CIB Forensic Science Center
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SWGDAM Guidelines

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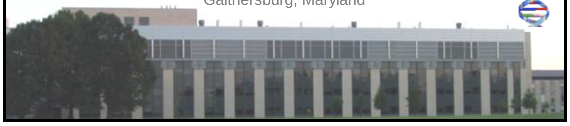

Scientific Working Group on DNA Analysis Methods (SWGDAM)

- 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

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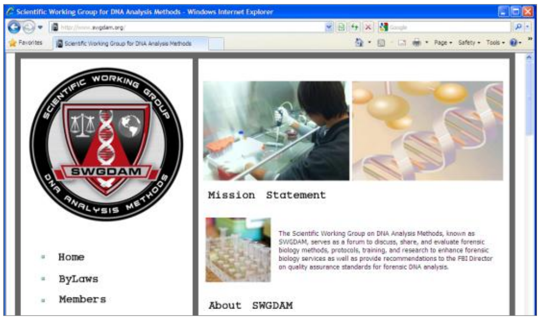
SWGDAM Website

www.swgdam.org



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.

SWGDAM

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.).

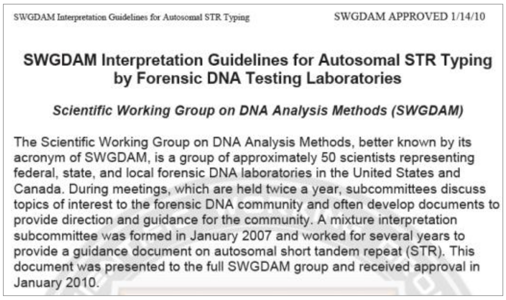
http://www.swgdam.org/

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)**

- Quality Assurance Standards for DNA Databasing Laboratories (2009, 2011)
  - Audit Document for DNA Databasing Laboratories (2009, 2011)
- Quality Assurance Standards for Forensic DNA Testing Laboratories (2009, 2011)
  - Audit Document for Forensic DNA Testing Laboratories (2009, 2011)

SWGDAM Autosomal STR Interpretation Guidelines



SWGDAM Interpretation Guidelines for Autosomal STR Typing SWGDAM APPROVED 1/14/10

SWGDAM Interpretation Guidelines for Autosomal STR Typing by Forensic DNA Testing Laboratories

Scientific Working Group on DNA Analysis Methods (SWGDAM)

The Scientific Working Group on DNA Analysis Methods, better known by its acronym of SWGDAM, is a group of approximately 50 scientists representing federal, state, and local forensic DNA laboratories in the United States and Canada. During meetings, which are held twice a year, subcommittees discuss topics of interest to the forensic DNA community and often develop documents to provide direction and guidance for the community. A mixture interpretation subcommittee was formed in January 2007 and worked for several years to provide a guidance document on autosomal short tandem repeat (STR). This document was presented to the full SWGDAM group and received approval in January 2010.

http://www.swgdam.org/Interpretation_Guidelines_January_2010.pdf

Process of Creating SWGDAM Guidelines

- Recognized need and/or request for guidance on a particular topic received (e.g., mixture interpretation)
- Jan 2007 — A committee is formed and individuals selected to participate (the committee selects a chair that directs the efforts)
- **Committee works to produce a document**
- July 2009 — Committee product provided to full SWGDAM for comment
- Oct 2009 — Committee revises document based on comments received
- Jan 2010 — Full SWGDAM group evaluates and discusses the document
- SWGDAM approves based on a membership vote
- Apr 2010 — Guidance document released to the public usually through the FBI website (*Forensic Science Communications*)

Because of 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)
- **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)

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

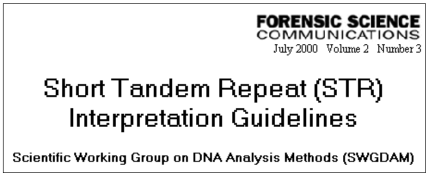
With 15 members, we represented almost one-third of SWGDAM

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

Previous SWGDAM (2000) STR Interpretation Guidelines

http://www.fbi.gov/hq/lab/fsc/backissu/july2000/strig.htm



FORENSIC SCIENCE COMMUNICATIONS
July 2000 Volume 2 Number 3

Short Tandem Repeat (STR) Interpretation Guidelines

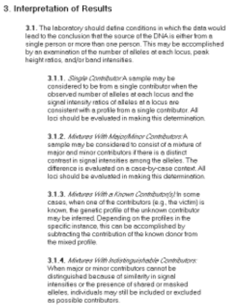
Scientific Working Group on DNA Analysis Methods (SWGDAM)

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

STR Interpretational Guidelines (2000)

1066 words
4 pages

7 sentences
on mixtures



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 context. 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.

(9862 words
28 pages
for the new
guidelines)

Needed Revisions After a Decade…

Quality Assurance Standards (1998/1999) → **Quality Assurance Standards (2009)**

http://www.fbi.gov/hq/lab/fsc/backissu/july2000/strig.htm

http://www.fbi.gov/hq/lab/html/codis_swgdam.pdf

STR Interpretation Guidelines (2000) → **STR Interpretation Guidelines (2010)**

1066 words (4 pages)

9862 words (28 pages)

Purpose and Scope of Document (1)

SWGDAM Interpretation Guidelines for Autosomal STR Typing by Forensic DNA Testing Laboratories

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.**

http://www.swgdam.org/Interpretation_Guidelines_January_2010.pdf

Purpose and Scope of Document (2)

SWGDAM Interpretation Guidelines for Autosomal STR Typing by Forensic DNA Testing Laboratories

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.

http://www.swgdam.org/Interpretation_Guidelines_January_2010.pdf

Purpose and Scope of Document (3)

SWGDAM Interpretation Guidelines for Autosomal STR Typing by Forensic DNA Testing Laboratories

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**.

http://www.swgdam.org/Interpretation_Guidelines_January_2010.pdf

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 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 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.

