DNA Mixture Interpretation: State-of-the-Art

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Presentation Outline

- Challenges that exist with DNA mixtures
  - Stacking, number of contributors (relatives), drop-out with low level
- NIST interlaboratory studies (MIX05 & MIX13)
- Concerns raised with MIX13 study
  - Why does this range of results exist?
- Comparison to other fields (e.g., cell line authentication)
- Accreditation issues
  - The QAS is not specific enough to provide much help here
- Validation needs for the community
  - Can we reliably extrapolate from 2-person mixture studies?
- Closing thoughts: where do we go from here?
Challenges that Exist with DNA Mixtures
Challenges with Complex DNA Mixtures

Complex mixture = more than two individuals contribute to a biological sample; often with low amounts of DNA

• **Interpretation uncertainty increases** (errors are more likely)
  – Allele stacking because mixture contributors have peaks in common

\[\text{Single-source}\]
Challenges with Complex DNA Mixtures

Complex mixture = more than two individuals contribute to a biological sample; often with low amounts of DNA

- **Interpretation uncertainty increases** (errors are more likely)
  - Allele stacking because mixture contributors have peaks in common
  - Number of contributors becomes more uncertain

<table>
<thead>
<tr>
<th>Configuration</th>
<th>$N$ contributor mixture</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Existing CODIS</td>
<td></td>
<td>1.93E-41</td>
<td>1.81E-09</td>
<td>8.21E-02</td>
<td><strong>0.8739</strong></td>
<td><strong>0.9993</strong></td>
</tr>
<tr>
<td>Proposed CODIS with SE33</td>
<td>6</td>
<td>0</td>
<td>4.58E-21</td>
<td>1.18E-04</td>
<td>0.3303</td>
<td>0.9384</td>
</tr>
<tr>
<td>Proposed CODIS without SE33</td>
<td></td>
<td>1.87E-69</td>
<td>7.71E-18</td>
<td>2.24E-03</td>
<td>0.6963</td>
<td>0.9981</td>
</tr>
<tr>
<td>Fusion</td>
<td></td>
<td>1.50E-81</td>
<td>3.76E-22</td>
<td>1.59E-04</td>
<td>0.5220</td>
<td>0.9937</td>
</tr>
</tbody>
</table>
Challenges with Complex DNA Mixtures

Complex mixture = more than two individuals contribute to a biological sample; often with low amounts of DNA

• **Interpretation uncertainty increases** (errors are more likely)
  – Allele stacking because mixture contributors have peaks in common
  – Number of contributors becomes more uncertain
  – Drop-out issues where allele information is lost when you attempt to work with low amounts of DNA

**Contributors**

A = 15, 15  
B = 14, 15  
C = 12, 14
Thinking in Terms of Genotypes vs Alleles

• CPI – The probability that a random, unrelated person would be included as a contributor to the observed DNA mixture.

• CPI provides a simple statistical model for equating observed alleles with all possible genotypes.

• The focus is on ALLELES and not GENOTYPES
Thinking in Terms of Genotypes vs Alleles

Would you include or exclude a reference sample that is 13,14 and 28,30 at these two loci?

A cautionary note on using CPI when drop-out is possible

\[
PI = (f_9 + f_{10} + f_{12} + f_{13} + f_{14})^2
\]

\[
PI = (0.049+0.051+0.361+0.384+0.141)^2
\]

\[
PI = 0.986 \text{ or 1 in 1.01}
\]

\[
PI = (f_{10} + f_{12})^2
\]

\[
PI = (0.051 + 0.361)^2
\]

\[
PI = 0.169 \text{ or 1 in 5.92}
\]

Drop-out \textbf{inflates} your statistics for CPI (not conservative!)
### 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
  - USACIL
MIX13 Participants from 108 Laboratories

46 states had at least one lab participate

Green = participants
Gray = no data returned

Federal Labs
FBI (DOJ)
ATF (DOJ)
USACIL (DOD)

Canada
RCMP
CFS
Montréal

52 state labs
(40 states)
49 local labs
3 federal
3 non-U.S.
## Purpose of MIX13 Cases

