DNA Mixture Interpretation:
Principles and Practice in Component Deconvolution and Statistical Analysis

Principles in Mixture Interpretation

AAFS 2008 Workshop #16
Washington, DC
February 19, 2008

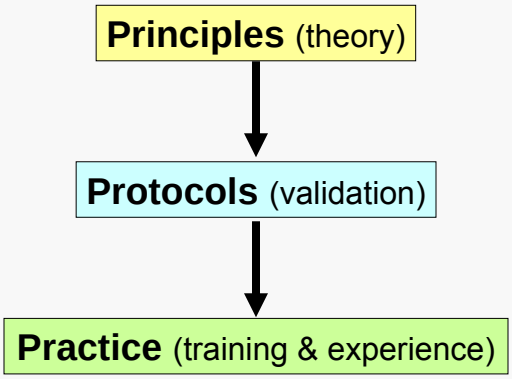
John M. Butler

john.butler@nist.gov

Outline

- Elements of Mixture Interpretation
- **ISFG Recommendations on Mixtures**
  - UK Response
  - German mixture classification categories
- Flowchart to aid interpretation

Elements of DNA Mixture Interpretation



Two Parts to Mixture Interpretation

- Determination of alleles present in the evidence and **deconvolution of mixture components** where possible
  - Many times through comparison to victim and suspect profiles
- **Providing some kind of statistical answer** regarding the weight of the evidence
  - There are multiple approaches and philosophies

Software tools can help with one or both of these…

Steps in the Interpretation of Mixtures

(Clayton *et al.* 1998)

Step #1 Identify the Presence of a Mixture

↓

Step #2 Designate Allele Peaks

↓

Step #3 Identify the Number of Potential Contributors

↓

Step #4 Estimate the Relative Ratio of the Individuals Contributing to the Mixture

↓

Step #5 Consider All Possible Genotype Combinations

↓

Step #6 Compare Reference Samples


ELSEVIER

Forensic Science International

Forensic Science International 91 (1998) 55–70

Analysis and interpretation of mixed forensic stains using DNA STR profiling

T.M. Clayton[a,*], J.P. Whitaker[a], R. Sparkes[b], P. Gill[b]

[a]Forensic Science Service, Wetherby Laboratory, Sandbeck Way, Audby Lane, Wetherby, West Yorkshire LS22 4DN, UK

[b]Forensic Science Service, Priory House, Gooch Street North, Birmingham B56QQ, UK

Received 13 May 1997; received in revised form 9 October 1997; accepted 27 October 1997

Clayton *et al.* (1998) *Forensic Sci. Int.* 91:55-70

Will be covered in next section of workshop on mixture deconvolution

Statistical Approaches with Mixtures

See Ladd *et al.* (2001) *Croat Med J.* 42:244-246

- **Inferring Genotypes of Contributors** - Separate major and minor components into individual profiles and compute the random match probability estimate as if a component was from a single source
- **Calculation of Exclusion Probabilities** - CPE/CPI (RMNE) – The probability that a random person (unrelated individual) would be excluded as a contributor to the observed DNA mixture
- **Calculation of Likelihood Ratio Estimates** – Comparing the probability of observing the mixture data under two (or more) alternative hypotheses; in its simplest form LR = 1/RMP

DAB Recommendations on Statistics

February 23, 2000
Forensic Sci. Comm. 2(3); available on-line at
http://www.fbi.gov/hq/lab/fsc/backissu/july2000/dnastat.htm

"The DAB finds either one or both PE or LR calculations acceptable and strongly recommends that one or both calculations be carried out whenever feasible and a mixture is indicated"

- Probability of exclusion (PE)
  - Devlin, B. (1992) Forensic inference from genetic markers. *Statistical Methods in Medical Research*, 2, 241–262.
- Likelihood ratios (LR)
  - Evett, I. W. and Weir, B. S. (1998) *Interpreting DNA Evidence*. Sinauer, Sunderland, Massachusetts.

ISFG DNA Commission on Mixture Interpretation

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

UPDATED SLIDE

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

Available online at www.sciencedirect.com
SCIENCE DIRECT
ELSEVIER
Forensic Science International 160 (2006) 90–101
Forensic Science International
www.elsevier.com/locate/forsciint

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

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

[a] Forensic Science Service, Trident Court, 2960 Solihull Parkway, Birmingham, UK
[b] Forensic Science Group, School of Public Health, University of California, Berkeley, CA 510-339-1911, USA
[c] ESR, Private Bag 92021, Auckland, New Zealand
[d] Institute of Legal Medicine, Faculty of Medicine, University of Santiago de Compostela, 15705 Santiago de Compostela, Spain
[e] Institute of Medical Informatics and Statistics, Kiel, Germany
[f] Division of Blood Group Serology, Medical University of Vienna, Austria
[g] Department of Forensic Genetics, Institute of Forensic Medicine, University of Copenhagen, Copenhagen, Denmark
[h] Office of the Chief Medical Examiner, Department of Forensic Biology, 520 First Avenue, New York, NY 10016, USA
[i] Institute of Legal Medicine University Clinic of Cologne, Melatengürtel, 60-62 D 50823 Köln, Germany
[j] University of Washington, Department of Biostatistics, Box 357232, Seattle, WA 98195, USA