"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

3.6 Comparison of DNA Typing Results

3.5. Interpretation of DNA Typing Results for Mixed Samples

3.5.1. Use of PHR to determine major/minor

3.5.2. Document any assumptions used in mixture deconvolution

3.5.3. Use of mixture ratios to distinguish contributor profiles

3.5.4. Mixtures with single major contributor
3.5.5. Mixtures with multiple major contributors
3.5.6. Mixtures with indistinguishable contributors

3.5.7. Use of "known" contributors to refine interpretation

3.5.8. **Interpretation of potential stutter peaks in a mixed sample**

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.

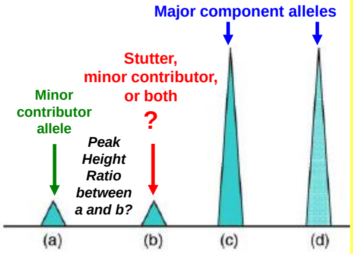
3.5.8.3. If a peak is at or below this expectation, it is generally designated as a stutter peak. However, it should also be considered as a possible allelic peak, particularly if the peak height of the potential stutter peak(s) is consistent with (or greater than) the heights observed for any allelic 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…**

Gill *et al.* (2006) DNA Commission of the International Society of Forensic Genetics: Recommendations on the interpretation of mixtures. *Forensic Sci. Int.* 160: 90-101

Consideration of Peak in Stutter Position



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

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

Gill *et al.* (2006) DNA Commission of the International Society of Forensic Genetics: Recommendations on the interpretation of mixtures. *Forensic Sci. Int.* 160: 90-101

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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/uninterpretable 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

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**.”

Buckleton, J. and Curran, J. (2008) A discussion of the merits of random man not excluded and likelihood ratios. *Forensic Sci. Int. Genet.* 2: 343-348.

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**.”

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

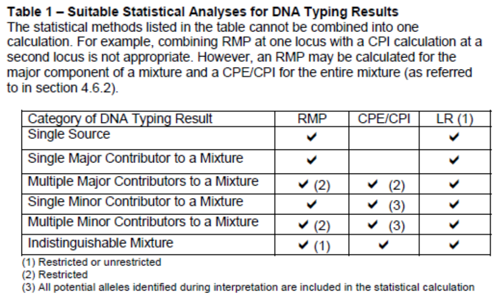


Table 1 – Suitable Statistical Analyses for DNA Typing Results
The statistical methods listed in the table cannot be combined into one calculation. For example, combining RMP at one locus with a CPI calculation at a second locus is not appropriate. However, an RMP may be calculated for the major component of a mixture and a CPE/CPI for the entire mixture (as referred to in section 4.6.2).

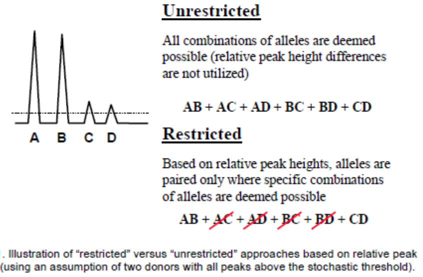
Category of DNA Typing Result	RMP	CPE/CPI	LR (1)
Single Source	✓		✓
Single Major Contributor to a Mixture	✓		✓
Multiple Major Contributors to a Mixture	✓ (2)	✓ (2)	✓
Single Minor Contributor to a Mixture	✓	✓ (3)	✓
Multiple Minor Contributors to a Mixture	✓ (2)	✓ (3)	✓
Indistinguishable Mixture	✓ (1)	✓	✓

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

http://www.swgdam.org/Interpretation_Guidelines_January_2010.pdf

Restricted vs Unrestricted

Are relative peak heights considered?



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

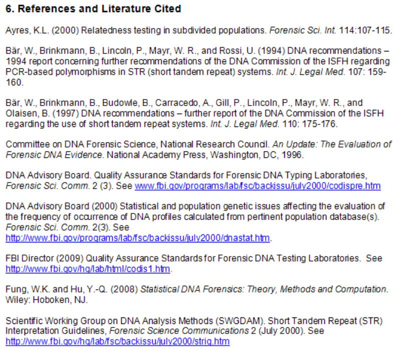
AB + ~~AC~~ + ~~AD~~ + ~~BC~~ + ~~BD~~ + CD

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).

http://www.swgdam.org/Interpretation_Guidelines_January_2010.pdf

Articles Cited in the Guidelines

9 total



6. References and Literature Cited

Ayres, K.L. (2000) Relatedness testing in subdivided populations. *Forensic Sci. Int.* 114:107-115.

Bär, W., Brinkmann, B., Lincoln, P., Mayr, W. R., and Rossi, U. (1994) DNA recommendations – 1994 report concerning further recommendations of the DNA Commission of the ISFH regarding PCR-based polymorphisms in STR (short tandem repeat) systems. *Int. J. Legal Med.* 107: 159-160.