<table>
<thead>
<tr>
<th>Case</th>
<th>Challenge provided to study responses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Case 1</td>
<td>~1:1 mixture (2-person)</td>
</tr>
<tr>
<td>Case 2</td>
<td><strong>Low template</strong> profile with potential dropout (3-person)</td>
</tr>
<tr>
<td>Case 3</td>
<td>Potential <strong>relative</strong> involved (3-person)</td>
</tr>
<tr>
<td>Case 4</td>
<td>Minor component (2-person)</td>
</tr>
<tr>
<td>Case 5</td>
<td>Complex mixture (&gt;3-person) with <strong># of contributors</strong>; 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?
RMP 68%
CPI 19%
LR 12%
No Stat 1%
MIX13 Study (Case 02)

• Summary – Mock handgun (touch DNA), 3 person 6:1.5:1 mixture, total DNA amplified was 300 pg, potential for drop-out with the 2 low-level contributors. An additional contributor profile (suspect D) was provided, but is not in the mixture.

• Purpose – How many labs would consider this mixture as too complex to interpret?
Primary Goals

• Most labs – CPI for some combination of Suspects A, B and C using a limited number of loci.

• One lab included Suspect D (Not in the mixture).
Suspect 2A

Exclude

Range = 100M to 1.5 Quad

RMP

Suspect 2B

RMP

Exclude

CPI

Inc.

Suspect 2C

Exclude

CPI

Range = 2.8 to 15K

Inc.
~ 1 in 35
# 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></td>
<td></td>
<td>Excluded</td>
</tr>
<tr>
<td>2</td>
<td>6.74 Quad</td>
<td><strong>23.6</strong></td>
<td>Excluded</td>
<td>Excluded</td>
</tr>
<tr>
<td>3</td>
<td>Inconclusive - A, B, C</td>
<td></td>
<td></td>
<td>Excluded</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td><strong>9.4</strong></td>
<td></td>
<td>Excluded</td>
</tr>
<tr>
<td>5</td>
<td>4.1 Quint</td>
<td><strong>37</strong></td>
<td>Excluded</td>
<td>Excluded</td>
</tr>
<tr>
<td>6</td>
<td></td>
<td><strong>230</strong></td>
<td>Inconclusive</td>
<td>Excluded</td>
</tr>
<tr>
<td>7</td>
<td><strong>9.4</strong> for A, B</td>
<td></td>
<td>Excluded</td>
<td>Excluded</td>
</tr>
<tr>
<td>8</td>
<td><strong>37.3</strong> for A, B</td>
<td></td>
<td>Excluded</td>
<td>Excluded</td>
</tr>
</tbody>
</table>
MIX13 Study (Case 03)

• Summary – Mock sexual assault, 3 person 7:2:1 mixture, The two minor contributors are brothers, An additional contributor profile (suspect 3B) was provided, but is not in the mixture.

• Most of the suspected brother’s alleles are masked in the mixture

• Purpose – Given the relatedness of the individuals in the mixture, is this too complex for interpretation?
Primary Goals

• Only one lab included Suspect B (Not in the mixture)

• Most labs are using CPI stats for this case…
RMNE

- Random Man Not Excluded (CPE/CPI) – The probability that a *random person* (unrelated individual) would be excluded as a contributor to the observed DNA mixture.

- Only a few labs have stated this – “Due to the relatedness of the exemplars submitted for comparison, a statistical analysis cannot be provided at this time.”
Case 03

- CPI (44%)
- RMP (20%)
- Inconclusive (27%)
- Exclude (8%)
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?

• With all labs using the AT/ST – how much variation is expected?
Statistical Evaluation

- RMP 58%
- CPI 27%
- LR 15%
Intra-Laboratory Results (n = 8)

Log_{10}(LR)

 Analyst

CPI

9.3

RMP

18.9
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?
MIX13 Case 5 Outcomes with Suspect C (whose genotypes were not present in the mixture)

<table>
<thead>
<tr>
<th># Labs</th>
<th>Report Conclusions</th>
<th>Reasons given</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>Exclude Suspect C</td>
<td>detailed genotype checks (ID+); TrueAllele negative LR (ID+); assumed major/minor and suspects did not fit (ID+); 4 of 18 labs noted Penta E missing allele 15 (PP16HS)</td>
</tr>
<tr>
<td>3</td>
<td>Inconclusive</td>
<td>All these labs used PP16HS</td>
</tr>
<tr>
<td></td>
<td>with C only (A &amp; B included)</td>
<td></td>
</tr>
<tr>
<td>22</td>
<td>Inconclusive</td>
<td></td>
</tr>
<tr>
<td></td>
<td>for A, B, and C</td>
<td></td>
</tr>
<tr>
<td>76</td>
<td>Include &amp; provide CPI statistics</td>
<td>All over the road…</td>
</tr>
</tbody>
</table>