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

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

ISFG Recommendations on Mixture Interpretation

July 13, 2006 issue of *Forensic Science International*

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

ELSEVIER
Forensic Science International 160 (2006) 90–101
www.elsevier.com/locate/forsciint

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

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

[a] Forensic Science Service, Trident Court, 2960 Solihull Parkway, Birmingham, UK
[b] Forensic Science Group, School of Public Health, University of California, Berkeley, CA 510-339-1911, USA

Abstract

The DNA commission of the International Society of Forensic Genetics (ISFG) was convened at the 21st congress of the International Society for Forensic Genetics held between 13 and 17 September in the Azores, Portugal. The purpose of the group was to agree on guidelines to encourage best practice that can be universally applied to assist with mixture interpretation. In addition the commission was tasked to provide guidance on low copy number (LCN) reporting. Our discussions have highlighted a significant need for continuing education and research into this area. We have attempted to present a consensus from experts but to be practical we do not claim to have conveyed a clear vision in every respect in this difficult subject. For this reason, we propose to allow a period of time for feedback and reflection by the scientific community. Then the DNA commission will meet again to consider further recommendations.

Keywords: STR typing; Biostatistical analysis; Likelihood ratio; Probability of exclusion; Mixtures; ISFG DNA commission

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

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

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
- ENFSI Policy Statement
  - Morling *et al.* (2007) *FSI Genetics* 1(3):291–292
- SWGDAM – nothing yet…
  - a Mixture Interpretation subcommittee was started Jan 2007

UPDATED SLIDE

SWGDAM Mixture Interpretation Subcommittee

- **John Butler** (NIST) - chair
- **Gary Sims** (CA DOJ) - co-chair
- Mike Adamowicz (CT)
- Jack Ballantyne (UCF/NCFS)
- George Carmody (Carleton U)
- Cecelia Crouse (PBSO)
- Allison Eastman (NYSP)
- Roger Frappier (CFS-Toronto)
- Ann Gross (MN BCA)
- Phil Kinsey (MT)
- Jeff Modler (RCMP)
- Gary Shutler (WSP)

Everyone not at every meeting…

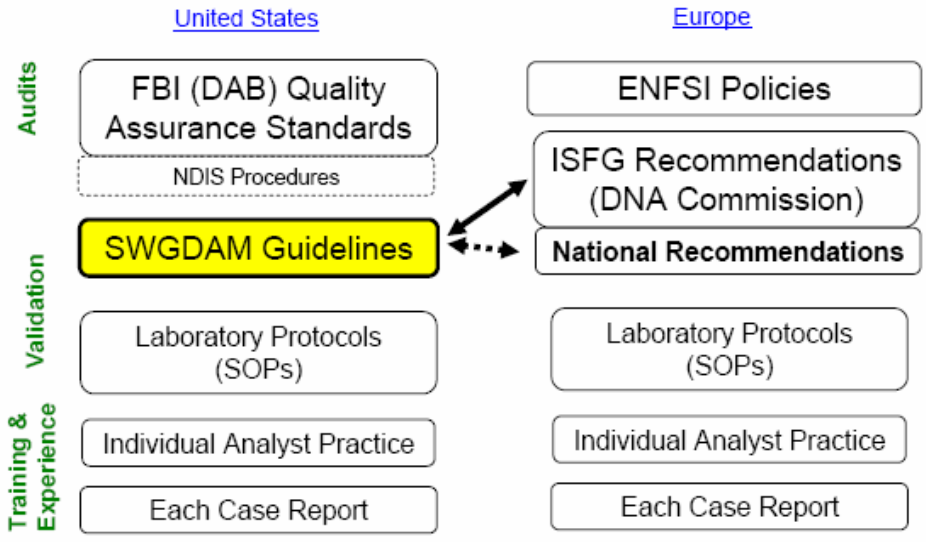
Have met 3 times:
Jan 2007
July 2007
Jan 2008

Additional Participants (Jan 2008)
Bruce Heidebrecht (MD)
Steve Lambert (SC)

