11,12  15,15  9,11  12,13  11,12
Cons Partner  12,13  17,21  6,8  13,16  10,12
Suspect 03A  12,13  17,21  8,9  13,16  10,12

The Brothers
For D16 and FGA – two alleles of the suspected brother are present in the epg

Markers  D16S539  FGA
Victim 03A  9,12  20,26
Cons Partner  10,10  26,27
Suspect 03A  8,9  23,27

95 RFU

Case 03

9 labs – false exclusion
1 lab – false inclusion
Statistical Concerns

Only 4 loci have alleles obligate to the suspected brother – ALL are below ST

Case 04 – IDPlus

MIX13 Study (Case 04)

• Summary – Mock sexual assault, 2 person 3.5:1 mixture, minor component has alleles below the ST of 150 (required by all labs!)

• Purpose – How many labs would attempt to separate the two components?

• No false exclusions.
Statistical Concerns

Intra-Laboratory Results (n = 8)

Case 05 – IDPlus
No more than 4 alleles at a locus

- Suggests a 2 person mixture

- Peak Height information does not agree

MIX13 Study (Case 05)

- Summary – Mock bank robbery with ski mask evidence (touch DNA), 4 person \(1:1:1:1\) mixture.

- However – this mixture had no more than 4 alleles at any locus (appears as a \(2p\) mixture). 2 of the 4 contributors were provided along with a non-contributor.

- Purpose – How many labs would consider this mixture as too complex to interpret?

Case 05 – 3 Suspects

<table>
<thead>
<tr>
<th>Individual</th>
<th>Included</th>
<th>Included</th>
<th>Not in the mixture</th>
</tr>
</thead>
<tbody>
<tr>
<td>Suspect 5A</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Suspect 5B</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Suspect 5C</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
CPI Stats ranged from 1 in 9 to 1 in 344,000

Concluding Thoughts

• Despite the improvements in protocols and interpretation guidelines since 2010, mixture interpretation is still all over the place.
• Some of this is a consequence of using a statistical approach that is inappropriate for complex mixture interpretation – CPI is often being used as a substitute for interpretation, and has the risk of including a non-contributor.

Concluding Thoughts

• Better training and improved validations for mixture interpretation is needed!
• Software solutions may assist in mixture interpretation, statistical evaluation, and removal of bias – however, “black box” solutions are not helpful for the analyst who will need to explain the process to the trier of fact.
Thank you for your attention

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