Complex Mixtures

The more you know the harder they get!

Charlotte J. Word, Ph.D.
Two-Person Mixtures

- Lots of experience and familiarity with two-person mixtures, literature, validation studies, training samples
- Published guidelines for interpretation
- Well developed SOPs for interpretation
- Routine amount of input DNA in amplification generally leads to nice profiles
Two-Person Mixtures

High Certainty Leads to High Confidence

- Only two contributors present
- Distinguishing stutter/artifacts from true alleles
- Use stochastic threshold to assess if all alleles are likely present vs. LT DNA with stochastic effects
- Assessing mixture ratio (distinguishable/major:minor or indistinguishable mixture)
- Deducing second contributor if one contributor is known
Two-Person Mixtures

Assume number of contributors is two:

- Aids in allele association at each locus based on peak height ratios
- May aid in genotype association for full profile based on mixture ratio
- Statistics calculations often straightforward
Complex Mixtures

- Multiple contributors
  - 3- & 4- person (or more!)

- Relatives in Mixtures
Complex Mixture Interpretation

Is hard because the parameters used to interpret two-person mixtures often may not be directly applicable to complex mixtures.
How many contributors assumed for interpretation?

Can this be interpreted?

Is there a major contributor?
Complex Mixture – Allele Summary

- 6 alleles at 2 loci
- 5 alleles at 3 loci
- 4 alleles at 7 loci
- 3 alleles at 2 loci
- 2 alleles at 1 locus
- 1 allele at 0 loci
- 63 total alleles
Two-Person Mixtures

<table>
<thead>
<tr>
<th>Observed profile</th>
<th>4 alleles</th>
<th>3 alleles</th>
<th>2 alleles</th>
<th>1 allele</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>All heterozygotes and non-overlapping alleles</td>
<td>Heterozygote + heterozygote, one overlapping allele</td>
<td>Heterozygote + homozygote, no overlapping alleles</td>
<td>Homozygote + homozygote, overlapping allele</td>
</tr>
<tr>
<td><img src="image1" alt="Observed profile A" /></td>
<td><img src="image2" alt="Observed profile B" /></td>
<td><img src="image3" alt="Observed profile A" /></td>
<td><img src="image4" alt="Observed profile A" /></td>
<td><img src="image5" alt="Observed profile A" /></td>
</tr>
<tr>
<td><img src="image6" alt="Observed profile A" /></td>
<td><img src="image7" alt="Observed profile B" /></td>
<td><img src="image8" alt="Observed profile A" /></td>
<td><img src="image9" alt="Observed profile A" /></td>
<td><img src="image10" alt="Observed profile A" /></td>
</tr>
<tr>
<td><img src="image11" alt="Observed profile A" /></td>
<td><img src="image12" alt="Observed profile B" /></td>
<td><img src="image13" alt="Observed profile A" /></td>
<td><img src="image14" alt="Observed profile A" /></td>
<td><img src="image15" alt="Observed profile A" /></td>
</tr>
<tr>
<td><img src="image16" alt="Observed profile A" /></td>
<td><img src="image17" alt="Observed profile B" /></td>
<td><img src="image18" alt="Observed profile A" /></td>
<td><img src="image19" alt="Observed profile A" /></td>
<td><img src="image20" alt="Observed profile A" /></td>
</tr>
</tbody>
</table>

14 total combinations
<table>
<thead>
<tr>
<th>Alleles</th>
<th>Combinations</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>All heterozygotes and non-overlapping alleles</td>
</tr>
<tr>
<td>5</td>
<td>Two heterozygotes and one homozygote&lt;br&gt;Three heterozygotes, one overlapping allele</td>
</tr>
<tr>
<td>4</td>
<td>Six combinations of heterozygotes, homozygotes and overlapping alleles</td>
</tr>
<tr>
<td>3</td>
<td>Eight combinations of heterozygotes, homozygotes, and overlapping alleles</td>
</tr>
<tr>
<td>2</td>
<td>Five combinations of heterozygotes, homozygotes, and overlapping alleles</td>
</tr>
<tr>
<td>1</td>
<td>All homozygotes, overlapping allele</td>
</tr>
</tbody>
</table>