Started in January 2007

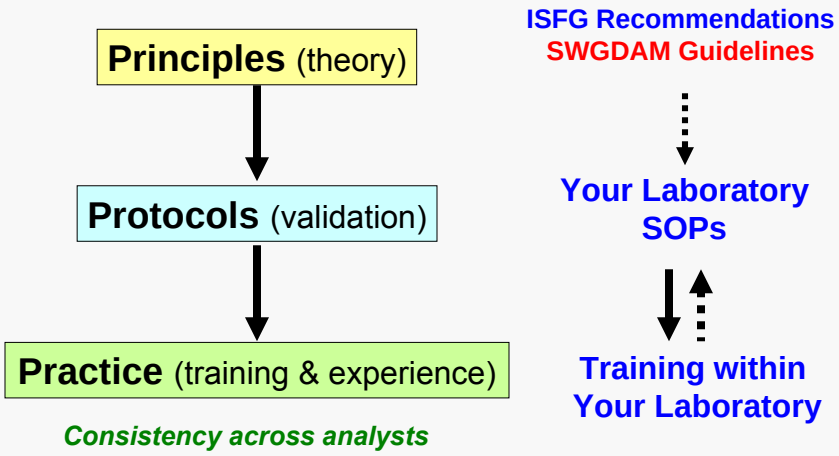
My perspective…

Hierarchy of Rules for Forensic DNA Labs



Hopefully each conforms to the levels above it…

Elements of DNA Mixture Interpretation



ISFG (2006) Recommendations

- Recommendation 1: **The likelihood ratio is the preferred approach to mixture interpretation.** The RMNE (probability of exclusion) approach is restricted to DNA profiles where the profiles are unambiguous. If the DNA crime stain profile is low level and some minor alleles are the same size as stutters of major alleles, and/or if drop-out is possible, then the RMNE method may not be conservative.

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

UK Response

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

Recommendation 1:

- **RMNE is a recognized and advocated interpretation method.** The likelihood ratio and match probability methods are interchangeable—however, the wording of the match probability is equally acceptable for understanding in court. In addition, a frequency calculation can be used, e.g. ‘‘I have calculated that the chance of observing this combination of DNA markers is about in 1 in X of the UK population’’ or ‘‘the chance that a person picked at random from the general UK population would have this combination of DNA markers is about 1 in X’’.

Recommendation 1 (cont): **UK Response**; Gill *et al.* (2008) *FSI Genetics* 2(1): 76–82

- **If a profile can be identified with confidence from a mixture then the match probability statement may be preferable.** A non-exhaustive list of examples is as follows:

  (a) There is a major/minor mixture where the major contributor can be easily separated from the minor contributor(s) by virtue of the differences in peak height/area of the alleles.

  (b) **It may be possible to condition on one contributor, e.g. a victim, and to subtract this profile from the mixture, to leave a single contributor that can be reported separately.** The contributors may be even, or major/minor. If the evidential profile is not major then it is inevitable that the conditioned major profile will mask some of the minor contributor alleles. Consequently, if a match probability is reported, some of the minor contributor alleles will be masked by the major contributor. The LR method may be preferred if this is the case.

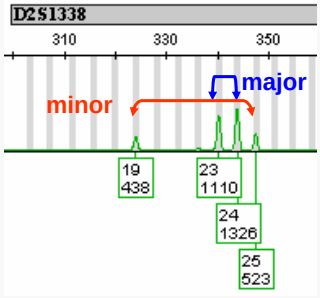
  (c) **When conditioning is used to subtract a profile, then this should be made clear in the statement**. If conditioning is challenged, then it may be appropriate to recalculate the strength of the evidence using the LR approach. A caveat can be included in the statement to make this point clear.

Example 1

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

If a profile can be identified with confidence from a mixture then the match probability statement may be preferable.

(a) There is a major/minor mixture where the major contributor can be easily separated from the minor contributor(s) by virtue of the differences in peak height/area of the alleles.



Major Contributor
23,24

23: 1110 RFUs
24: 1326 RFUs

Minor Contributor
19,25

19: 438 RFUs
25: 523 RFUs

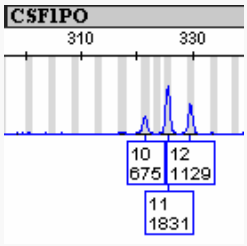
523/1110 = 0.47 which is less than 0.60 PHR

Example 2

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

If a profile can be identified with confidence from a mixture then the match probability statement may be preferable.

(b) It may be possible to condition on one contributor, e.g. a victim, and to subtract this profile from the mixture, to leave a single contributor that can be reported separately.



If Victim = 11,12

Then other component would be 10,11

ISFG (2006) Recommendations

- Recommendation 2: Even if the legal system does not implicitly appear to support the use of the likelihood ratio, it is recommended that the scientist is trained in the methodology and routinely uses it in case notes, advising the court in the preferred method before reporting the evidence in line with the court requirements. The scientific community has a responsibility to support improvement of standards of scientific reasoning in the court-room.

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

UK Response

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

Recommendation 2:

- **Accepted**—albeit we prefer to think in terms of advising the justice system rather than the court or court-room.

ISFG (2006) Recommendations

- Recommendation 3: The methods to calculate likelihood ratios of mixtures (not considering peak area) described by Evett *et al.* (*J. Forensic Sci. Soc.* 1991;31:41-47) and Weir *et al.* (*J. Forensic Sci.* 1997;42:213-222) are recommended.

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

UK Response

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

Recommendation 3:

- All laboratories in the UK consider peak height/area in their assessments. The formulae are fundamental to all mixture interpretation with or without peak height/area consideration.