Range of CPI stats for Caucasian population:
FBI allele frequencies: 1 in 9 (Labs 12 & 54) to 1 in 344,000 (Lab 107)
Concerns raised with MIX13 study
Concerns raised with MIX13 (1)

• From my perspective – labs are substituting CPI for Interpretation when it comes to mixtures – even for simple mixtures.

Case 04 – D16 locus
If 10% stutter from the 12 allele (163 RFU) is part of the 11 allele, then the remaining peak (70 RFU) would be below the ST

No CPI labs excluded D16 from the stat (N = 22)

POI = 11, 12
Concerns raised with MIX13 (2)

- Another example – Case 02, D19 locus

**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
Concerns raised with MIX13 (3)

• Labs using RMP, LR – all over the place

• 4.6.2. It is not appropriate to calculate a composite statistic using multiple formulae for a multi-locus profile. For example, the CPI and RMP cannot be multiplied across loci in the statistical analysis of an individual DNA profile because they rely upon different fundamental assumptions about the number of contributors to the mixture.
One Lab’s Interpretation

Diagram showing a series of markers labeled RMP, CPI, and 2P, with associated numbers and values.
Switch to JOHN
Confronting Irreproducibility

**Cell line authentication** example:

- This field now has a **standard** (ANSI/ATCC ASN-0002-2011, Authentication of Human Cell Lines: Standardization of STR Profiling), which NIST scientists Margaret Kline and John Butler helped write

  - Yet many laboratories are not following it and some labs even deny there is a problem!

  - **Compliance is estimated to be only 15-30%**

See C&E News article (Dec 15, 2014) Vol. 92 (50) pp. 28-30

Remember that these labs are interpreting the same MIX05 electropherograms.

Some Differences in Reporting Statistics

<table>
<thead>
<tr>
<th>LabID</th>
<th>Kits Used</th>
<th>Case1</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Caucasians</td>
<td>African Americans</td>
<td>Hispanics</td>
<td></td>
</tr>
<tr>
<td>90</td>
<td>ProPlus/Cofiler</td>
<td>1.18E+15</td>
<td>2.13E+14</td>
<td>3.09E+15</td>
<td></td>
</tr>
<tr>
<td>34</td>
<td>ProPlus/Cofiler</td>
<td>2.40E+11</td>
<td>7.00E+09</td>
<td>9.80E+10</td>
<td></td>
</tr>
<tr>
<td>33</td>
<td>ProPlus/Cofiler</td>
<td>2.94E+08</td>
<td>1.12E+08</td>
<td>1.74E+09</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>ProPlus/Cofiler</td>
<td>40,000,000</td>
<td>3,500,000</td>
<td>280,000,000</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>ProPlus/Cofiler</td>
<td>4.14E+07</td>
<td>1.97E+07</td>
<td>1.54E+08</td>
<td></td>
</tr>
<tr>
<td>79</td>
<td>ProPlus/Cofiler</td>
<td>930,000</td>
<td>47,900</td>
<td>1,350,000</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>ProPlus/Cofiler</td>
<td>434,600</td>
<td>31,710</td>
<td>399,100</td>
<td></td>
</tr>
</tbody>
</table>

~10 orders of magnitude difference (10^5 to 10^{15}) based on which alleles were deduced and reported.

Slide presented to SWGDAM (Jan 2007) regarding MIX05 variation.
Further Examination of These 7 Labs

Possible Reasons for Variability in Reported Statistics:

- Different types of calculations (CPE vs RMP)
- Different loci included in calculations (due to different thresholds used)
- Different allele frequency population databases (most use PopStats)
- Use of victim (e.g., major component in Case 1) profile stats
DNA Mixture Workshop Attendees

50 states and >25 other countries

Butler (2015) Interpretation book Table 6.5 details 51 workshops with >7,000 attendees given by the author and colleagues from Sept 2005 to Feb 2014

Federal Labs
FBI
ATF
AFDIL
USACIL

AAFS 2006 (N=200)
AAFS 2008 (N=200)
AAFS 2011 (N=230)
TL Summit
Nov 20-21, 2013 (N=550)

