SWGDAM Guidelines

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Scientific Working Group on DNA Analysis Methods (SWGDAM)

• Organized originally by FBI Laboratory as Technical Working Group on DNA Analysis Methods (TWGDAM) in 1988
• Meets semiannually – each January and July
• Membership consists of voting members and invited guests (usually ~50 attend) from public forensic DNA laboratories around the U.S. & Canada
• Current chair is Anthony Onorato (FBI Laboratory)
• Currently organized into eight subcommittees:
  • CODIS, Enhanced Detection Methods & Interpretation, Mass Spectrometry & mtDNA, Missing Persons & Mass Disasters, Mixture Interpretation, Quality Assurance, Rapid DNA, ad hoc Y-STR

SWGDAM has previously issued guidance documents on validation and data interpretation

SWGDAM Website
www.swgdam.org

Mission Statement

• The Scientific Working Group on DNA Analysis Methods, known as SWGDAM, serves as a forum to discuss, share, and evaluate forensic biology methods, protocols, training, and research to enhance forensic biology services as well as provide recommendations to the FBI Director on quality assurance standards for forensic DNA analysis.
• The group meets each January and July to address issues of importance to the DNA community (ranging from familial searches, partial matches, recent court cases, audit issues, kits and reagents, etc.).

http://www.swgdam.org/

SWGDAM Guidelines and FBI Quality Assurance Standards

• STR Interpretation (2000)
• Training (2001)
• mtDNA Nucleotide Sequence Interpretation (2003)
• Revised Validation (2004)
• Y-STR Interpretation (2009)
• STR Autosomal Interpretation (2010)
  • Audit Document for DNA Databasing Laboratories (2009, 2011)
  • Audit Document for Forensic DNA Testing Laboratories (2009, 2011)

SWGDAM Autosomal STR Interpretation Guidelines

Process of Creating SWGDAM Guidelines

- Recognized need and/or request for guidance on a particular topic received (e.g., mixture interpretation)
- A committee is formed and individuals selected to participate (the committee selects a chair that directs the efforts)
- Committee works to produce a document
- Committee product provided to full SWGDAM for comment
- Committee revises document based on comments received
- Full SWGDAM group evaluates and discusses the document
- SWGDAM approves based on a membership vote
- Guidance document released to the public usually through the FBI website (Forensic Science Communications)

Because most work is done only during semiannual meetings*, it can take several years to complete this process.

In some cases, phone conferences, WebEx, or additional in-person meetings are conducted.

Members of SWGDAM Mixture Committee over the time period of Jan 2007 to Jan 2010

- 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)
- Tamya Moretti (FBI DNA Unit I)
- George Carmody (Carleton U)
- Roger Frappier (CFS-Toronto)
- Jack Ballantyne (UCF/NCFS)

*In some cases, phone conferences, WebEx, or additional in-person meetings are conducted.

Committee Member Backgrounds

- State Lab – CA (x2), OR, WA, MT, MN, CT, MA, MD
- State/Local Lab – CFS Toronto (early on PBSO)
- Canadian Labs – RCMP, CFS Toronto
- Federal Lab/Agency – FBI, NIST
- Academic – Jack Ballantyne, George Carmody

With 15 members, we represented almost one-third of SWGDAM

Previous SWGDAM (2000) STR Interpretation Guidelines


1. Preliminary Evaluation of Data
2. Designation
3. Interpretation of Results
4. Conclusions
5. Statistical Interpretation
6. References/Suggested Readings

STR Interpretational Guidelines (2000)

1. Preliminary Evaluation of Results
2. Designation
3. Interpretation of Results
4. Conclusions
5. Statistical Interpretation
6. References/Suggested Readings

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, mitochondrial DNA testing, or CODIS entries
- Forensic DNA Testing Laboratories
  - Databasing labs may have different issues since they are working with known single source samples
Purpose and Scope of Document (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 of Document (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 of Document (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.