ISFG (2006) Recommendations

- Recommendation 4: If peak height or area information is used to eliminate various genotypes from the unrestricted combinatorial method, this can be carried out by following a sequence of guidelines based on Clayton *et al.* (*Forensic Sci. Int.* 1998;91:55-70).

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

UK Response

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

Recommendation 4:

- Accepted.

ISFG (2006) Recommendations

- Recommendation 5: The probability of the evidence under H_p is the province of the prosecution and the probability of the evidence under H_d is the province of the defense. The prosecution and defense both seek to maximize their respective probabilities of the evidence profile. To do this both H_p and H_d require propositions. **There is no reason why multiple pairs of propositions may not be evaluated**.

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

UK Response

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

Recommendation 5:

- Accepted.

Thoughts by Peter Gill on Recommendation #5

(ENFSI meeting, Krakow, Poland, April 19, 2007)

- Prosecution and defense each want to maximize their respective probabilities
- Recommendation 5 places ownership for each hypothesis.
- In order to perform the LR calculation(s), the forensic scientist decides on both the prosecution and defense hypotheses.
- Since the forensic scientists usually cannot discover the defense hypothesis before the trial (as they are typically working with the prosecution if the DNA matches…), assumptions must be clearly stated with the important caveat that you cannot perform calculations on the stand! (For example, you need three weeks warning to make and check calculations.)
- By anchoring the respective hypotheses to each side, the defense can change their hypothesis but the prosecution does not need to change theirs…
- It is worth noting that the likelihood ratio always goes up if the defense lowers their hypothesis (H_d gets lower with more possible combinations)

ISFG (2006) Recommendations

- 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.** Under these circumstances alleles in stutter positions that do not support H_p should be included in the assessment.
- In general, stutter percentage is <15%

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

NEW SLIDE

Consideration of Peak in Stutter Position

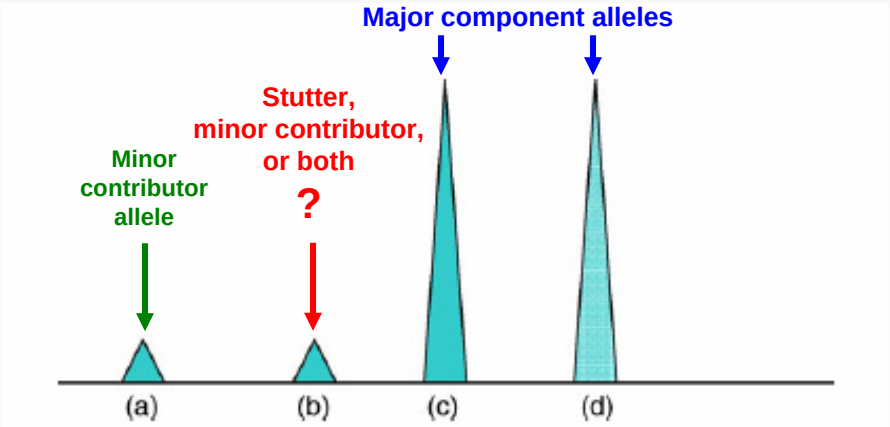


Fig. 4. *c* and *d* are unambiguous alleles, *b* is a minor allele in a stutter position and *a* is an unambiguous minor allele.

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

UK Response

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

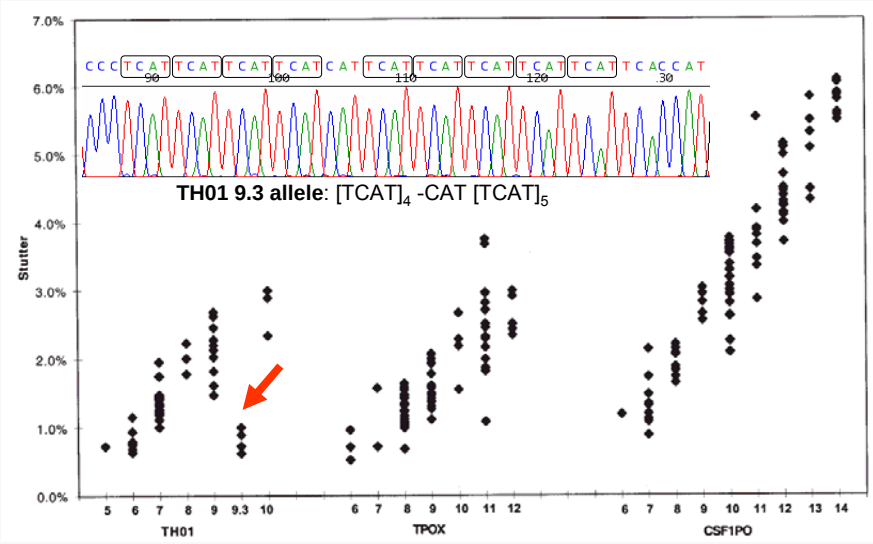
Recommendation 6:

- Stutters are locus-dependent…
- It is recommended that laboratories make their own maximum experimentally observed stutter sizes per locus determinations since the effects may be technique dependent.
- It is recommended that [maximum stutter percentages be] evaluated per locus.