4 regional workshops (N=200)

Alaska
Hawaii
Puerto Rico

ISHI 2010 (N=200)
ISHI 2011 (N=160)
ISHI 2012 (N=145)

NIST Webinar
April 12, 2013
>1000 continuing education certificates

Green = participants
# DNA Mixture Information Coverage in *Forensic DNA Typing* Textbooks

<table>
<thead>
<tr>
<th>Edition</th>
<th>Year</th>
<th>Pages</th>
<th>Pages</th>
<th>Pages</th>
<th>Pages</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st Edition</td>
<td>Jan 2001</td>
<td>335</td>
<td>13</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2nd Edition</td>
<td>Feb 2005</td>
<td>688</td>
<td>25</td>
<td></td>
<td></td>
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<tr>
<td>(3 volumes)</td>
<td>Aug 2011</td>
<td>704</td>
<td></td>
<td>1 p.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Oct 2014</td>
<td>608</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Chapters 6, 7, 12, 13
Appendix 4 (low-level, 2-person example)
People Know They Can Pass Audits and Still Be Doing Something Scientifically Invalid

- Within the past two years, I received a phone call during an ASCLD/LAB audit from a laboratory director (and the next day from DNA TL) sharing a concern raised by an auditor about how their laboratory was performing DNA mixture interpretation (they were doing what is known as “suspect-driven CPI”)
- When I explained that what their laboratory was doing was not scientifically sound, the response was “I don’t care because I can still pass my audit! We have a protocol and we are following it.”
### Audit Document with Current FBI Quality Assurance Standards (2011)

<table>
<thead>
<tr>
<th>Standard</th>
<th>Question</th>
<th>Yes</th>
<th>No</th>
<th>N/A</th>
</tr>
</thead>
<tbody>
<tr>
<td>9.6</td>
<td>Does the laboratory <strong>have and follow written guidelines for the interpretation of data?</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9.6.1</td>
<td>Does the laboratory <strong>verify that all control results meet the laboratory’s interpretation guidelines for all reported results?</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9.6.2</td>
<td>Has the <strong>1996 National Research Council report</strong> and/or a court-directed method been used for the statistical interpretation of a DNA profile for a given population and/or hypothesis or relatedness, and are these calculations derived from an established population database(s) appropriate for the calculation?</td>
<td>NRC II (1996), p. 130 emphasizes LR over CPI as a better approach for DNA mixtures</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9.6.3</td>
<td>Does the laboratory <strong>have and follow specific documented statistical interpretation guidelines</strong> if genetic analyses that are not addressed by Standard 9.6.2 are being performed?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9.6.4</td>
<td>Does the laboratory <strong>have and follow documented procedures for mixture interpretation</strong> to include the following:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>a. Major and minor contributors?</td>
<td>Yes</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td></td>
<td>b. Inclusions and exclusions?</td>
<td>Yes</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td></td>
<td>c. Policies for reporting results and statistics?</td>
<td>Yes</td>
<td>No</td>
<td></td>
</tr>
</tbody>
</table>

From FBI QAS Audit Document

Discussion (following Section 9.6)

A laboratory shall have and follow written guidelines for the interpretation of data that are **supported through its validation**. A laboratory shall verify that all control results meet the laboratory’s interpretation guidelines for all reported results. A documented method must exist to demonstrate that control values are verified when used (e.g., check-off, technical review).

The statistical interpretation of autosomal loci shall be made following recommendations 4.1, 4.2, or 4.3, as deemed applicable, of the National Research Council report titled “The Evaluation of Forensic DNA Evidence” (1996) and/or a court-directed method. **The laboratory shall provide documentation for the interpretation method being used.** These calculations shall be derived from a documented population database(s) appropriate for the calculation.

If a laboratory is performing genetic analyses not addressed by Standard 9.6.2, (e.g., Y-chromosome, mtDNA), the laboratory shall have and follow documented statistical interpretation guidelines for that testing.

**A laboratory shall have and follow a documented procedure for mixture interpretation supported by its validation.** Based upon a laboratory’s validation, it shall have and follow procedures to discern major and minor contributors, inclusions and exclusions, and policies for reporting results and applicable statistics.
SWGDAM Guidelines are Always Not Followed

• How many labs ignore the SWGDAM guidelines because they are not auditable standards?
  – Example: does a laboratory have and use a stochastic threshold (ST) that is necessary when using a CPI approach to mixtures?