Overview of these SWGDAM Guidelines

1. Preliminary evaluation of 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 ratios
   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

"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 Interpretation 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.

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

“Should” used for (most) other guidelines

• The FBI QAS do not address a requirement regarding peak height ratios.
• SWGDAM Interpretation Guidelines 3.3.1:
  – The laboratory should establish PHR requirements based on empirical data for interpretation of DNA typing results from single-source samples...

Interpretation of Evidence Completed before Comparison to Knowns

• “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.”
  – While the FBI QAS do not address this issue, this is an example of an issue felt by the committee members to be of such importance that it warranted a “must.”

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
  3.5. Comparison of DNA Typing Results
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.

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.

3.5.8.3. 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).
ISFG (2006) Mixture Recommendation

- **Recommendation 6**: If the crime profile is a major/minor mixture, where minor alleles are the same size (height or area) as stutters of major alleles, then stutters and minor alleles are indistinguishable...


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**4. Statistical Analysis of DNA Typing Results**

*Genetic loci and assumptions used for stats calculations must be documented*

4.1. Stats required in support of any inclusion

4.2. Stats to come from evidentiary items not from knowns

4.3. Must not use inconclusive/interpretible data in stats

4.4. Exclusionary conclusions do not require stats

4.5. Must document population database used

4.6. Must document statistical formulae used
   4.6.1. Selection of suitable statistical approach
   4.6.2. A composite statistic is not appropriate
   4.6.3. CPE/CPI alleles below stochastic threshold may not be used to support an inclusion

4.7. Source attribution criteria must be established

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**Stats Required for Inclusions**

SWGDAM Interpretation Guideline 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.”


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**No Composite Statistics**

SWGDAM Interpretation Guideline 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.”

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**Summary of Statistical Analysis Sections**

- Guidelines do not state a preference for one statistical method over another

- Some worked examples for various statistical formulae are provided in Section 5

- These guidelines provide information as to the appropriate ways to apply various statistical methods, and their limitations (see Table 1)
All Statistical Approaches Are Considered

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 CPI/CR 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>CPI/CR</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>✓</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>✓</td>
<td></td>
</tr>
<tr>
<td>Indistinguishable Mixture</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
</tbody>
</table>

(1) Restricted or unrestricted
(2) If potential alleles identified during interpretation are included in the statistical calculation


Restricted vs Unrestricted
Are relative peak heights considered?

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

Unrestricted

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

AB = AC + AD + BC + BD + CD

Figure 1. Illustration of restricted versus unrestricted approaches based on relative peak heights (using an assumption of two donors with all peaks above the stochastic threshold).


Articles Cited in the Guidelines

9 total

6. References and Literature Cited

Useful Articles for Further Information

34 total

1. Additional Suggested Readings

What the document does not include

- Report writing statements
- Worked examples
- Flowcharts of how or when to make decisions during interpretation

The SWGDAM mixture committee has discussed the possibility of creating a separate training document to include additional helpful information.
Summary

- SWGDAM guidelines for autosomal STR interpretation were developed with a lot of thought and discussion and are now available.
- Key elements of allelic and statistical interpretation are included with guidance on what needs to be documented when analyzing DNA mixtures.

Further Training Materials

- Training materials with worked examples are needed to help analysts better appreciate what is being conveyed with specific points in these SWGDAM Guidelines.
  http://www.cstl.nist.gov/biotech/strbase/mixture/SWGDAM-mixture-info.htm
- Slides from several mixture interpretation workshops are available on the NIST STRBase website.