NEW SLIDE

Measured Stutter Percentages

Variable by Allele Length and Composition



Holt CL, Buoncristiani M, Wallin JM, Nguyen T, Lazaruk KD, Walsh PS. TWGDAM validation of AmpFlSTR PCR amplification kits for forensic DNA casework. *J Forensic Sci* 2002; 47(1): 66-96.

UK Response

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

- ***Characterization of +4 base stutters***

  We agreed to review +4 bp stutters, however, we note that their presence often relates to over-amplified samples. Preliminary experimental work suggests that they are low level and **generally less then 4% the size of the progenitor allele** (Rosalind Brown, personal communication). Note that 4 bp and +4 bp stutter cannot be distinguished from genetic somatic mutation without experimental work—furthermore, somatic mutations may give rise to peaks that are larger than those caused by stutter artifacts.

ISFG (2006) Recommendations

- Recommendation 7: If drop-out of an allele is required to explain the evidence under H_p: (S = ab; E = a), then the allele should be small enough (height/area) to justify this. Conversely, if a full crime stain profile is obtained where alleles are well above the background level, and the probability of drop-out approaches Pr(D) ≈ 0, then H_p is not supported.

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

UK Response

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

Recommendation 7:

- We recommend slight rewording…[with mention of companion allele]
- If a full crime-stain profile is obtained where alleles are well above the background level, and the probability of dropout Pr(D) approaches zero, then H_p is not supported (Figure 6).

Hypothetical Examples

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

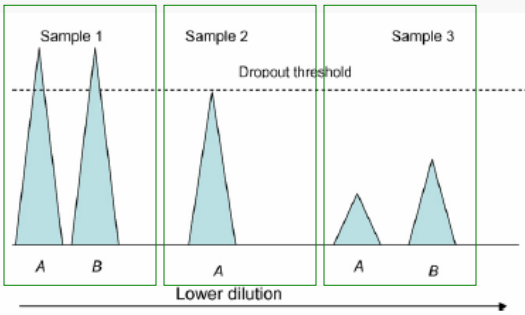


Fig. 4. Results from serial dilutions of the same sample genotype *AB*. The first result (sample 1) shows a locus where both alleles are represented in the profile. One or both of these alleles are above the dropout threshold and consequently are always present in the epg. The second result shows a result where dropout has occurred – the survivor allele is just below the dropout threshold hence this is a rare event, but not impossible. If *A* was just above the dropout threshold we would determine it to be a homozygote *AA* genotype. In the third sample, both alleles are well below the dropout threshold – it is an unambiguous, albeit unbalanced heterozygote. If only one allele was present, then we would have to consider the possibility of dropout of the partner. The same rationale can be applied to any analytical regime, e.g. 28 and 34 PCR cycles.

If Below Dropout Threshold…

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

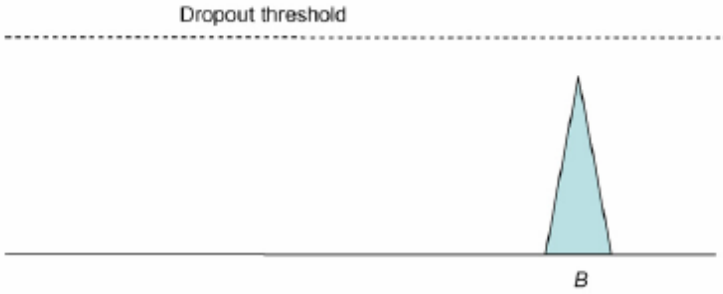


Fig. 5. In this example allele *B* is below the dropout threshold, hence we cannot be confident that it is from a homozygote *BB* individual. It could also be from an individual who is heterozygote, where the missing allele is any other allele. The probability $B|\text{unknown}$, H_d is $2\Pr(BF)$, where the '*F*' designation is assigned a probability of 1 to take account of the possibility that any allele could have dropped out.

If Above Dropout Threshold…

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

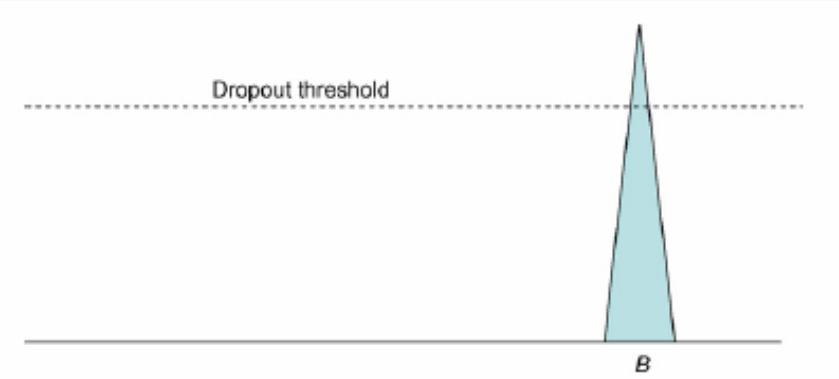


Fig. 6. In this example allele *B* is above the dropout threshold, hence we can be confident that it is from a homozygote *BB* individual. The probability of B|unknown, H_d is $\Pr(B)^2$.