Bär, W., Brinkmann, B., Budowle, B., Carracedo, A., Gill, P., Lincoln, P., Mayr, W. R., and Olaisen, B. (1997) DNA recommendations – further report of the DNA Commission of the ISFH regarding the use of short tandem repeat systems. *Int. J. Legal Med.* 110: 175-176.

Committee on DNA Forensic Science, National Research Council. *An Update: The Evaluation of Forensic DNA Evidence*. National Academy Press, Washington, DC, 1996.

DNA Advisory Board. Quality Assurance Standards for Forensic DNA Typing Laboratories, *Forensic Sci. Comm.* 2 (3). See www.fbi.gov/programs/lab/fsc/backissu/july2000/codispre.htm

DNA Advisory Board (2000) Statistical and population genetic issues affecting the evaluation of the frequency of occurrence of DNA profiles calculated from pertinent population database(s). *Forensic Sci. Comm.* 2(3). See http://www.fbi.gov/programs/lab/fsc/backissu/july2000/dnastat.htm.

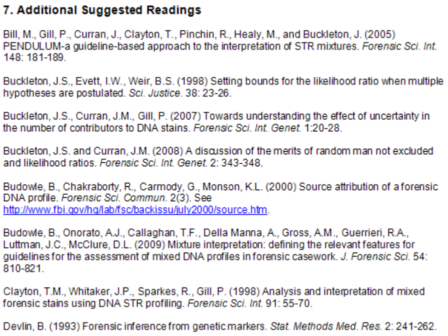
FBI Director (2009) Quality Assurance Standards for Forensic DNA Testing Laboratories. See http://www.fbi.gov/hq/lab/html/codis1.htm.

Fung, W.K. and Hu, Y.-Q. (2008) *Statistical DNA Forensics: Theory, Methods and Computation*. Wiley: Hoboken, NJ.

Scientific Working Group on DNA Analysis Methods (SWGDAM). Short Tandem Repeat (STR) Interpretation Guidelines, *Forensic Science Communications* 2 (July 2000). See http://www.fbi.gov/hq/lab/fsc/backissu/july2000/strig.htm

Useful Articles for Further Information

34 total



7. Additional Suggested Readings

Bill, M., Gill, P., Curran, J., Clayton, T., Pinchin, R., Healy, M., and Buckleton, J. (2005) PENDULUM-a guideline-based approach to the interpretation of STR mixtures. *Forensic Sci. Int.* 148: 181-189.

Buckleton, J.S., Evett, I.W., Weir, B.S. (1998) Setting bounds for the likelihood ratio when multiple hypotheses are postulated. *Sci. Justice*. 38: 23-26.

Buckleton, J.S., Curran, J.M., Gill, P. (2007) Towards understanding the effect of uncertainty in the number of contributors to DNA stains. *Forensic Sci. Int. Genet.* 1:20-28.

Buckleton, J.S. and Curran, J.M. (2008) A discussion of the merits of random man not excluded and likelihood ratios. *Forensic Sci. Int. Genet.* 2: 343-348.

Budowle, B., Chakraborty, R., Carmody, G., Monson, K.L. (2000) Source attribution of a forensic DNA profile. *Forensic Sci. Commun.* 2(3). See http://www.fbi.gov/hq/lab/fsc/backissu/july2000/source.htm.

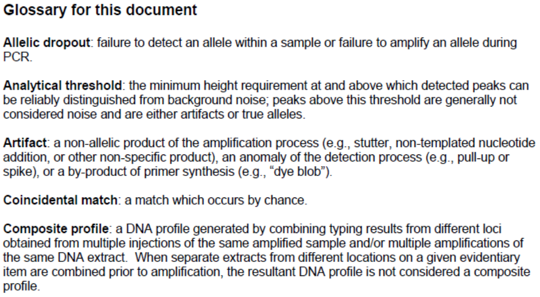
Budowle, B., Onorato, A.J., Callaghan, T.F., Della Manna, A., Gross, A.M., Guerrieri, R.A., Luttman, J.C., McClure, D.L. (2009) Mixture interpretation: defining the relevant features for guidelines for the assessment of mixed DNA profiles in forensic casework. *J. Forensic Sci.* 54: 810-821.

Clayton, T.M., Whitaker, J.P., Sparkes, R., Gill, P. (1998) Analysis and interpretation of mixed forensic stains using DNA STR profiling. *Forensic Sci. Int.* 91: 55-70.

Devlin, B. (1993) Forensic inference from genetic markers. *Stat. Methods Med. Res.* 2: 241-262.

Glossary with Defined Terms

46 total



Glossary for this document

Allelic dropout: failure to detect an allele within a sample or failure to amplify an allele during PCR.

Analytical threshold: the minimum height requirement at and above which detected peaks can be reliably distinguished from background noise; peaks above this threshold are generally not considered noise and are either artifacts or true alleles.

Artifact: a non-allelic product of the amplification process (e.g., stutter, non-templated nucleotide addition, or other non-specific product), an anomaly of the detection process (e.g., pull-up or spike), or a by-product of primer synthesis (e.g., "dye blob").

Coincidental match: a match which occurs by chance.

Composite profile: a DNA profile generated by combining typing results from different loci obtained from multiple injections of the same amplified sample and/or multiple amplifications of the same DNA extract. 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.