Hierarchy of Rules for Forensic DNA Labs

<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>SWGDAM Guidelines</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>

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)
- Bio-statistics 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
Angé Carracedo
(Madrid, Spain)

Treasurer
Wolfgang Mayr
(Vienna, Austria)

Secretary
Leonor Gusmão
(Porto, Portugal)

Authors of ISFG Mixture Article

Peter Gill
Pioneer of forensic DNA techniques and applications
University of Strathclyde (Apr 2008 – present)

The Mathematicians/Statisticians

Charles Brenner
Berkeley, CA, USA

John Buckleton
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

Gill et al. (2006) DNA Commission of the International Society of Forensic Genetics:
Recommendations on the interpretation of mixtures

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
4. Follow Clayton et al. (1998) guidelines when deducing component genotypes
5. Prosecution determines H₀ and defense determines H₁, 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**
  - Gill et al. (2008) *FSI Genetics* 2(1): 76–82

- **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** — *Autosomal STR Interpretation Guidelines* (2010)

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**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.

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**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.

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**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

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

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) \[\text{Signal} > 3\times \text{sd of noise}\]

Noise (N)
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](image)

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

Setting Thresholds

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

What is a peak?
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).

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.
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](image)

### 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.

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).

3.3. 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](image)
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%).

New Program from NIST (Dave Duewer)

Welcome to STR_AleleFreq!

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

Required input data format...

Words all go here

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>Signal aided with 31 PCR cycles</th>
<th>Peak Heights (RFUs)</th>
<th>Average PHR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>FGA-22</td>
<td>FGA-25</td>
</tr>
<tr>
<td>100 pg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Good balance</td>
<td>1692</td>
<td>1517</td>
</tr>
<tr>
<td></td>
<td>1915</td>
<td>864</td>
</tr>
<tr>
<td></td>
<td>1239</td>
<td>909</td>
</tr>
<tr>
<td>50 pg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Severe imbalance</td>
<td>992</td>
<td>260</td>
</tr>
<tr>
<td></td>
<td>1422</td>
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</tr>
<tr>
<td></td>
<td>895</td>
<td>805</td>
</tr>
<tr>
<td>10 pg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Allele dropout</td>
<td>54</td>
<td>107</td>
</tr>
<tr>
<td></td>
<td>130</td>
<td>219</td>
</tr>
<tr>
<td>All levels performed in triplicate...</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

3.3. Peak Height Ratio

- 3.3.2. PHR requirements are only applicable to allelic peaks that meet or exceed the stochastic threshold.
3. Interpretation of DNA Typing Results

3.1. Non-Allelic Peaks

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

**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>
<td>TPOX</td>
<td>8 265.2</td>
<td>86</td>
<td>2.1</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>9 269.2</td>
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<td>2.9</td>
<td>0.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>11 277.2</td>
<td>75</td>
<td>3.6</td>
<td>0.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>12 281.2</td>
<td>14</td>
<td>4.3</td>
<td>0.4</td>
<td></td>
</tr>
<tr>
<td>Avg</td>
<td>195</td>
<td>3.3</td>
<td>0.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SD</td>
<td>0.9</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Mutation Rate:** 0.01%
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.

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).

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.

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
  - Stutter product
  - True allele
  - 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.

- 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.

3.4.1. 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.

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

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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.
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

<table>
<thead>
<tr>
<th>Type A</th>
<th>Type B</th>
<th>Type C</th>
</tr>
</thead>
<tbody>
<tr>
<td>no obvious major contributor, no evidence of stochastic effects</td>
<td>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</td>
<td>mixtures without major contributor(s), evidence for stochastic effects</td>
</tr>
</tbody>
</table>

Schneider et al. (2006) and SWGDAM

- 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.

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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 Heidebrecht

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Test for various possibilities for mixture deconvolution

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

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 453 rfu
(This locus was not identified as having stochastic issues)

Example courtesy of Bruce Heidebrecht

---

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 15,17,
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

---

Example courtesy of Bruce Heidebrecht
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
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- 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.
- 3.5.3.1. Differential degradation of the contributors to a mixture may impact the mixture ratio across the entire profile.

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.”

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.”

4.4. 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).

4.2. 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.

4.6. Exclusionary conclusions do not require statistical analysis.

4.6.1 Selection of the suitable statistical approach

4.6.2. 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

- 4.6. 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. CPI/CPE 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.

5. Statistical Formulae

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

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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