SWGDAM Autosomal STR Interpretation Guidelines

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This presentation will be made available on STRBase:
http://www.cstl.nist.gov/biotech/strbase/NISTpub.htm

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 Ted Staples (Georgia Bureau of Investigation)
- **Currently organized into six subcommittees**:
  - CODIS, LTDNA, mtDNA, Missing Persons & Mass Disasters Mass Spectrometry, **Mixture Interpretation**

SWGDAM has previously issued guidance documents on validation and data interpretation

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 STR Guidelines

- The January 14, 2010 approved SWGDAM STR Guidelines were **publicly released April 8, 2010** on the FBI website for the CODIS group: http://www.fbi.gov/hq/lab/html/codis1.htm
  (underneath the Audit document information).
  - The direct links are:
  - http://www.fbi.gov/hq/lab/html/codis_swgdam.htm
    (html text version)
  - http://www.fbi.gov/hq/lab/html/codis_swgdam.pdf
    (pdf version)

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

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

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

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

1066 words (4 pages)

9862 words (28 pages)

http://www.cstl.nist.gov/biotech/strbase/NISTpub.htm

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.fbi.gov/hq/lab/html/codis_swgdam.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.fbi.gov/hq/lab/html/codis_swgdam.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.fbi.gov/hq/lab/html/codis_swgdam.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."

Points to Address Today Related to Mixtures

Stutter and Stats

- Stutter peaks – accounting for them statistically in mixtures
- Statistics – required use with inclusions and appropriate use of different statistical approaches

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

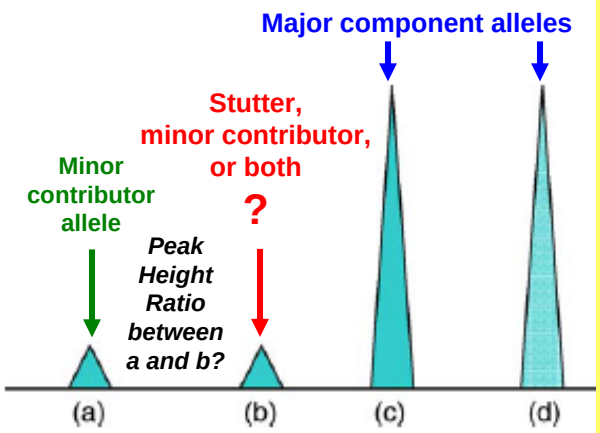
3.5.7. Use of "known" contributors to refine interpretation

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.

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

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).

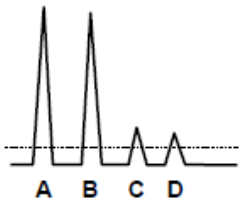
Category of DNA Typing Result	RMP	CPE/CPI	LR (1)
Single Source	✔		✔
Single Major Contributor to a Mixture	✔		✔
Multiple Major Contributors to a Mixture	✔ (2)	✔ (2)	✔
Single Minor Contributor to a Mixture	✔	✔ (3)	✔
Multiple Minor Contributors to a Mixture	✔ (2)	✔ (3)	✔
Indistinguishable Mixture	✔ (1)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

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

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

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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.

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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.

http://www.cstl.nist.gov/biotech/strbase/NISTpub.htm

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

Future Directions

- **Training materials with worked examples are needed** to help analysts better appreciate what is being conveyed with specific points in these SWGDAM Guidelines
- **An NIJ-sponsored workshop on mixture interpretation will be conducted October 11, 2010** at the International Symposium on Human Identification (Promega meeting) in San Antonio, TX
  - **Registration for 175 State and Local Crime Laboratory personnel is provided at no charge** through a cooperative agreement from the National Institute of Justice, Office of Justice Programs, U.S. Department of Justice, #2008-DN-BX-K158

Acknowledgments

- **Mixture committee members** for their hard work through many long hours of discussing and writing these guidelines
- Ted Staples for his support as SWGDAM chair
- Bruce Heidebrecht (for some of the slides)
- NIJ Funding to our NIST Group through NIST OLES

http://www.cstl.nist.gov/biotech/strbase/NISTpub.htm
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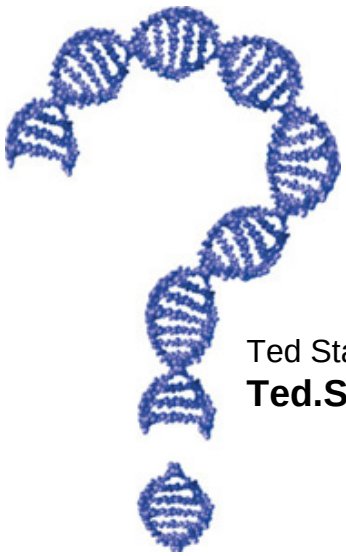

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