• In June 2013 I received a phone call from a Los Angeles prosecutor who expressed concern that the two labs she used were getting different results from DNA mixtures (LASD uses a ST while LAPD does not → both are ASCLD/LAB accredited for DNA testing)
ISO/IEC 17025 validation requirement

5.4.5 Validation of methods

- 5.4.5.2 The laboratory shall validate ... standard methods used outside their intended scope [e.g., CPI stats on complex DNA mixtures] ... to confirm that the methods are fit for the intended use. The validation shall be as extensive as is necessary to meet the needs of the given application...

Note 2 The techniques used for the determination of the performance of a method should be one of, or a combination of, the following:
- (a) calibration using reference standards or materials;
- (b) comparison of results achieved with other methods;
- (c) interlaboratory comparisons;
- (d) systematic assessment of the factors influencing the result;
- (e) assessment of the uncertainty of the results based on scientific understanding of the theoretical principles of the method and practical experience.
The Perils of “Validation Extrapolation”

“Laboratories cannot adequately understand performance characteristics of low-template, complex DNA mixtures from having run a few high-template, simple DNA mixtures such as a few mixtures of 9947A and 9948. Attempts at validation extrapolation, where a simple two-person mixture study is expected to provide guidance for proper interpretation of less optimal mixtures, will not enable creation of robust protocols that provide consistent, reliable results. Every DNA interpretation protocol should be based on validation data, the scientific literature, and experience (SWGDAM 2010). Empirical data are always needed to establish limitations for a technique.”


PCR
Total DNA amplified
13, 17
Genotype
13, 14
Mixture Ratio of Components

Validation establishes variation and limits in the processes involved

Potential STR alleles
12, 13, 14, 15, 16, 17, 18, 19

D18S51
portion of a CE electropherogram

Extraction
PCR
CE Injection
CE Detection

Infer possible genotypes & determine sample components
From available data

False Sample Components
Sample Processing
DNA Data Obtained

Potential Allele Overlap & Stacking

Number of Contributors (sample components)

Potential Sample
Components

Total DNA amplified

Validation

Extraction
PCR
DNA Data Obtained

Female
13, 17

Male
13, 14
in a complex mixture. Of course, if peaks are observed below a stochastic threshold in a complex mixture, then allele drop-out of a sister allele is possible as with simple two-person mixtures and the detected allele could be a false homozygote.

Validation Needs to Match Sample Types

If a laboratory desires to develop appropriate protocols that will enable reliable interpretation of DNA from low-level DNA or mixtures involving three or more contributors, then validation studies need to be performed with known samples that mimic the amounts of DNA and complexity of profiles where stochastic effects and allele dropout are expected. In short, three- or four-person mixtures of known genotypes should be mixed at specific ratios and amplified multiple times. Then these complex mixture profiles should be subjected to interpretation approaches to see if a true contributor is appropriately associated with the mixture and if non-contributors are appropriately excluded.

In my opinion, a laboratory cannot run a single two-person mixture series (e.g. 9:1, 5:1, 3:1, 1:1, 1:3, 1:5, and 1:9) and feel confident that minimum requirements for “mixture validation” have been met. This type of a limited validation may simply be able to help determine that a minor contributor can be detected down to a certain level. Determining that a mixture exists is not the same as fully interpreting a mixture. Developing robust interpretation protocols will require considering more samples — especially ones that go beyond a cursory combination of control samples 9947A and 9948 (D.N.A. Box 7.1).
“If a laboratory desires to develop appropriate protocols that will enable reliable interpretation of DNA from low-level DNA or mixtures involving three or more contributors, then validation studies need to be performed with known samples that mimic the amounts of DNA and complexity of profiles where stochastic effects and allele dropout are expected. In short, three- or four-person mixtures of known genotypes should be mixed at specific ratios and amplified multiple times. Then these complex mixture profiles should be subjected to interpretation approaches to see if a true contributor is appropriately associated with the mixture and if non-contributors are appropriately excluded.”