*Observed profile: 3 alleles*
<table>
<thead>
<tr>
<th>Observed profile</th>
<th>4-Person Mixtures</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>8 alleles</td>
</tr>
<tr>
<td></td>
<td>All heterozygotes and non-overlapping alleles</td>
</tr>
<tr>
<td></td>
<td>7 alleles</td>
</tr>
<tr>
<td></td>
<td>Several combinations of heterozygotes, homozygotes, and overlapping alleles</td>
</tr>
<tr>
<td></td>
<td>6 alleles</td>
</tr>
<tr>
<td></td>
<td>Many combinations</td>
</tr>
<tr>
<td></td>
<td>5 alleles</td>
</tr>
<tr>
<td></td>
<td>Many combinations</td>
</tr>
<tr>
<td></td>
<td>4 alleles</td>
</tr>
<tr>
<td></td>
<td>Many combinations</td>
</tr>
<tr>
<td></td>
<td>3 alleles</td>
</tr>
<tr>
<td></td>
<td>Many combinations</td>
</tr>
<tr>
<td></td>
<td>2 alleles</td>
</tr>
<tr>
<td></td>
<td>Many combinations</td>
</tr>
<tr>
<td></td>
<td>1 allele</td>
</tr>
<tr>
<td></td>
<td>All homozygotes, overlapping allele</td>
</tr>
</tbody>
</table>
## Two-Person Simulated Mixtures – SGM\(^+\)
### Number of Alleles at each Locus

**Table 1**
The probability of observing a given number of alleles in a two-person mixtures for simulated profiles at the SGM\(^{+\text{TM}}\) loci

<table>
<thead>
<tr>
<th>Loci</th>
<th>No. of alleles</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>D3</td>
<td>0.011</td>
</tr>
<tr>
<td>vWA</td>
<td>0.008</td>
</tr>
<tr>
<td>D16</td>
<td>0.016</td>
</tr>
<tr>
<td>D2</td>
<td>0.003</td>
</tr>
<tr>
<td>D8</td>
<td>0.011</td>
</tr>
<tr>
<td>D21</td>
<td>0.007</td>
</tr>
<tr>
<td>D18</td>
<td>0.003</td>
</tr>
<tr>
<td>D19</td>
<td>0.020</td>
</tr>
<tr>
<td>THO</td>
<td>0.016</td>
</tr>
<tr>
<td>FGA</td>
<td>0.003</td>
</tr>
</tbody>
</table>
### Table 2

Number of Alleles at each Locus

The probability of observing a given number of alleles in a three-person mixtures for simulated profiles at the SGM$^+\text{TM}$ loci.

<table>
<thead>
<tr>
<th>Loci</th>
<th>No. of alleles showing</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>D3</td>
<td>0.000</td>
</tr>
<tr>
<td>vWA</td>
<td>0.000</td>
</tr>
<tr>
<td>D16</td>
<td>0.001</td>
</tr>
<tr>
<td>D2</td>
<td>0.000</td>
</tr>
<tr>
<td>D8</td>
<td>0.001</td>
</tr>
<tr>
<td>D21</td>
<td>0.000</td>
</tr>
<tr>
<td>D18</td>
<td>0.000</td>
</tr>
<tr>
<td>D19</td>
<td>0.003</td>
</tr>
<tr>
<td>THO</td>
<td>0.001</td>
</tr>
<tr>
<td>FGA</td>
<td>0.000</td>
</tr>
</tbody>
</table>

Estimating the number of contributors to two-, three-, and four-person mixtures containing DNA in high template and low template amounts

Perez et al., Croat Med J. 2011; 52:314-26
Two-Person Mixture Studies
Summary

- **Always** recognized as a mixture – no risk of confusing as a single-source
  - Loci with 3 or 4 alleles
  - Peak height ratio imbalance at loci with 2 alleles
- Observe more loci with 2 or 3 alleles than 4 alleles – even when DNA from two heterozygous individuals were mixed
- 49 or fewer total alleles

Three-Person Mixture Studies
Summary

• No risk of confusing as a single-source
• Small risk of confusing with two-person mixture
  – Observe at least one locus with 5 or 6 alleles in ~97% of profiles (3% have ≤4 alleles)
  – 3% profiles look like 2-person mixture
  – Risk if LT-DNA, degradation, inhibition, primer mutation to look like 2-person mixture
• Most loci have 3 or 4 alleles
• 52-59 total alleles
Four-Person Mixture Studies
Summary

• No risk of confusing as a single-source
• Very small risk of confusing with two-person mixture
  – Likely to have peak height imbalance
• Very small number of loci with 8 alleles and very few with 7 alleles
  – High risk of confusing with three-person mixture
  – Risk if LT-DNA, degradation, inhibition, primer mutation
• ≥65 total alleles

Four-Person Mixture Studies Summary

>70% of 4-person mixtures would NOT be recognized as 4-person mixtures based on allele count

Five-, Six- Person Mixture Studies Summary

• >99% of 5 person mixtures would look like 4 person mixtures (~60%) or 3-person mixtures (~40%)

• Most 6 person mixtures would look like 5 person mixture (6%), 4-person mixtures (80%) or 3-person mixtures (14%)

Complex Mixture – Allele Summary

- 6 alleles at 2 loci
- 5 alleles at 3 loci
- 4 alleles at 7 loci
- 3 alleles at 2 loci
- 2 alleles at 1 locus
- 1 allele at 0 loci
- 63 total alleles

