Current SWGDAM Guidelines
<table>
<thead>
<tr>
<th>United States</th>
<th>Europe</th>
</tr>
</thead>
<tbody>
<tr>
<td>FBI (DAB) Quality Assurance Standards</td>
<td>ENFSI Policies</td>
</tr>
<tr>
<td>NDIS Procedures</td>
<td>ISFG Recommendations (DNA Commission)</td>
</tr>
<tr>
<td><strong>SWGDAM Guidelines</strong></td>
<td>National Recommendations</td>
</tr>
<tr>
<td>Laboratory Protocols (SOPs)</td>
<td>Laboratory Protocols (SOPs)</td>
</tr>
<tr>
<td>Individual Analyst Practice</td>
<td>Individual Analyst Practice</td>
</tr>
<tr>
<td>Each Case Report</td>
<td>Each Case Report</td>
</tr>
</tbody>
</table>

**Validation**

Training & Experience

DNA Mixture Interpretation  
Current SWGDAM Guidelines  
2
A brief review of the literature...
ISFG DNA Commission on Mixture Interpretation

ISFG DNA Commission on Mixture Interpretation

Who is the ISFG and why do their recommendations matter?
International Society of Forensic Genetics

http://www.isfg.org/

• An international organization responsible for the promotion of scientific knowledge in the field of genetic markers analyzed with forensic purposes.

• Founded in 1968 and represents more than 1100 members from over 60 countries.

• DNA Commissions regularly offer recommendations on forensic genetic analysis.
DNA Commission of the ISFG

- DNA polymorphisms (1989)
- PCR based polymorphisms (1992)
- Naming variant alleles (1994)
- Repeat nomenclature (1997)
- Mitochondrial DNA (2000)
- Y-STR use in forensic analysis (2001)
- Mixture Interpretation (2006)
- Disaster Victim Identification (2007)
- Biostatistics for Parentage Analysis (2007)
- Non-human (animal) DNA (2010)

http://www.isfg.org/Publications/DNA+Commission
**ISFG Executive Committee**

**President**  
Niels Morling  
(Copenhagen, Denmark)

**Vice-President**  
Peter Schneider  
(Köln, Germany)

**Working Party Representative**  
Mecki Prinz  
(New York City, USA)

**Treasurer**  
Leonor Gusmão  
(Porto, Portugal)

**Secretary**  
Wolfgang Mayr  
(Vienna, Austria)

Angel Carracedo  
FSI Genetics Editor-in-Chief  
(former ISFG President, VP)  
(Santiago de Compostela, Spain)
Authors of ISFG Mixture Article

Peter Gill
Pioneer of forensic DNA techniques and applications
UK’s Forensic Science Service (1978-2008)
University of Strathclyde (Apr 2008 – present)

The Mathematicians/Statisticians

Charles Brenner
DNA-View, Berkeley, CA, USA

John Buckleton
ESR, Auckland, New Zealand

Michael Krawczak
Christian-Albrechts-University, Kiel, Germany

Bruce Weir
U. Washington, Seattle, USA
Available for download from the ISFG Website: http://www.isfg.org/Publication;Gill2006

DNA commission of the International Society of Forensic Genetics: Recommendations on the interpretation of mixtures

P. Gill a,*, C.H. Brenner b, J.S. Buckleton c, A. Carracedo d, M. Krawczak e, W.R. Mayr f, N. Morling g, M. Prinz h, P.M. Schneider i, B.S. Weir j

a Forensic Science Service, Trident Court, 2960 Solihull Parkway, Birmingham, UK

“Our discussions have highlighted a significant need for continuing education and research into this area.”

Received 4 April 2006; accepted 10 April 2006
Available online 5 June 2006

“...These recommendations have been written to serve two purposes: to define a generally acceptable mathematical approach for typical mixture scenarios and to address open questions where practical and generally accepted solutions do not yet exist. This has been done to stimulate the discussion among scientists in this field. The aim is to invite proposals and criticism in the form of comments and letters to the editors of this journal... We are hoping to continue the process to allow the DNA Commission to critically revise or extend these recommendations in due time...”
Summary of ISFG Recommendations on Mixture Interpretation

1. The likelihood ratio (LR) is the preferred statistical method for mixtures over RMNE.
2. Scientists should be trained in and use LRs.
3. Methods to calculate LRs of mixtures are cited.
5. Prosecution determines $H_p$ and defense determines $H_d$ and multiple propositions may be evaluated.
6. When minor alleles are the same size as stutters of major alleles, then they are indistinguishable.
7. Allele dropout to explain evidence can only be used with low signal data.
8. No statistical interpretation should be performed on alleles below threshold.
9. Stochastic effects limit usefulness of heterozygote balance and mixture proportion estimates with low level DNA.
Responses to ISFG DNA Commission Mixture Recommendations

- **UK Response**

- **German Stain Commission**
  - Schneider *et al.* (2006) *Rechtsmedizin* 16:401-404 (German version)
Allgemeine Empfehlungen der Spurenkommission zur Bewertung von DNA-Mischspuren

The German Stain Commission: recommendations for the interpretation of mixed stains

P. M. Schneider · R. Fimmers · W. Keil · G. Molsberger · D. Patzelt · W. Pflug · T. Rothämel · H. Schmitter · H. Schneider · B. Brinkmann
Responses to ISFG DNA Commission Mixture Recommendations