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

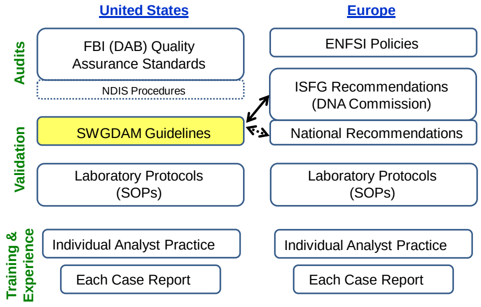
Further Training Materials

- **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
  - **http://www.cstl.nist.gov/biotech/strbase/mixture.htm**

Hierarchy of Rules for Forensic DNA Labs



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)
- Additional Y-STRs - nomenclature (2006)
- Mixture Interpretation (2006)
- Disaster Victim Identification (2007)
- Biostatistics for Parentage Analysis (2007)
- Non-human (animal) DNA (2010)

http://www.isfg.org/Publications/DNA+Commission

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University of Strathclyde (Apr 2008 – present)

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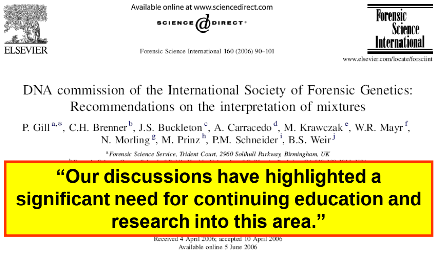
Available for download from the ISFG Website:
http://www.isfg.org/Publication;Gill2006



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

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

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Gill *et al.* (2006) DNA Commission of the International Society of Forensic Genetics: Recommendations on the interpretation of mixtures. *Forensic Sci. Int.* 160: 90-101

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Editorial

Editorial on the recommendations of the DNA commission of the ISFG on the interpretation of mixtures

"…**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
4. Follow Clayton et al. (1998) guidelines when deducing component genotypes
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
  - Gill *et al.* (2008) *FSI Genetics* 2(1): 76–82
- German Stain Commission
  - Schneider *et al.* (2006) *Rechtsmedizin* 16:401-404 (German version)
  - Schneider *et al.* (2009) *Int. J. Legal Med.* 123: 1-5 (English version)

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Allgemeine Empfehlungen der Spurenkommission zur Bewertung von DNA-Mischspuren

Int J Legal Med (2009) 123:1–5
DOI 10.1007/s00414-008-0244-4

REVIEW ARTICLE

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
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  - Schneider *et al.* (2009) *Int. J. Legal Med.* 123: 1-5 (English version)
- ENFSI Policy Statement
  - Morling *et al.* (2007) *FSI Genetics* 1(3):291–292
- New Zealand/Australia Support Statement
  - Stringer *et al.* (2009) *FSI Genetics*
- SWGDAM – **Autosomal STR Interpretation Guidelines (2010)**

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.

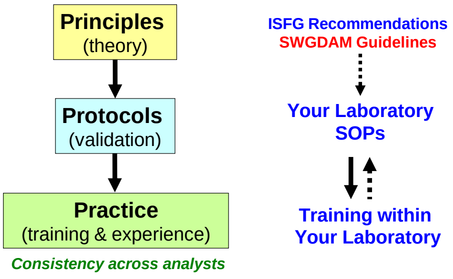
Purpose and Scope (3)

- 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

---

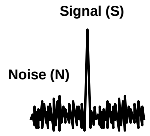
1. Preliminary Evaluation of Data

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 > 3x sd of noise

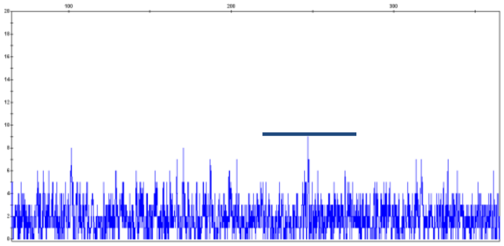
---

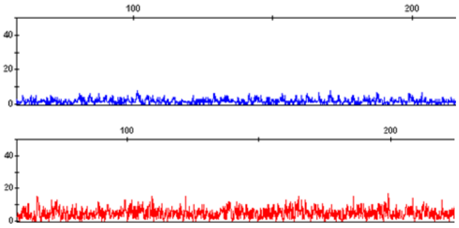
1. Preliminary Evaluation of Data

- 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

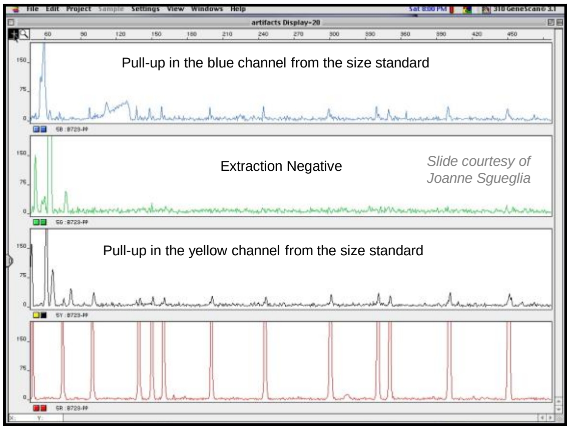
- 2.1. The laboratory establishes criteria to assign allele designations to appropriate peaks.
- 2.1.2.2. The laboratory establishes guidelines for the designation of alleles containing an incomplete repeat motif (i.e., an off-ladder allele falling within the range spanned by the ladder alleles).
- 2.1.2.3. 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).