Setting Thresholds

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

- **Dropout (stochastic) threshold**
  - Dependent on biological sensitivity
  ~150-200 RFU
  - Impacted by assay and injection parameters

Determining the Dropout (Stochastic) Threshold

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

- The dropout threshold can be determined experimentally for a given analytical technique from a series of pre-PCR dilutions of extracts of known genotype technique (it will probably vary between analytical methods). These samples can be used to determine the point where allelic dropout of a heterozygote is observed relative to the size of the survivor companion allele. The threshold is the maximum size of the companion allele observed. This is also the point where Pr(D) approaches zero (Fig. 4).

Dropout threshold will change depending on instrument and assay conditions (e.g., longer CE injection will raise dropout threshold)

ISFG (2006) Recommendations

- Recommendation 8: If the alleles of certain loci in the DNA profile are at a level that is dominated by background noise, then a biostatistical interpretation for these alleles should not be attempted.

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

UK Response

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

Recommendation 8:

- If there is a band below the experimental threshold where background noise might be prevalent, and it is distinct and clear from the background, then it should be recorded and available on the case file.

ISFG (2006) Recommendations

- Recommendation 9: In relation to low copy number, stochastic effects limit the usefulness of heterozygous balance and mixture proportion estimates. In addition, allelic drop-out and allelic drop-in (contamination) should be taken into consideration of any assessment.

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

UK Response

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

Recommendation 9:

- Case pre-assessment is necessary in order to determine the best scientific method to process a sample. To facilitate this, it is recommended that wherever possible, this should include quantification. Quantification is used to determine the optimum method to process—if low-level DNA, a sample would benefit from procedures to enhance sensitivity of detection. There may be reasons where quantification is not practicable, especially if low levels of DNA are expected, since the result itself may be compromised if a portion of the sample is sacrificed. At low DNA levels, the accuracy of the quantification test itself may be inefficient.

UK Response

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

Recommendation 9 (cont):

- It is possible that a given DNA profile may simultaneously comprise both 'conventional' and 'low-level' loci: for example, if degradation has occurred then low molecular weight loci may be above the dropout threshold, whereas high molecular weight loci may be below the dropout threshold.
- Similarly, if the sample is a mixture, then at a given locus there may be some alleles that are above the dropout threshold (from a major contributor) and others that are below the dropout threshold (from a minor contributor), i.e. different interpretation rationale may be simultaneously applied to different contributors within a locus.

German Stain Commission on DNA Mixtures

Rechtsmedizin 2006, 16 : 401 - 404

Rechtsmedizin 2006 · 16:401–404
DOI 10.1007/s00194-006-0411-1
Online publiziert: 16. November 2006

Article in German (English summary in handout)

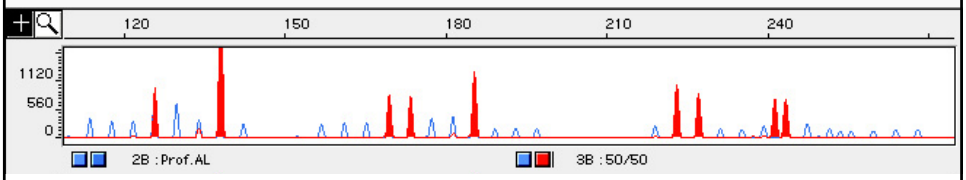
P. M. Schneider[1] · R. Fimmers[4] · W. Keil[2] · G. Molsberger[5] · D. Patzelt[6] · W. Pflug[7] · T. Rothämel[8] · H. Schmitter[3] · H. Schneider[9] · B. Brinkmann[10]
[1] Institut für Rechtsmedizin, Universitätsklinikum, Köln
[2] Institut für Rechtsmedizin, Ludwig-Maximilians-Universität, München
[3] Bundeskriminalamt, Wiesbaden
[4] Institut für Medizinische Biometrie, Informatik und Epidemiologie, Bonn
[5] Landeskriminalamt Nordrhein-Westfalen, Düsseldorf
[6] Institut für Rechtsmedizin, Bayr. Julius-Maximilians-Universität, Würzburg
[7] Landeskriminalamt Baden-Württemberg, Stuttgart
[8] Institut für Rechtsmedizin der Medizinischen Hochschule, Hannover
[9] Hessisches Landeskriminalamt, Wiesbaden
[10] Vorsitzender der Spurenkommission, Institut für Rechtsmedizin, Münster

Danksagung. Für die konstruktive Mitarbeit an den hier vorgestellten Empfehlungen zur Bewertung von DNA-Mischspuren sei K. Anslinger (München), P. Berschick (Düsseldorf), M. Eckert (Wiesbaden), C. Hohoff (Münster), S. Jung (Würzburg) und J. Schnee-Griese (Stuttgart) herzlich gedankt.