- **UK Response**

- **German Stain Commission**
  - Schneider *et al.* (2006) *Rechtsmedizin* 16:401-404 (German version)

- **ENFSI Policy Statement**

- **New Zealand/Australia Support Statement**
  - Stringer *et al.* (2009) *FSI Genetics*

- **SWGDAM** — Mixture Interpretation Subcommittee initiated in Jan. 2007
SWGDAM Mixture Interpretation
Subcommittee

- John Butler (NIST) – chair
- Mike Adamowicz (CT)
- Terry Coons (OR)
- Jeff Modler (RCMP)
- Phil Kinsey (MT)
- Todd Bille (ATF)
- Allison Eastman (NYSP)
- Bruce Heidebrecht (MD)
- Tamyra Moretti (FBI DNA Unit I)
- George Carmody (Carleton U)
- Roger Frappier (CFS-Toronto)
- Jack Ballantyne (UCF/NCFS)

Gary Sims (CA DOJ) - co-chair
Joanne Sgueglia (MA)
Gary Shutler (WA)
Cecelia Crouse (PBSO)
Hiron Poon (RCMP)
Steve Lambert (SC)
Steven Myers (CA DOJ)
Ann Gross (MN BCA)

The 15 members in bold font were involved with most of the writing (July-Oct 2009)
SWGDAM STR Guidelines

- Guidelines were approved at the January 14, 2010 SWGDAM meeting. The guidelines were publically released on April 8, 2010 on the FBI website for the CODIS group:

  http://www.fbi.gov/hq/lab/html/codis1.htm
  (under “Quality Assurance” information)

  http://www.fbi.gov/about-us/lab/codis/swgdam.pdf (PDF)

  http://www.fbi.gov/about-us/lab/codis/swgdam-interpretation-guidelines (HTML text)
SWGDAM Interpretation Guidelines for Autosomal STR Typing by Forensic DNA Testing Laboratories

- Guidelines
  - Not Standards
  - No lab should be audited against this document

- Autosomal STR Typing
  - This document does not address Y-STRs, mtDNA testing, or CODIS entries

- Forensic DNA Testing Laboratories
  - Databasing labs may have different issues since they are working with known single source samples
3. Interpretation of Results

3.1. The laboratory should define conditions in which the data would lead to the conclusion that the source of the DNA is either from a single person or more than one person. This may be accomplished by an examination of the number of alleles at each locus, peak height ratios, and/or band intensities.

3.1.1. Single Contributor: A sample may be considered to be from a single contributor when the observed number of alleles at each locus and the signal intensity ratios of alleles at a locus are consistent with a profile from a single contributor. All loci should be evaluated in making this determination.

3.1.2. Mixtures With Major/Minor Contributors: A sample may be considered to consist of a mixture of major and minor contributors if there is a distinct contrast in signal intensities among the alleles. The difference is evaluated on a case-by-case basis. All loci should be evaluated in making this determination.

3.1.3. Mixtures With a Known Contributor(s): In some cases, when one of the contributors (e.g., the victim) is known, the genetic profile of the unknown contributor may be inferred. Depending on the profiles in the specific instance, this can be accomplished by subtracting the contribution of the known donor from the mixed profile.

3.1.4. Mixtures With Indistinguishable Contributors: When major or minor contributors cannot be distinguished because of similarity in signal intensities or the presence of shared or masked alleles, individuals may still be included or excluded as possible contributors.
Purpose and Scope (1)

- This document provides guidelines for the interpretation of DNA typing results from short tandem repeats (STR) and supersedes the Scientific Working Group on DNA Analysis Methods (SWGDAM) Short Tandem Repeat (STR) Interpretation Guidelines (2000). The revised guidelines are not intended to be applied retroactively.
Purpose and Scope (2)

- Guidance is provided for forensic casework analyses on the identification and application of thresholds for allele detection and interpretation, and appropriate statistical approaches to the interpretation of autosomal STRs with further guidance on mixture interpretation.
Purpose and Scope (3)

- Laboratories are encouraged to review their standard operating procedures and validation data in light of these guidelines and to update their procedures as needed. It is anticipated that these guidelines will evolve further as future technologies emerge. Some aspects of these guidelines may be applicable to low level DNA samples. However, this document is not intended to address the interpretation of analytical results from enhanced low template DNA techniques.
Purpose and Scope (4)

- Due to the multiplicity of forensic sample types and the potential complexity of DNA typing results, *it is impractical and infeasible to cover every aspect of DNA interpretation by a preset rule.* However, the laboratory should utilize written procedures for interpretation of analytical results with the understanding that specificity in the standard operating protocols will enable greater consistency and accuracy among analysts within a laboratory.
Elements of DNA Mixture Interpretation

Principles (theory)

Protocols (validation)

Practice (training & experience)

Consistency across analysts

ISFG Recommendations

SWGDAM Guidelines

Your Laboratory

SOPs

Training within Your Laboratory

Periodic training will aid accuracy and efficiency within your laboratory.
Overview of the SWGDAM Guidelines