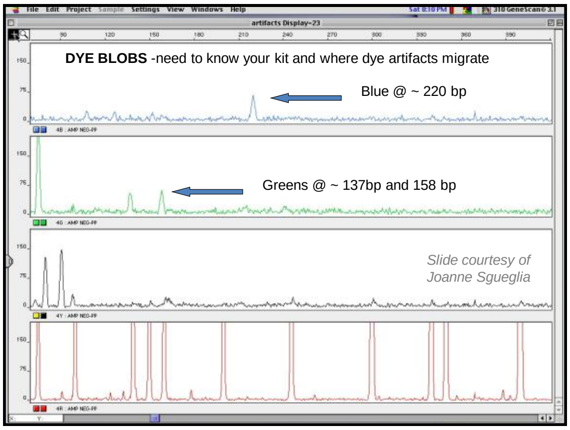


Pull-up in the blue channel from the size standard

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

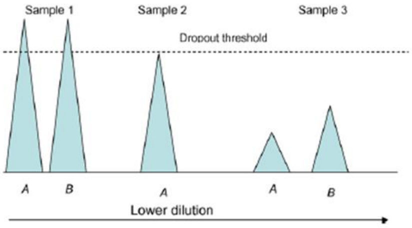
- A threshold value can be applied to alert the DNA analyst that all of the DNA typing information may not have been detected for a given sample.
- This threshold, referred to as a stochastic threshold, is defined as the value above which it is reasonable to assume that allelic dropout has not occurred 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

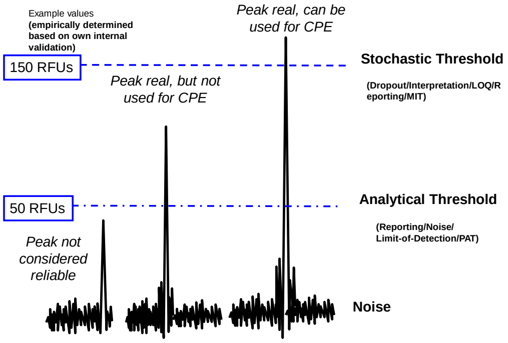
Gill *et al.* (2008) *FSI Genetics* 2(1): 76–82



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



Setting Thresholds

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

what is a peak?

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

what is reliable PCR data?

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. Interpretation of DNA Typing Results

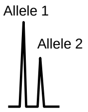
- 3.2.1.1. If measures are used to enhance detection sensitivity (i.e., allelic height), the laboratory should perform additional studies to establish independent criteria for application of a separate stochastic threshold(s). Such measures may include but not be limited to increased amplification cycle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

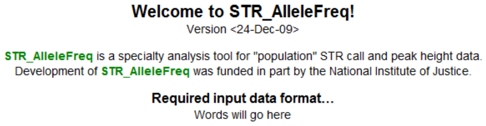


PHR consistent with single source Typically above 60%

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%).

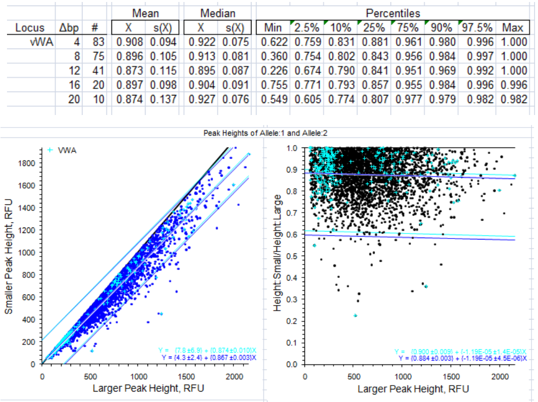
New Program from NIST (Dave Duewer)



Welcome to STR_AlleleFreq!
Version <24-Dec-09>

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

Required input data format…
Words will go here



Locus	Δbp	#	Mean X	Mean s(X)	Median X	Median s(X)	Percentiles Min	2.5%	10%	25%	75%	90%	97.5%	Max
vWA	4	83	0.908	0.094	0.922	0.075	0.622	0.759	0.831	0.881	0.961	0.980	0.996	1.000
	8	75	0.896	0.105	0.913	0.081	0.360	0.754	0.802	0.843	0.956	0.984	0.997	1.000
	12	41	0.873	0.115	0.895	0.087	0.226	0.674	0.790	0.841	0.951	0.969	0.992	1.000
	16	20	0.897	0.098	0.904	0.091	0.755	0.771	0.793	0.857	0.955	0.984	0.996	0.996
	20	10	0.874	0.137	0.927	0.076	0.549	0.605	0.774	0.807	0.977	0.979	0.982	0.982

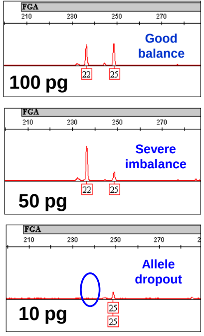
Peak Heights of Allele:1 and Allele:2

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

Signal aided with 31 PCR cycles



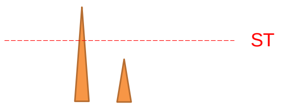
Peak Heights (RFUs)

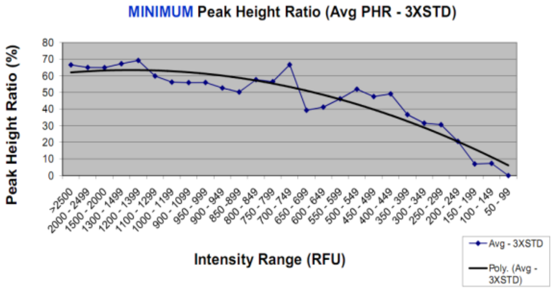
	FGA-22	FGA-25	PHR	Average PHR
(1)	1692	1517	**0.90**	**0.69** (0.23)
(2)	1915	864	0.45	
(3)	1239	909	0.73	
(1)	992	260	**0.26**	**0.49** (0.36)
(2)	1422	419	0.29	
(3)	895	805	0.90	
(1)	--	66	**0**	**0.37** (0.32)
(2)	54	107	0.50	
(3)	130	219	0.59	

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.