A 4-person mixture @ 1:1:1:2 ratio!!
Complex Mixtures

Mixtures with Relatives

Parent-Child
Sibling-Sibling
**Parent + Child**

- **1 allele**
  - Homozygote + homozygote, one shared allele

- **2 alleles**
  - Heterozygote + heterozygote, two shared alleles
  - Heterozygote + homozygote, one shared allele

- **Maximum: 3 alleles**
  - Both heterozygote, one shared allele

**Mixture DNA Profile Pattern**

**ALLELE SHARE AT EACH LOCUS**
<table>
<thead>
<tr>
<th>Genotypes of Children</th>
<th>% Sibling Allele Sharing</th>
</tr>
</thead>
<tbody>
<tr>
<td>AC or AD or BC or BD</td>
<td>0%, 50% or 100%</td>
</tr>
<tr>
<td>AB or AC or BB or BC</td>
<td>0%, 50% or 100%</td>
</tr>
<tr>
<td>AB/BA or AA or BB</td>
<td>0%, 50% or 100%</td>
</tr>
<tr>
<td>AC or BC</td>
<td>50% or 100%</td>
</tr>
<tr>
<td>AA or BA</td>
<td>50% or 100%</td>
</tr>
<tr>
<td>AB</td>
<td>100%</td>
</tr>
<tr>
<td>AA</td>
<td>100%</td>
</tr>
</tbody>
</table>

*P1 = Parent 1; P2 = Parent 2*
Simulated profiles with Profiler Plus

315 mother-child pairs

91 full-sib pairs

Mixtures with Relatives – Summary

Parent-Child

• Expect at least 50% allele share
• Expect at least one shared allele at each locus
• Maximum 3 alleles per locus (in absence of mutation)
• If test X loci, expect >X allele shares (9-14 Profiler Plus; 13-20 CODIS)
Mixtures with Relatives – Summary

Sibling-Sibling

- Expect at least 50% allele share overall, but variable: 7-16 Profiler Plus; 12-22 CODIS ($\geq X-1$)
- Expect 0, 50 or 100% allele share at each locus
- Expect at least one allele share at 9-13 loci (CODIS data)
Are the contributors to this profile related?
Mixtures with Relatives – Working Backwards from Mixed DNA Profile

- With mixed DNA profile from unknowns, may not know if alleles are shared
- Data in the graphs are not helpful

11,12 + 11,13
or
11,11 + 12,13

Relative?  
Parent-Child?  
Sibs?

Unrelated?
INCREASED COMPLEXITY

HIGH UNCERTAINTY

LACK OF CONFIDENCE
Complex Mixtures

More Uncertainty and Lack of Confidence

- Peak vs. Artifacts
- Stutter?
- Pull-up?
- True Allele?
Complex Mixtures

More Uncertainty and Lack of Confidence

- High likelihood that DNA from one or more contributors is below optimal range
- LT DNA = stochastic effects
- Missing alleles? (allele drop out)
- Elevated Stutter? True allele vs. Stutter?
- Allele drop-in?
Complex Mixtures

More Uncertainty and Lack of Confidence

- Stochastic threshold
  - Only meaningful for the peaks below the value – may be missing sister allele
  - All alleles present?
Complex Mixtures

More Uncertainty and Lack of Confidence

- Stochastic threshold
- NO meaning for peaks above the value –
  - Major contributor?
  - Shared alleles? How many shares? Relatives or unrelated

Major vs. Shared alleles
Complex Mixtures

More Uncertainty and Lack of Confidence

- Peak height ratios have no meaning at most or all loci
  - Cannot use to associate alleles into genotypes
  - Ability to deduce other contributors decreased even if know one contributor
Complex Mixtures

More Uncertainty and Lack of Confidence

- Mixture ratio cannot be calculated
- Different amount from each contributor likely with no way to determine
- Cannot use to associate genotypes into profiles
Complex Mixtures

More Uncertainty and Lack of Confidence

- Number of contributors – maximum allele/minimum number often an underestimate
- What number to assume?
- May need to interpret under multiple assumptions (especially if the conclusion changes)
Complex Mixtures

False Inclusions
- Increased risk as # of alleles increase
- Cannot assign meaningful statistical frequency

Exclusions less likely
- Can anyone be excluded if LT DNA present?
- Partial “inclusions”

Inconclusive reporting increased
Conclusions

• Criteria routinely used in crime laboratories for the interpretation of two-person mixtures may not apply for most complex mixtures

• LT-DNA, degradation, inhibition play more significant role

• Additional complex mixtures need to be generated and evaluated for establishment of interpretation guidelines
Thank you!

Charlotte J. Word, Ph.D.

cjword@comcast.net

301-527-1350