In my opinion, a laboratory cannot run a single two-person mixture series (e.g. 9:1, 5:1, 3:1, 1:1, 1:3, 1:5, and 1:9) and feel confident that minimum requirements for “mixture validation” have been met. This type of a limited validation may simply be able to help determine that a minor contributor can be detected down to a certain level. Determining that a mixture exists is not the same as fully interpreting a mixture. Developing robust interpretation protocols will require considering more samples – especially ones that go beyond a cursory combination of control samples 9947A and 9948 (D.N.A. Box 7.1).
D.N.A. Box 7.2  
**Number of Possible Genotype Combinations**

<table>
<thead>
<tr>
<th>Contributors</th>
<th>1 allele</th>
<th>2 alleles</th>
<th>3 alleles</th>
<th>4 alleles</th>
<th>5 alleles</th>
<th>6 alleles</th>
<th>7 alleles</th>
<th>8 alleles</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 contributor</td>
<td>(1 hom, 0 het)</td>
<td>(2 hom, 1 het)</td>
<td>(3 hom, 3 het)</td>
<td>(4 hom, 6 het)</td>
<td>(5 hom, 10 het)</td>
<td>(6 hom, 15 het)</td>
<td>(7 hom, 21 het)</td>
<td>(8 hom, 28 het)</td>
</tr>
<tr>
<td>2 contributors</td>
<td>(2 hom, 1 shared)</td>
<td>(3 hom, 1 shared)</td>
<td>(4 hom, 1 shared)</td>
<td>(5 hom, 2 shared)</td>
<td>(6 hom, 2 shared)</td>
<td>(7 hom, 2 shared)</td>
<td>(8 hom, 2 shared)</td>
<td>(9 hom, 2 shared)</td>
</tr>
<tr>
<td>3 contributors</td>
<td>(6 hom, 3 shared)</td>
<td>(11 hom, 5 shared)</td>
<td>(16 hom, 8 shared)</td>
<td>(21 hom, 11 shared)</td>
<td>(26 hom, 14 shared)</td>
<td>(31 hom, 17 shared)</td>
<td>(36 hom, 20 shared)</td>
<td>(41 hom, 23 shared)</td>
</tr>
<tr>
<td>4 contributors</td>
<td>(10 hom, 6 shared)</td>
<td>(15 hom, 9 shared)</td>
<td>(20 hom, 12 shared)</td>
<td>(25 hom, 15 shared)</td>
<td>(30 hom, 18 shared)</td>
<td>(35 hom, 21 shared)</td>
<td>(40 hom, 24 shared)</td>
<td>(45 hom, 27 shared)</td>
</tr>
</tbody>
</table>

*Complexity Increases with More Contributors*
Some Key Principles

• Everything in **science involves** mapping observed data to **models** (“hypotheses”)
  – Hardy Weinberg Equilibrium (HWE) models expected genotype frequencies ($p^2$ or $2pq$) assuming unrelated individuals
  – Theta corrections ($\theta=0.01$ or $\theta=0.03$) model potential variation from assumptions of unrelated individuals

• All **models require assumptions**, some of which are more reliable than others depending on data obtained

• **Validation studies generate data that inform the model being used** or enable a model to be constructed
  – For example, a test for HWE is comparing population (validation) data to a model to see goodness-of-fit

• **Genotypes—not alleles—matter** in deciphering mixtures

• **Probabilistic genotyping involves modeling observed data** against potential genotype combinations
Why are we where we are today?

• The incredible success of DNA has lead to more sensitive methods and more “touch-evidence” samples being provided which has led to more complex mixtures (we are pushing the envelope)
  – Lower template DNA profiles have more uncertainty associated with them in terms of allele peak height variation

• Statistical interpretation techniques have not kept pace with the methodology improvements
  – Much of the U.S. forensic DNA community is effectively using a 1992 statistical tool on 21st century data
What does the scientific literature say?

• ISFG DNA Commission (2006) states that likelihood ratios (LRs) are preferred over use of the combined probability of inclusion (CPI) [termed the random man not excluded, RMNE] because LRs address the question about whether a specific suspect’s profile may be included in a mixture; NRC II (1996) p. 130 also supports ISFG.

• Yet most U.S. labs still use CPI in large measure because of the DAB 2000 statement that use of either CPI or LR is okay (I would argue though that this was made in the context of simple two-person mixtures, primarily sexual assault evidence being done at the time).