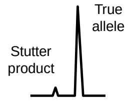


Slide courtesy of Todd Bille (ATF)

Stutter

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;



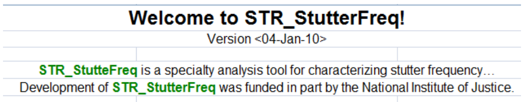
Stutter typically below 15%

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>

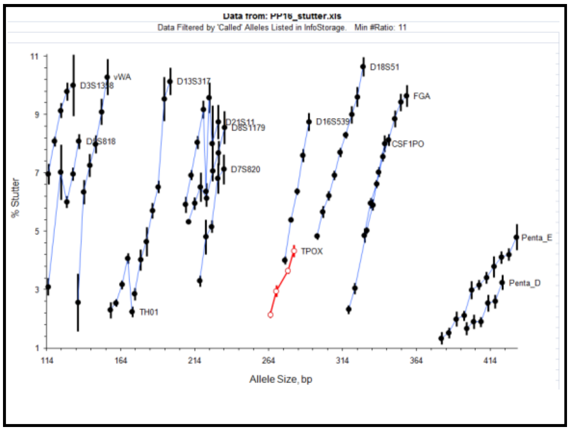
STR_StutteFreq is a specialty analysis tool for characterizing stutter frequency…

Development of **STR_StutterFreq** was funded in part by the National Institute of Justice.

TPOX – [AATG]$_N$

			Stutter		
Locus	Allele	Size	#	Median	MADe
TPOX	8	265.2	86	2.1	0.5
	9	269.2	21	2.9	0.4
	11	277.2	75	3.6	0.4
	12	281.2	14	4.3	0.4
		Avg	196	3.3	0.4
		SD		0.9	

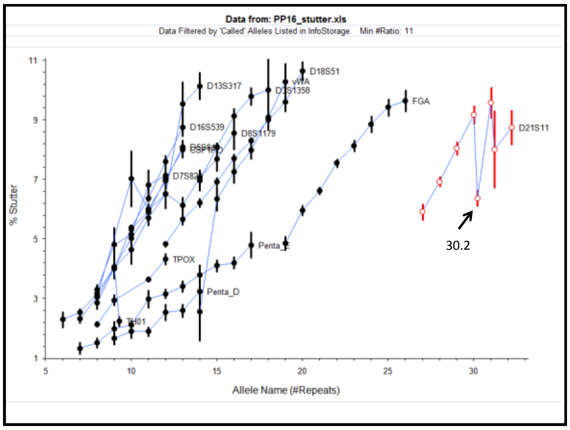
Mutation Rate: 0.01%



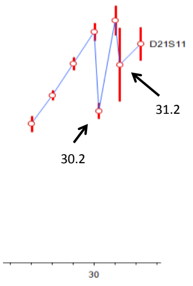
D21S11 – a complex repeat

			Stutter		
Locus	Allele	Size	#	Median	MADe
D21S11	27	207.8	20	5.9	0.6
	28	211.8	69	6.9	0.7
	29	215.8	59	8.0	0.8
	30	219.9	66	9.2	1.2
→	30.2	221.9	11	6.4	0.5
	31	223.9	21	9.6	1.2
	31.2	226.0	28	8.0	3.4
	32.2	230.0	33	8.7	1.7
		Avg	307	7.8	1.6
		SD		1.3	

[TCTA]$_N$ [TCTG]$_N$
[TCTA]$_N$ TA
[TCTA]$_N$ TCA
[TCTA]$_N$ TCCATA
[TCTA]$_N$


Data from: PP16_stutter.xls
Data Filtered by 'Called' Alleles Listed in InfoStorage. Min #Ratio: 11

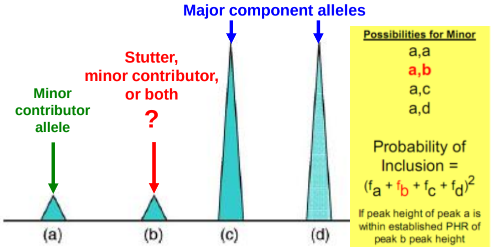
			Stutter		
Locus	Allele	Size	#	Median	MADe
D21S11	27	207.8	20	5.9	0.6
	28	211.8	69	6.9	0.7
	29	215.8	59	8.0	0.8
	30	219.9	66	9.2	1.2
	30.2	221.9	11	6.4	0.5
	31	223.9	21	9.6	1.2
→	31.2	226.0	28	8.0	3.4
	32.2	230.0	33	8.7	1.7
		Avg	307	7.8	1.6
		SD		1.3	



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



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

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

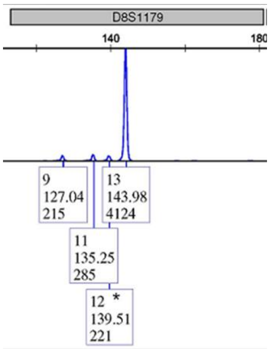
Gill *et al.* (2006) DNA Commission of the International Society of Forensic Genetics: Recommendations on the interpretation of mixtures. *Forensic Sci. Int.* 160: 90-101

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

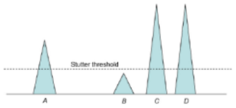


Fig. 2. A two person mixture with major peaks *C*, *D* and minor peaks *A*. There is an additional peak present in a stutter position (*B*).