1. Preliminary evaluation of the data – is something a peak and is the analysis method working properly?
2. Allele designation – calling peaks as alleles
3. Interpretation of DNA typing results – using the allele information to make a determination about the sample
   - 1. Non-allelic peaks
   - 2. Application of peak height thresholds to allelic peaks
   - 3. Peak height ratio
   - 4. Number of contributors to a DNA profile
   - 5. Interpretation of DNA typing results for mixed samples
   - 6. Comparison of DNA typing results
4. Statistical analysis of DNA typing results – assessing the meaning (rarity) of a match

Other supportive material: statistical formulae, references, and glossary
“Must” (used 29 times) VS. “Should” (used 41 times)

“Must” used when the FBI revised Quality Assurance Standards (2009) cover the topic:

- FBI QAS Standard 9.6.1:
  - The laboratory *shall verify* that all control results meet the laboratory’s interpretation guidelines for all reported results.

- SWGDAM Interpretational Guidelines 1.3.1:
  - The laboratory *must establish* criteria for evaluation of the following controls, including but not limited to: reagent blank and positive and negative amplification controls.

DNA Mixture Interpretation

Current SWGDAM Guidelines
1. Preliminary Evaluation of Data

The laboratory should develop criteria to determine whether an instrumental response represents the detection of DNA fragment(s) rather than instrument noise.
1.1. Analytical threshold

- The Laboratory should establish an analytical threshold based on signal-to-noise analyses of internally derived empirical data.

**Peak detection threshold**

- Signal ($S$) > 3x standard deviation of noise ($N$)

Current SWGDAM Guidelines
1. Preliminary Evaluation of Data

• An analytical threshold defines the minimum height requirement at and above which detected peaks can be reliably distinguished from background noise. Because the analytical threshold is based upon a distribution of noise values, it is expected that occasional, non-reproducible noise peaks may be detected above the analytical threshold.
1. Preliminary Evaluation of Data

- An analytical threshold should be sufficiently high to filter out noise peaks. Usage of an exceedingly high analytical threshold increases the risk of allelic data loss which is of potential exclusionary value.
Analytical Thresholds can be determined for each dye channel.
Setting Thresholds

- **Analytical (detection) threshold**
  - Dependent on instrument sensitivity
  - ~50 RFU
  - Impacted by instrument baseline noise

*what is a peak?*
2. Allele Designation

- 2.1. The laboratory establishes criteria to assign allele designations to appropriate peaks.

- 2.1.2.2. The laboratory establishes guidelines for the designation of alleles containing an incomplete repeat motif (i.e., an off-ladder allele falling within the range spanned by the ladder alleles).

- 2.1.2.3. The laboratory establishes criteria for designating alleles that fall above the largest or below the smallest allele of the allelic ladder (or virtual bin).
3. Interpretation of DNA Typing Results

- 3.1. Non-Allelic Peaks
- 3.2. Application of Peak Height Thresholds to Allelic Peaks
- 3.3. Peak Height Ratio
- 3.4. Number of Contributors to a DNA Profile
- 3.5. Interpretation of DNA Typing Results for Mixed Samples
- 3.6 Comparison of DNA Typing Results
3. Interpretation of DNA Typing Results

3.1. Non-Allelic Peaks

Non-allelic peaks may be PCR products (e.g., stutter, non-template dependent nucleotide addition, and non-specific amplification product), analytical artifacts (e.g., spikes and raised baseline), instrumental limitations (e.g., incomplete spectral separation resulting in pull-up or bleed-through), or may be introduced into the process (e.g., disassociated primer dye).
Pull-up in the blue channel from the size standard

Extraction Negative

Slide courtesy of Joanne Sgueglia

Pull-up in the yellow channel from the size standard
DYE BLOBS - need to know your kit and where dye artifacts migrate

Blue @ ~220 bp

Greens @ ~137bp and 158 bp

Slide courtesy of Joanne Sgueglia
3. Interpretation of DNA Typing Results

- A threshold value can be applied to alert the DNA analyst that all of the DNA typing information may not have been detected for a given sample.

- This threshold, referred to as a stochastic threshold, is defined as the value above which it is reasonable to assume that allelic dropout has not occurred within a single-source sample.
3.2. Application of Peak Height Thresholds to Allelic Peaks

• 3.2.1. The laboratory establishes a stochastic threshold based on empirical data derived within the laboratory and specific to the quantitation and amplification systems (e.g., kits) and the detection instrumentation used.
Hypothetical Examples

Gill et al. (2008) FSI Genetics 2(1): 76–82
3.2. Application of Peak Height Thresholds to Allelic Peaks

- It is noted that a stochastic threshold may be established by assessing peak height ratios across multiple loci in dilution series of DNA amplified in replicate. The RFU value above which it is reasonable to assume that, at a given locus, allelic dropout of a sister allele has not occurred constitutes a stochastic threshold.
Different Thresholds

Example values (empirically determined based on own internal validation)

150 RFUs

Peak real, but not used for CPE

Stochastic Threshold

(Dropout/Interpretation/LOQ/Reporting/MIT)

50 RFUs

Peak not considered reliable

Analytical Threshold

(Reporting/Noise/Limit-of-Detection/PAT)