General recommendations of the stain commission on the interpretation of DNA results from mixed stains

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

Mixtures



- Mixed stain: more than two alleles per locus in at least two DNA systems
- Inference on the number of contributors:
  - up to 4 alleles: at least 2 contributors
  - up to 6 alleles: at least 3 contributors
  - more than 6 alleles: no meaningful interpretation possible

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

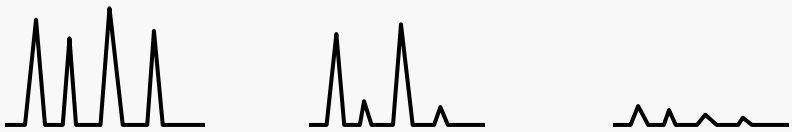
Adapted from Peter Schneider slide (presented at EDNAP meeting in Krakow in April 2007)

Mixture Classification Scheme

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

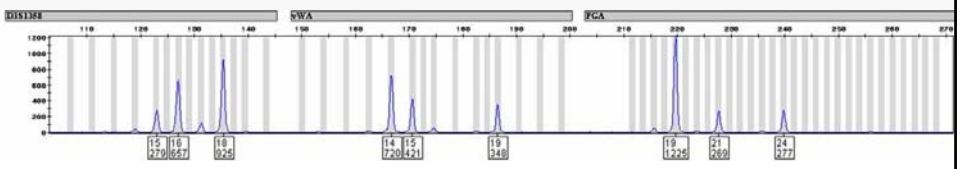
(German Stain Commission, 2006):

- **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



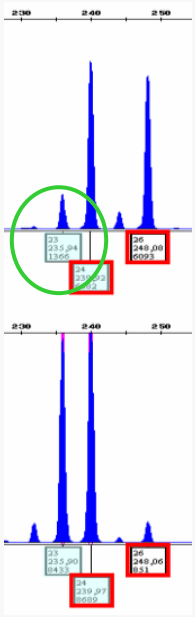
Type A **Type B** **Type C**

Stochastic phenomena



- May lead to allele and locus drop-out and drop-in effects
- Occur when using „low copy number“ conditions
  - e,.g. with increased no. of PCR cycles,
  - BUT ALSO using standard conditions and DNA amounts < 200pg (e.g. as minor component in a mixture!)

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



Stutter effects

- The following criteria have to be considered in case of *stutter peaks*:
  - the relative *stutter* intensities within the alleles of a locus, as well as between loci of a multiplex amplification,
  - the possibility that a stain allele is in the position of a *stutter peak*.

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

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)

Type of mixture and interpretation

- **Type A:** Mixed profile without stochastic effects, a biostatistical analysis has to be performed
- **Type B:** Profile of a major contributor can be unambiguously described and interpreted as a profile from an unmixed stain
- **Type C:** due to the complexity of the mixture, the occurrence of stochastic effects such as allele and locus drop-outs have to be expected:
  - a clear decision to include or exclude a suspect may be difficult to reach, thus a biostatistical interpretation is not appropriate.

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

Biostatistical approaches

- Calculation of the **probability of exclusion** for a randomly selected stain donor* [P(E)]
  (*RMNE - "random man not excluded")
- Calculation of the **likelihood ratio** [LR] based on defined hypotheses for the origin of the mixed stain

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

Which approach should be used?

- If the basis for clearly defined and mutually exclusive hypotheses is given, i.e.:
  - the number of contributors to the stain can be determined,
  - unambiguous DNA profiles across all loci are observed (type A mixtures, or type B, if the person considered as "unknown" contributor is part of the minor component of the mixture),

  then the calculation of a likelihood ratio is appropriate.

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

Which approach should be used?

- If major/minor contributors cannot be identified based on unambiguous DNA profiles, or if the the number of contributors cannot be determined, then the calculation of the probability of exclusion is appropriate.
- The calculation of P(E) is always possible for type A and type B mixtures.

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

Not acceptable …

- … is the inclusion of a genotype frequency of a non-excluded suspect into the report, if the given mixed stain does not allow a meaningful biostatistical interpretation.
  - this would lead to the wrongful impression that this genotype frequency has any evidentiary value regarding the role of the suspect as a contributor to the mixed stain in question.

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

Conclusions

- The likelihood ratio has a significant weight of evidence, as it relates directly to the role of the suspect in the context of the origin of the stain.
- The exclusion probability makes a general statement without relevance to the role of the suspect.
- However, this does not imply that P(E) is always more "conservative" in the sense that the weight of evidence is not as strong compared to the LR.