Likely a AA

(homozygote)

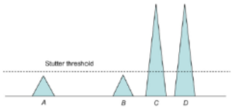


Fig. 3. A two person mixture with major peaks *C*, *D* and minor peaks *A*, *B*, where *B* is in a stutter position.

Possibly AB

(heterozygote)

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

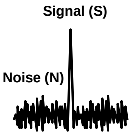
Stutter effects

- 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)

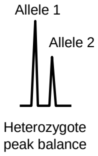
What is a true peak (allele)?

Analytical threshold



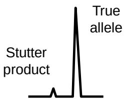
Signal > 3x sd of noise

Peak height ratio (PHR)



PHR consistent with single source Typically above 60%

Stutter percentage



Stutter location below 15%

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3. Interpretation of DNA Typing Results

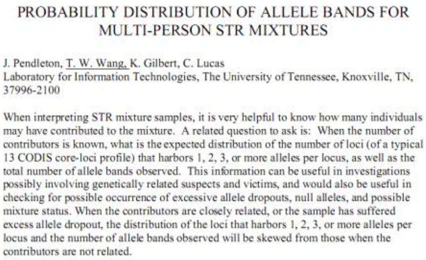
- 3.1. Non-Allelic Peaks
- 3.2. Application of Peak Height Thresholds to Allelic Peaks
- 3.3. Peak Height Ratio
- 3.4. Number of Contributors to a DNA Profile
- 3.5. Interpretation of DNA Typing Results for Mixed Samples
- 3.6 Comparison of DNA Typing Results

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

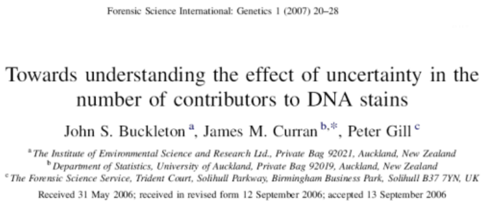
When interpreting STR mixture samples, it is very helpful to know how many individuals may have contributed to the mixture. A related question to ask is: When the number of contributors is known, what is the expected distribution of the number of loci (of a typical 13 CODIS core-loci profile) that harbors 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?

- D.R. Paoletti, T.E. Doom, C.M. Krane, M.L. Raymer, D.E. Krane, "Empirical analysis of the STR profiles resulting from conceptual mixtures", J. Forensic Sci. 50 (2005) 1361–1366.
- 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.



Forensic Science International: Genetics 1 (2007) 20–28

Towards understanding the effect of uncertainty in the number of contributors to DNA stains

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Received 31 May 2006; received in revised form 12 September 2006; accepted 13 September 2006

Buckleton et al. (2007)

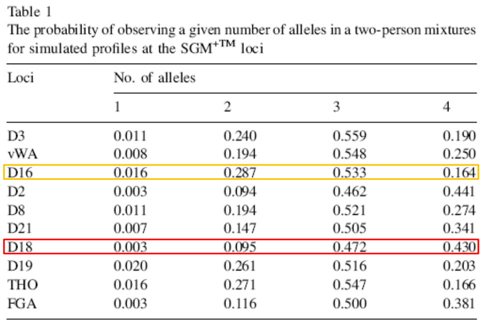

Table 1
The probability of observing a given number of alleles in a two-person mixtures for simulated profiles at the SGM^{+TM} loci

Loci	No. of alleles			
	1	2	3	4
D3	0.011	0.240	0.559	0.190
vWA	0.008	0.194	0.548	0.250
D16	0.016	0.287	0.533	0.164
D2	0.003	0.094	0.462	0.441
D8	0.011	0.194	0.521	0.274
D21	0.007	0.147	0.505	0.341
D18	0.003	0.095	0.472	0.430
D19	0.020	0.261	0.516	0.203
THO	0.016	0.271	0.547	0.166
FGA	0.003	0.116	0.500	0.381

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.

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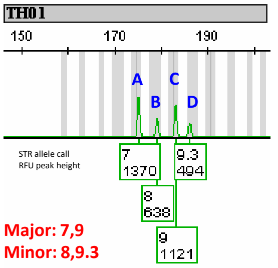
4 Allele Locus: TH01

PHRs



Major: 7,9
Minor: 8,9.3

Consider all possible combinations:

B/A = 638/1370 = 0.466

B/C = 638/1121 = 0.569

C/A = 1121/1370 = 0.818 **major**

D/B = 494/648 = 0.774 **minor**

D/C = 494/1121 = 0.441

All other combinations <0.60 PHR

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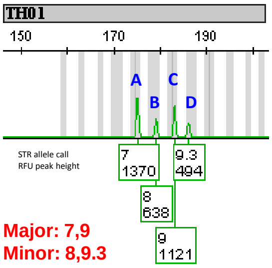
4 Allele Locus: TH01

Mix Ratio



Major: 7,9
Minor: 8,9.3

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**

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.