Noise

50 RFUs

Peak real, can be used for CPE

Current SWGDAM Guidelines

DNA Mixture Interpretation
Setting Thresholds

- **Analytical (detection) threshold**
  - Dependent on *instrument sensitivity*
  - ~50 RFU
  - Impacted by instrument baseline noise

- **Stochastic (drop-out) threshold**
  - Dependent on *biological sensitivity*
  - ~150-200 RFU
  - Impacted by assay and injection parameters

Validation studies should be performed in each laboratory

**what is a peak?**

**what is reliable PCR data?**
3. Interpretation of DNA Typing Results

3.2. Application of Peak Height Thresholds to Allelic Peaks

Amplification of low-level DNA samples may be subject to stochastic effects, where two alleles at a heterozygous locus exhibit considerably different peak heights (i.e., peak height ratio generally <60%) or an allele fails to amplify to a detectable level (i.e., allelic dropout).
3. Interpretation of DNA Typing Results

3.2.1.1. If measures are used to enhance detection sensitivity (i.e., allelic height), the laboratory should perform additional studies to establish independent criteria for application of a separate stochastic threshold(s). Such measures may include but not be limited to increased amplification cycle number, increased injection time, and post-amplification purification/concentration of amplified products.

More on this topic later...
3.3. Peak Height Ratio

- Intra-locus peak height ratios (PHR) are calculated for a given locus by dividing the peak height of an allele with a lower RFU value by the peak height of an allele with a higher RFU value, and then multiplying this value by 100 to express the PHR as a percentage.

**Peak height ratio (PHR)**

PHR consistent with single source
Typically above 60%
3.3. Peak Height Ratio

3.3.1. The laboratory should establish PHR requirements based on empirical data for interpretation of DNA typing results from single-source samples. Different PHR expectations can be applied to individual loci (e.g., 70% for D3S1358, 65% for vWA, etc.); alternatively, a single PHR expectation can be applied to multiple loci (e.g., 60%).
Welcome to STRAlleleFreq!
Version <24-Dec-09>

STRAlleleFreq is a specialty analysis tool for "population" STR call and peak height data. Development of STRAlleleFreq was funded in part by the National Institute of Justice.

Required input data format…
Words will go here
<table>
<thead>
<tr>
<th>Locus</th>
<th>Δbp</th>
<th>#</th>
<th>Mean X</th>
<th>s(X)</th>
<th>Median X</th>
<th>s(X)</th>
<th>Percentiles</th>
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<tbody>
<tr>
<td>vWA</td>
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<td>0.908</td>
<td>0.094</td>
<td>0.922</td>
<td>0.075</td>
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<td></td>
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<td>0.087</td>
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<tr>
<td></td>
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<td>0.091</td>
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<td>0.076</td>
<td>0.549 0.605 0.774 0.807 0.977 0.979 0.982 0.982</td>
</tr>
</tbody>
</table>

**Peak Heights of Allele:1 and Allele:2**

![Graph showing scatter plots and linear relationships between peak heights](image-url)
3.3. Peak Height Ratio

• 3.3.1.1. The laboratory may evaluate PHRs at various DNA template levels (e.g., dilution series of DNA). It is noted that different PHR expectations at different peak height ranges may be established.
Peak Height Ratio Measurements

<table>
<thead>
<tr>
<th>Allele</th>
<th>Signal aided with 31 PCR cycles</th>
<th>Peak Heights (RFUs)</th>
<th>Average PHR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>FGA-22</td>
<td>FGA-25</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(1) 1692</td>
<td>1517</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(2) 1915</td>
<td>864</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(3) 1239</td>
<td>909</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(1) 992</td>
<td>260</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(2) 1422</td>
<td>419</td>
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<td>(3) 895</td>
<td>805</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(1) --</td>
<td>66</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(2) 54</td>
<td>107</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(3) 130</td>
<td>219</td>
</tr>
</tbody>
</table>

All levels performed in triplicate...
3.3. Peak Height Ratio

- 3.3.2. PHR requirements are only applicable to allelic peaks that meet or exceed the stochastic threshold.
The slide shows a graph titled "MINIMUM Peak Height Ratio (Avg PHR - 3XSTD)". The x-axis represents the intensity range (RFU) and the y-axis represents the peak height ratio (%). The graph plots various intensity ranges against their corresponding peak height ratios, with a trend line indicating the average - 3XSTD values. The graph is courtesy of Todd Bille (ATF).
3. Interpretation of DNA Typing Results

3.1. Non-Allelic Peaks

• Generally, non-allelic data such as stutter, nontemplate dependent nucleotide addition, disassociated dye, and incomplete spectral separation are reproducible;

\[ \text{Stutter typically below 15\%} \]
Stutter

3.1.1.1.