• SWGDAM 2010 Autosomal STR Guidelines provide guidance for use of CPI, LR, and deconvolution with RMP (again primarily with a focus on two-person mixtures that are not low-level touch evidence – see SWGDAM.org FAQ statement).
Historical Perspective on DNA Mixture Approaches

**Probabilistic genotyping software in development...**

LR commonly used in Europe and other labs around the world

- **1991**
  - Evett et al. describe LRs for mixtures

- **1992**
  - NRC I report (p.130) supports CPI

- **1996**
  - Weir et al. describe LRs for mixtures

- **1997**
  - NRC II report (p.130) supports LR

- **2000**
  - 2-person mixtures predominate

- **2006**
  - ISFG DNA Commission LR over CPI

- **2008**
  - NIJ burglary report increases touch evidence

- **2010**
  - ISFG DNA Commission LR with drop-out

- **2012**
  - SWGDAM guidelines (RMP, CPI, LR)

- **2013**
  - DNA TL Summit

- **2013**
  - Probabilistic genotyping software in development...

1985

- RMNE (CPI) used in paternity testing

2010

- DAB Stats (Feb 2000) CPI and LR okay

2012

- CPI becomes routine in U.S.

**LR = likelihood ratio**

**CPI = combined probability of inclusion**

**RMNE = random man not excluded**
Forensic Laboratories Accredited for Biology/DNA

<table>
<thead>
<tr>
<th>Accrediting Body</th>
<th># Labs</th>
<th>% total</th>
</tr>
</thead>
<tbody>
<tr>
<td>ASCLD/LAB</td>
<td>240</td>
<td>88.6%</td>
</tr>
<tr>
<td>FQS ANSI-ASQ National Accreditation Board (ANAB)</td>
<td>31</td>
<td>11%</td>
</tr>
<tr>
<td>A2LA</td>
<td>1</td>
<td>0.4%</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>271</strong></td>
<td></td>
</tr>
</tbody>
</table>

ASCLD/LAB 240 labs: 26 legacy (11%) + 214 international (89%)
FQS: about half government labs and half private labs

Based on searches of the following websites on 3 Jan 2015:
http://www.ascld-lab.org/accredited-laboratory-index/
http://www.a2la.org/dirsearchnew/searchbyspec.cfm?fieldpk=18&title=Forensic
Important Questions to Consider

• Do we have laboratories getting incorrect results in spite of being accredited by ASCLD/LAB (or others) to ISO/IEC 17025?
  – Most MIX05 and all MIX13 participants were from accredited forensic DNA laboratories…

• If being accredited does not lead to getting consistent and accurate DNA mixture results, will this observation erode confidence in the accreditation process?
  – Will more specificity in the QAS and other audit documents help in the future?
What does ASCLD/LAB want in terms of specificity in standards?

- SWGDAM and OSAC can work to produce documents with more specificity to aid interpretation but **these documents must be enforceable by accrediting bodies or they may be ignored by some**

- SWGDAM Autosomal STR Interpretation Committee is current revising the 2010 guidelines (see SWGDAM.org FAQ)
  - Current version including a detailed examples document is almost 100 pages long
Thank you for your attention

STRBase validation information available at: http://www.cstl.nist.gov/strbase/validation.htm
STRBase mixture information available at: http://www.cstl.nist.gov/strbase/mixture.htm

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**Postscript:** This slide was added after the meeting upon further thought about effective preparation to handle complex DNA mixtures

- **Forensic DNA testing** can be equated to different levels of **mathematics**:
  1. **Single-source samples** (e.g., reference samples) are like **basic arithmetic**
  2. **Two-person mixtures** (e.g., sexual assault evidence) are like **algebra**
  3. **Complex mixtures** (e.g., touch DNA) are like **calculus**

- **Validation studies** can be considered classroom instruction to help understand the topic and prepare for the casework “exams”

- **Proficiency tests** are like homework – a graded experience where feedback is received to prepare students for the casework exams when the true answers are not known to the test takers

- If homework is not challenging enough or if your classroom instruction is not to the level needed to be prepared, how can you hope to pass the test? Algebraic principles are necessary for calculus (just like two-person mixture principles apply to complex mixtures), but to truly solve calculus problems and complex mixtures a different level of thinking is required and more study is necessary. *Would you want to go into a calculus final with only instruction in algebra and experience doing only basic math homework problems?*