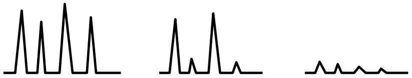
Adapted from Peter Schneider slide (presented at EDNAP meeting in Krakow in April 2007)

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



Type A **Type B** **Type C**

Schneider et al. (2009) and SWGDAM

Type A **Type B** **Type C**

"Indistinguishable" **"Distinguishable"** **"Uninterpretable"**

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.

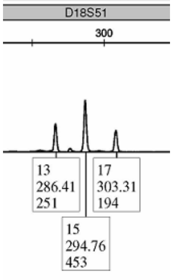
3.5. Interpretation of DNA Typing Results for Mixed Samples

- 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.

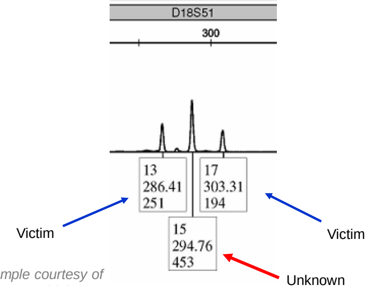
An Example – Stain on Victim's Underwear



Donor Mix
0.6 Victim to 1 Unknown

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

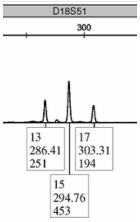
Example courtesy of Bruce Heidebrecht



Example courtesy of Bruce Heidebrecht

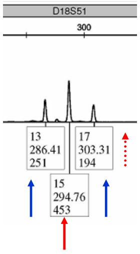
Test for various possibilities for mixture deconvolution

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



Example courtesy of Bruce Heidebrecht

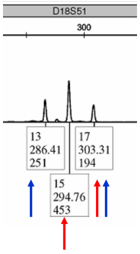
Test for various possibilities for mixture deconvolution



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)

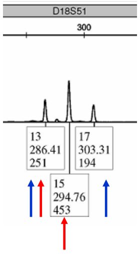
Test for various possibilities for mixture deconvolution



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

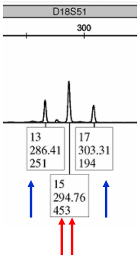
Test for various possibilities for mixture deconvolution



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 then that leaves the Victim with PHR of 77%
(194/251)

Calculation based on ratio of
0.6 Victim to 1 Unknown

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3. Interpretation of DNA Typing Results

- 3.1. Non-Allelic Peaks
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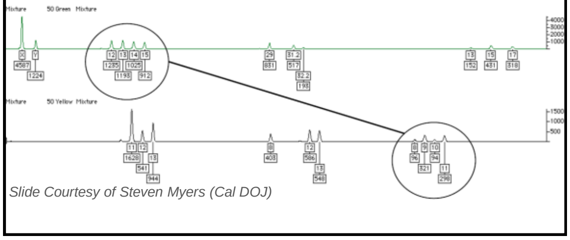
- 3.6 Comparison of DNA Typing Results

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. 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.



Slide Courtesy of Steven Myers (Cal DOJ)

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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**.”

Buckleton, J. and Curran, J. (2008) A discussion of the merits of random man not excluded and likelihood ratios. *Forensic Sci. Int. Genet.* 2: 343-348.

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).

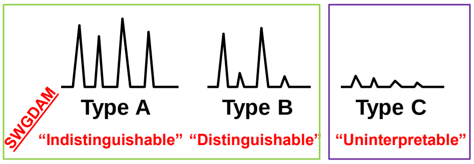
Category of DNA Typing Result	RMP	CPE/CPI	LR (1)
Single Source	✓		✓
Single Major Contributor to a Mixture	✓		✓
Multiple Major Contributors to a Mixture	✓ (2)	✓ (2)	✓
Single Minor Contributor to a Mixture	✓	✓ (3)	✓
Multiple Minor Contributors to a Mixture	✓ (2)	✓ (3)	✓
Indistinguishable Mixture	✓ (1)	✓	✓

(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



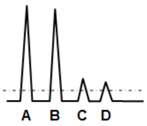
A statistical analysis must be performed

A statistical analysis <u>should not</u> 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

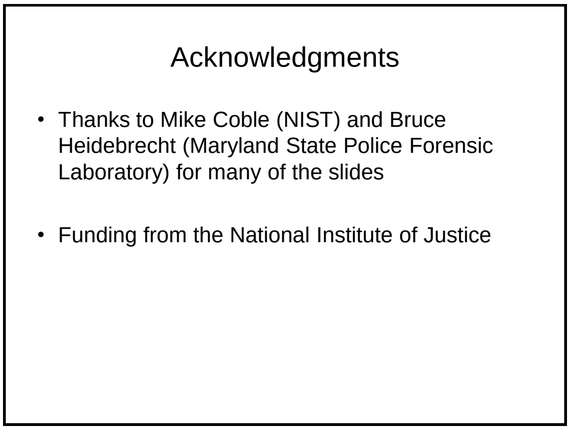
Restricted

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.



Acknowledgments

- Thanks to Mike Coble (NIST) and Bruce Heidebrecht (Maryland State Police Forensic Laboratory) for many of the slides

- Funding from the National Institute of Justice