In general, the empirical criteria are based on qualitative and/or quantitative characteristics of peaks. As an example, dye artifacts and spikes may be distinguished from allelic peaks based on morphology and/or reproducibility. **Stutter and non-template dependent nucleotide addition peaks may be characterized based on size relative to an allelic peak and amplitude.**
New Program from NIST (Dave Duewer)

Welcome to STR_StutterFreq!
Version <04-Jan-10>

STR_StutterFreq is a specialty analysis tool for characterizing stutter frequency…
Development of STR_StutterFreq was funded in part by the National Institute of Justice.
**TPOX – \([AATG]_N\)**

<table>
<thead>
<tr>
<th>Locus</th>
<th>Allele</th>
<th>Size</th>
<th>#</th>
<th>Median</th>
<th>MADe</th>
</tr>
</thead>
<tbody>
<tr>
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<td>265.2</td>
<td>86</td>
<td>2.1</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>269.2</td>
<td>21</td>
<td>2.9</td>
<td>0.4</td>
</tr>
<tr>
<td></td>
<td>11</td>
<td>277.2</td>
<td>75</td>
<td>3.6</td>
<td>0.4</td>
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<td>14</td>
<td>4.3</td>
<td>0.4</td>
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<tr>
<td><strong>Avg</strong></td>
<td></td>
<td></td>
<td>196</td>
<td>3.3</td>
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<tr>
<td><strong>SD</strong></td>
<td></td>
<td></td>
<td></td>
<td>0.9</td>
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</tbody>
</table>

**Mutation Rate: 0.01%**
**D21S11 – a complex repeat**

<table>
<thead>
<tr>
<th>Locus</th>
<th>Allele</th>
<th>Size</th>
</tr>
</thead>
<tbody>
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<td>27</td>
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<td></td>
<td>28</td>
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<td>29</td>
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<td></td>
<td>30</td>
<td>219.9</td>
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<td>32.2</td>
<td>230.0</td>
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</table>

**Stutter**

<table>
<thead>
<tr>
<th>#</th>
<th>Median</th>
<th>MADe</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
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</tr>
<tr>
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<td>0.7</td>
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<tr>
<td>59</td>
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<td>66</td>
<td>9.2</td>
<td>1.2</td>
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<tr>
<td>11</td>
<td>6.4</td>
<td>0.5</td>
</tr>
<tr>
<td>21</td>
<td>9.6</td>
<td>1.2</td>
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<tr>
<td>28</td>
<td>8.0</td>
<td>3.4</td>
</tr>
<tr>
<td>33</td>
<td>8.7</td>
<td>1.7</td>
</tr>
</tbody>
</table>

- \([\text{TCTA}]_N\)
- \([\text{TCTG}]_N\)
- \([\text{TCTA}]_N\) TA
- \([\text{TCTA}]_N\) TCA
- \([\text{TCTA}]_N\) TCCATA
- \([\text{TCTA}]_N\)
### Stutter

<table>
<thead>
<tr>
<th>Locus</th>
<th>Allele</th>
<th>Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>D21S11</td>
<td>27</td>
<td>207.8</td>
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<tr>
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<tr>
<td></td>
<td>31</td>
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<td></td>
<td>31.2</td>
<td>226.0</td>
</tr>
<tr>
<td></td>
<td>32.2</td>
<td>230.0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>#</th>
<th>Median</th>
<th>MADe</th>
</tr>
</thead>
<tbody>
<tr>
<td>D21S11</td>
<td>20</td>
<td>5.9</td>
<td>0.6</td>
</tr>
<tr>
<td></td>
<td>69</td>
<td>6.9</td>
<td>0.7</td>
</tr>
<tr>
<td></td>
<td>59</td>
<td>8.0</td>
<td>0.8</td>
</tr>
<tr>
<td></td>
<td>66</td>
<td>9.2</td>
<td>1.2</td>
</tr>
<tr>
<td></td>
<td>11</td>
<td>6.4</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>21</td>
<td>9.6</td>
<td>1.2</td>
</tr>
<tr>
<td></td>
<td>28</td>
<td>8.0</td>
<td>3.4</td>
</tr>
<tr>
<td></td>
<td>33</td>
<td>8.7</td>
<td>1.7</td>
</tr>
</tbody>
</table>

Avg: 307, SD: 1.3
3.5.8. Interpretation of Potential Stutter Peaks in a Mixed Sample

3.5.8.1. For mixtures in which minor contributors are determined to be present, a peak in stutter position (generally n-4) may be determined to be 1) a stutter peak, 2) an allelic peak, or 3) indistinguishable as being either an allelic or stutter peak. This determination is based principally on the height of the peak in the stutter position and its relationship to the stutter percentage expectations established by the laboratory.
Consideration of Peak in Stutter Position

Fig. 4. c and d are unambiguous alleles, b is a minor allele in a stutter position and a is an unambiguous minor allele.

Possibilities for Minor:
- a,a
- a,b
- a,c
- a,d

Probability of Inclusion = 
\((f_a + f_b + f_c + f_d)^2\)

If peak height of peak a is within established PHR of peak b peak height.
3.5.8. Interpretation of Potential Stutter Peaks in a Mixed Sample

• 3.5.8.2. Generally, when the height of a peak in the stutter position exceeds the laboratory’s stutter expectation for a given locus, that peak is consistent with being of allelic origin and should be designated as an allele.
3.5.8. Interpretation of Potential Stutter Peaks in a Mixed Sample

• 3.5.8.3. If a peak is at or below this expectation, it is generally designated as a stutter peak. However, it should also be considered as a possible allelic peak, particularly if the peak height of the potential stutter peak(s) is consistent with (or greater than) the heights observed for any allelic peaks that are conclusively attributed (i.e., peaks in non-stutter positions) to the minor contributor(s).
Slide courtesy of Bruce Heidebrect (MDSP)
**ISFG Recommendation #6 Example**

**Likely a AA**
(homozygote)

**Possibly AB**
(heterozygote)

Could also be AC, AD, AA, or A,? (dropout)
Stutter effects

- In case of doubt a suspicious peak in the position of a stutter band has to be considered as a true allele and part of the DNA profile, and should be included into the biostatistical interpretation.