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

GEDNAP 32

Mixture interpretation exercise:

- 3 person mixture without major contributor
- Person A from group of reference samples was not excluded
- Allele frequencies for eight German database systems provided for exercise
- German-speaking GEDNAP participants invited to participate based on published recommendations

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

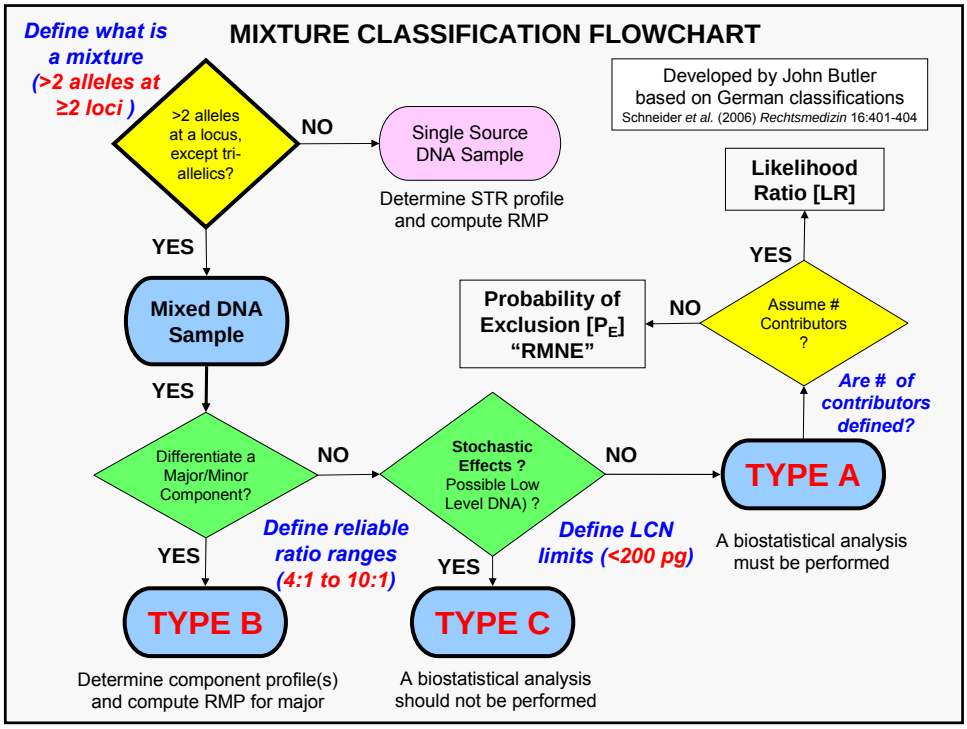
GEDNAP 32

Results:

- 22 labs submitted results (from approx. 80 German-speaking GEDNAP participants)
- Calculations submitted were all correct and consistent:
  - 15x LR approach:
    - Person A + 2 unknown vs. 3 unknown contributors
  - 11x RMNE calculation
- Will be offered again next time

Training and Specific Guidelines/Classification Schemes yielded consistent results among laboratories

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



Claims Have Been Made of No Consensus Regarding Mixture Interpretation

http://www.promega.com/geneticidproc/ussymp17proc/oralpresentations/Perlin.pdf

Scientific Validation of Mixture Interpretation Methods

Mark W. Perlin
Cybergenetics, Pittsburgh, PA

December 5, 2006

In the Proceedings of Promega's
Seventeenth International Symposium on Human Identification

- **Different laboratories follow different mixture interpretation guidelines.** Moreover, different examiners within the same laboratory who are following the same guidelines often infer different STR profiles.
- **Therefore, there is no concordance in current forensic practice on what constitutes a "correct" mixture solution.** Thus, it is not possible to conduct a mixture interpretation concordance study in order to validate a mixture interpretation method.
- **DNA mixture evidence currently fails the general acceptance test of both Frye and Daubert, since there are no generally accepted methods for interpreting mixed stains.**

Interpretation of DNA Mixtures – **European Consensus on Principles**

Morling *et al.* (2007) *FSI Genetics* 1(3):291–292

"We propose that the German paper and the UK response can provide a model for other countries to follow in formulating their local national recommendations."

"We consider this [support by a formal network of European and national forensic genetics, scientific organizations] to be **sufficient evidence of a scientific consensus** (or general agreement) to support the basic principles concerning the interpretation and formulation of the strength of evidence of DNA [mixture] results."

Interpretation of DNA Mixtures – **European Consensus on Principles**

Morling *et al.* (2007) *FSI Genetics* 1(3):291–292

"We would like to draw the attention to…the need for:

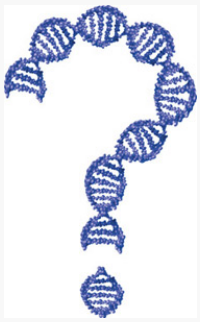
(1) clarification of working practices for the interpretation of DNA profiles based on accreditation according to recognized laboratory standards such as ISO 17025,

(2) education in the interpretation of the weight of the evidence of complicated DNA profiles, and

(3) development of computer based expert systems that can assist in the interpretation of complicated DNA profiles."

Thank you for your attention…

Questions
or Comments?



http://www.cstl.nist.gov/biotech/strbase
john.butler@nist.gov
301-975-4049

Our team publications and presentations are available at:
http://www.cstl.nist.gov/biotech/strbase/NISTpub.htm