Slide from Peter Schneider
(presented at EDNAP meeting in Krakow in April 2007)
What is a true peak (allele)?

**Analytical threshold**
- **Signal (S)**
- **Noise (N)**
- **Signal > 3x sd of noise**

**Peak height ratio (PHR)**
- **Allele 1**
- **Allele 2**
- **Heterozygote peak balance**
- **PHR consistent with single source**
- **Typically above 60%**

**Stutter percentage**
- **True allele**
- **Stutter product**
- **Stutter location below 15%**
3. Interpretation of DNA Typing Results

• 3.1. Non-Allelic Peaks

• 3.2. Application of Peak Height Thresholds to Allelic Peaks

• 3.3. Peak Height Ratio

• 3.4. Number of Contributors to a DNA Profile

• 3.5. Interpretation of DNA Typing Results for Mixed Samples

• 3.6 Comparison of DNA Typing Results
3.4. Number of Contributors to a DNA Profile

- A sample is generally considered to have originated from more than one individual if three or more alleles are present at one or more loci (excepting tri-allelic loci) and/or the peak height ratios between a single pair of allelic peaks for one or more loci are below the empirically determined heterozygous peak height ratio expectation.
3.4. Number of Contributors to a DNA Profile

3.4.1. For DNA mixtures, the laboratory should establish guidelines for determination of the minimum number of contributors to a sample. Alleles need not meet the stochastic threshold to be used in this assessment.
PROBABILITY DISTRIBUTION OF ALLELE BANDS FOR MULTI-PERSON STR MIXTURES

J. Pendleton, T. W. Wang, K. Gilbert, C. Lucas
Laboratory for Information Technologies, The University of Tennessee, Knoxville, TN, 37996-2100

When interpreting STR mixture samples, it is very helpful to know how many individuals may have contributed to the mixture. A related question to ask is: When the number of contributors is known, what is the expected distribution of the number of loci (of a typical 13 CODIS core-loci profile) that harbors 1, 2, 3, or more alleles per locus, as well as the total number of allele bands observed. This information can be useful in investigations possibly involving genetically related suspects and victims, and would also be useful in checking for possible occurrence of excessive allele dropouts, null alleles, and possible mixture status. When the contributors are closely related, or the sample has suffered excess allele dropout, the distribution of the loci that harbors 1, 2, 3, or more alleles per locus and the number of allele bands observed will be skewed from those when the contributors are not related.
Pendleton et al. Summary

- For single source samples, 99% of the American Caucasian population contains 20 to 26 allele bands in a 13 core CODIS loci profile with an average of 23 bands.
- For 2-person 13-loci mixtures, almost all samples will contain between 30 and 45 bands with a mean of 38 bands.
- For 3-person 13-loci mixtures, almost all samples will contain between 39 and 57 bands with a mean of 48 bands.
Is it possible to observe 3 people with 4 or less alleles per locus?


- It is estimated that about 3.2% to 3.4% of three person mixtures would present four or fewer alleles for the CODIS core loci.
Towards understanding the effect of uncertainty in the number of contributors to DNA stains

John S. Buckleton a, James M. Curran b, *, Peter Gill c

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b Department of Statistics, University of Auckland, Private Bag 92019, Auckland, New Zealand
c The Forensic Science Service, Trident Court, Solihull Parkway, Birmingham Business Park, Solihull B37 7YN, UK

Received 31 May 2006; received in revised form 12 September 2006; accepted 13 September 2006
**Buckleton et al. (2007)**

Table 1

The probability of observing a given number of alleles in a two-person mixtures for simulated profiles at the SGM\(^+\) 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><strong>D16</strong></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><strong>D18</strong></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>
3.4. Number of Contributors to a DNA Profile

- 3.4.3.1. If composite profiles (i.e., generated by combining typing results obtained from multiple amplifications and/or injections) are used, the laboratory should establish guidelines for the generation of the composite result. When separate extracts from different locations on a given evidentiary item are combined prior to amplification, the resultant DNA profile is not considered a composite profile.
3.5. Interpretation of DNA Typing Results for Mixed Samples

3.5.3. A laboratory may define other quantitative characteristics of mixtures (e.g., mixture ratios) to aid in further refining the contributors.
4 Allele Locus: TH01

**STR allele call**

- A
- B
- C
- D

**RFU peak height**

- PHRs

**Major:** 7,9

**Minor:** 8,9,3

Consider all possible combinations:

- \( B/A = \frac{638}{1370} = 0.466 \)
- \( B/C = \frac{638}{1121} = 0.569 \)
- \( C/A = \frac{1121}{1370} = 0.818 \)
- \( D/B = \frac{494}{648} = 0.774 \)
- \( D/C = \frac{494}{1121} = 0.441 \)

All other combinations <0.60 PHR

DNA Mixture Interpretation

Current SWGDAM Guidelines
4 Allele Locus: TH01

Mix Ratio

Total of all peak heights
= 1370 + 638 + 1121 + 494
= 3623 RFUs

Minor component:
(B+D)/total = (638+494)/3623
= 0.312

Major component:
(A+C)/total = (1370+1121)/3623
= 0.688

STR allele call
RFU peak height

Major: 7,9
Minor: 8,9.3
3.5. Interpretation of DNA Typing Results for Mixed Samples

- [If] a sample contains a predominance of one individual’s DNA, that individual’s DNA profile may be determined. This state results in a distinguishable mixture, whereby there is a distinct contrast in signal intensities (e.g., peak heights) among the different contributors’ alleles. In such instances, major and/or minor contributors may be determined.
3.5. Interpretation of DNA Typing Results for Mixed Samples

- Alternatively, if the amounts of biological material from multiple donors are similar, it may not be possible to further refine the mixture profile. When major or minor contributors cannot be distinguished because of similarity in signal intensities, the sample is considered to be an indistinguishable mixture.
Mixture Classification Scheme

Schneider et al. (2006) Rechtsmedizin 16:401-404

(German Stain Commission, 2006):

- **Type A**: no obvious major contributor, no evidence of stochastic effects
- **Type B**: clearly distinguishable major and minor contributors; consistent peak height ratios of *approximately 4:1* (major to minor component) for all heterozygous systems, no stochastic effects
- **Type C**: mixtures without major contributor(s), evidence for stochastic effects

Adapted from Peter Schneider slide (presented at EDNAP meeting in Krakow in April 2007)
Schneider et al. (2009) and SWGDAM

Not all mixtures are homogeneous for Types A, B and C
e.g. Predominantly “A” with some “C” loci
3.5. Interpretation of DNA Typing Results for Mixed Samples

- Evidence items taken directly from an intimate sample, as determined by the laboratory, are generally expected to yield DNA from the individual from whom the sample was taken.
3.5. Interpretation of DNA Typing Results for Mixed Samples

• 3.5.1. The laboratory should establish guidelines based on peak height ratio assessments for evaluating potential sharing of allelic peaks among contributors and for determining whether contributors to a mixed DNA typing result are distinguishable. When assessing peak height ratios, pair-wise comparison of all potential genotypic combinations should be evaluated.
3.5. Interpretation of DNA Typing Results for Mixed Samples

3.5.2.2. If assumptions are made as to the number of contributors, additional information such as the number of alleles at a given locus and the relative peak heights can be used to distinguish major and minor contributors.
An Example – Stain on Victim’s Underwear

**Donor Mix**
0.6 Victim to 1 Unknown

No stochastic issues with this locus
(ST = 150 RFUs)

Example courtesy of Bruce Heidebrect
Example courtesy of Bruce Heidebrecht
Test for various possibilities for mixture deconvolution

- Unknown donor may be 15,-- or 15,15 or 13,15 or 15,17

Example courtesy of Bruce Heidebrect
Test for various possibilities for mixture deconvolution

If unknown donor is 15,--, then that leaves the Victim with PHR of 77% \((194/251)\).

But it is unreasonable to assume dropout associated with peak of 453rfu

(This locus was not identified as having stochastic issues)
Test for various possibilities for mixture deconvolution

If unknown donor is 15, 17, then that splits the rfu for allele 17, leaving the Victim with PHR of 31% (78/251) and unknown donor with PHR of 26% (116/453)

Calculation based on ratio of 0.6 Victim to 1 Unknown
Test for various possibilities for mixture deconvolution

If unknown donor is 13, 15 then that splits the rfu for allele 13, leaving the Victim with PHR of 52% (100/194) and unknown donor with PHR of 33% (151/453)

Calculation based on ratio of 0.6 Victim to 1 Unknown
Test for various possibilities for mixture deconvolution

If unknown donor is 15,15 then that leaves the Victim with PHR of 77% (194/251)

Calculation based on ratio of 0.6 Victim to 1 Unknown
3. Interpretation of DNA Typing Results

- 3.1. Non-Allelic Peaks
- 3.2. Application of Peak Height Thresholds to Allelic Peaks
- 3.3. Peak Height Ratio
- 3.4. Number of Contributors to a DNA Profile
- 3.5. Interpretation of DNA Typing Results for Mixed Samples
- 3.6 Comparison of DNA Typing Results
3.5. Interpretation of DNA Typing Results for Mixed Samples

- 3.5.2. The laboratory should define and document what, if any, assumptions are used in a particular mixture deconvolution.
3.5. Interpretation of DNA Typing Results for Mixed Samples

- 3.5.3.1. Differential degradation of the contributors to a mixture may impact the mixture ratio across the entire profile.
3. Interpretation of DNA Typing Results

- 3.1. Non-Allelic Peaks
- 3.2. Application of Peak Height Thresholds to Allelic Peaks
- 3.3. Peak Height Ratio
- 3.4. Number of Contributors to a DNA Profile
- 3.5. Interpretation of DNA Typing Results for Mixed Samples
- 3.6 Comparison of DNA Typing Results
3.6 Comparison of DNA Typing Results

- The following determinations can be made upon comparison of evidentiary and known DNA typing results (and between evidentiary samples):
  - The known individual cannot be excluded (i.e., is included) as a possible contributor to the DNA obtained from an evidentiary item.
  - The known individual is excluded as a possible contributor.
  - The DNA typing results are inconclusive/uninterpretable.
  - The DNA typing results from multiple evidentiary items are consistent or inconsistent with originating from a common source(s).
3.6 Comparison of DNA Typing Results

• 3.6.1. The laboratory must establish guidelines to ensure that, to the extent possible, DNA typing results from evidentiary samples are interpreted before comparison with any known samples, other than those of assumed contributors.

• The SWGDAM committee felt that this was an issue of such importance that it deserved a “must.”
3.6 Comparison of DNA Typing Results

• 3.6.2. DNA typing results may not be obtained at all loci for a given evidentiary sample (e.g., due to DNA degradation, inhibition of amplification and/or low-template quantity); a partial profile thus results.

• 3.6.2.1. For partial profiles, the determination of which alleles/loci are suitable for comparison and statistical analysis should be made prior to comparison to the known profiles.
4. Statistical Analysis of DNA Typing Results

4.1. The laboratory must perform statistical analysis in support of any inclusion that is determined to be relevant in the context of a case, irrespective of the number of alleles detected and the quantitative value of the statistical analysis.

Buckleton & Curran (2008): “There is a considerable aura to DNA evidence. Because of this aura it is vital that weak evidence is correctly represented as weak or not presented at all.”

4. Statistical Analysis of DNA Typing Results

- 4.1. The laboratory must perform statistical analysis in support of any inclusion.

- 4.2. For calculating the CPE or RMP, any DNA typing results used for statistical analysis must be derived from evidentiary items and not known samples.

- 4.3. The laboratory must not use inconclusive/uninterpretable data (e.g., at individual loci or an entire multi-locus profile) in statistical analysis.
4. Statistical Analysis of DNA Typing Results

- 4.4. Exclusionary conclusions do not require statistical analysis.
- 4.5. The laboratory must document the source of the population database(s) used in any statistical analysis. *(for future analysts).*
4. Statistical Analysis of DNA Typing Results

• 4.6. The formulae used in any statistical analysis must be documented
  – 4.6.1 Selection of the suitable statistical approach
**Table 1 – Suitable Statistical Analyses for DNA Typing Results**

The statistical methods listed in the table cannot be combined into one calculation. For example, combining RMP at one locus with a CPI calculation at a second locus is not appropriate. However, an RMP may be calculated for the major component of a mixture and a CPE/CPI for the entire mixture (as referred to in section 4.6.2).

<table>
<thead>
<tr>
<th>Category of DNA Typing Result</th>
<th>RMP</th>
<th>CPE/CPI</th>
<th>LR (1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single Source</td>
<td>✓</td>
<td></td>
<td>✓</td>
</tr>
<tr>
<td>Single Major Contributor to a Mixture</td>
<td>✓</td>
<td></td>
<td>✓</td>
</tr>
<tr>
<td>Multiple Major Contributors to a Mixture</td>
<td>✓</td>
<td>(2)</td>
<td>✓</td>
</tr>
<tr>
<td>Single Minor Contributor to a Mixture</td>
<td>✓</td>
<td></td>
<td>✓</td>
</tr>
<tr>
<td>Multiple Minor Contributors to a Mixture</td>
<td>✓</td>
<td>(2)</td>
<td>✓</td>
</tr>
<tr>
<td>Indistinguishable Mixture</td>
<td>✓</td>
<td></td>
<td>✓</td>
</tr>
</tbody>
</table>

(1) Restricted or unrestricted  
(2) Restricted  
(3) All potential alleles identified during interpretation are included in the statistical calculation.
4. Statistical Analysis of DNA Typing Results

• 4.6. The formulae used in any statistical analysis must be documented
  – 4.6.1 Selection of the suitable statistical approach
  – 4.6.2. It is not appropriate to calculate a composite statistic using multiple formulae for a multi-locus profile. (no mix and match of RMP and CPI).
  – 4.6.3. CPE/CPI alleles below the stochastic threshold may not be used to support an inclusion.

• 4.7. If a laboratory uses source attribution statements, then it must establish guidelines for the criteria on which such a declaration is based.
Schneider et al. (2009) and SWGDAM

Type A  
Indistinguishable

Type B  
Distinguishable

Type C  
Uninterpretable

A statistical analysis must be performed

A statistical analysis should not be performed
5. *Statistical Formulae*

- 5.2. Random Match Probability (RMP)
- 5.3. Combined Probability of Inclusion (CPI) and Exclusion (CPE)
- 5.4. Likelihood Ratio (LR)
Unrestricted vs. Restricted

**Unrestricted**

All combinations of alleles are deemed possible (relative peak height differences are not utilized)

\[ AB + AC + AD + BC + BD + CD \]

**Restricted**

Based on relative peak heights, alleles are paired only where specific combinations of alleles are deemed possible

\[ AB + AC + AD + BC + BD + CD \]
Summary

• The new SWGDAM Guidelines are meant to provide guidance for forensic casework analyses to identify and apply thresholds for allele detection and interpretation, and determine the appropriate statistical approaches to the interpretation of autosomal STRs with further guidance on mixture interpretation.

• It is hoped that laboratories will be encouraged to review their SOPs and validation data in light of these guidelines and to update their procedures as needed.
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