Communicating Forensic Findings Workshop: Current Practices and Future Directions

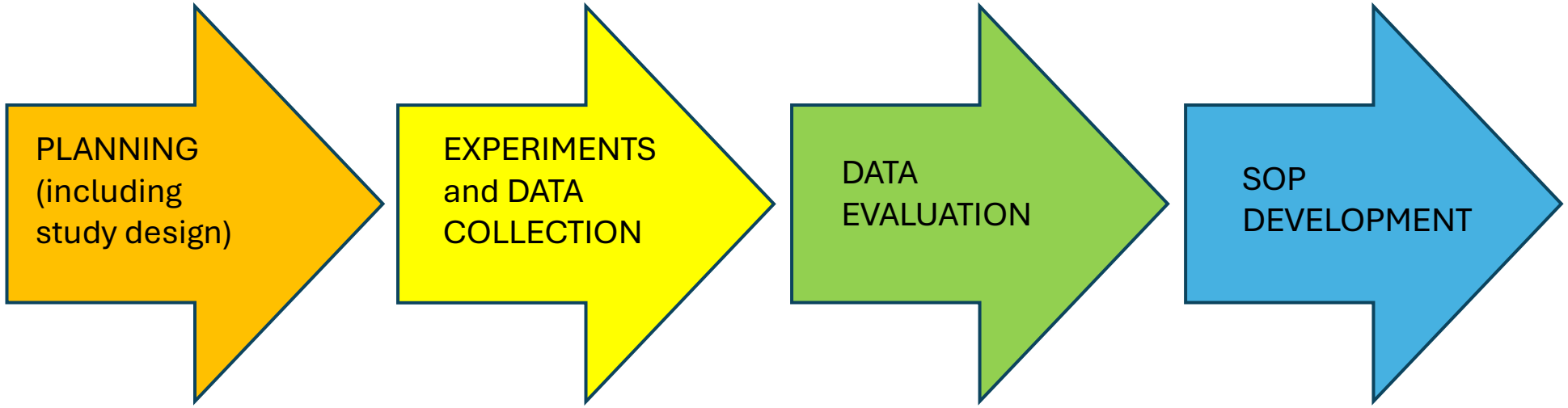
Session 5: Gaps and How to Fill Them

Identifying Gaps & Limitations via SOPs

Kate Philpott

"A primary purpose for validation studies then is to push the system until it fails in order to understand the potential limitations – to define the scope of method (and interpretation) reliability"

(Butler, Validation Webinar (2014), slide 12)



Clearly defined criteria and limitations tightly connected to validation data promote interpretations that are:

- Justifiable
- Complete
- Understandable *(ala Bill T)*

Recommendation 3.8: Forensic science service providers' standard operating procedures should provide criteria for assessing and documenting when a probabilistic genotyping interpretation should be rejected.

HOW TO “PUSH THE SYSTEM UNTIL IT FAILS”

First step: understanding what factors challenge the system

The factors that make DNA mixture interpretation challenging are well known:

- ✓a large and/or unknown **number of contributors**;
- ✓sub-optimal amounts of **template DNA** (i.e. stochastic effects);
- ✓skewed/evenly-distributed **mixture ratios**;
- ✓**allele sharing** between two or more contributors to a mixture (as well as between true contributors and non-contributors); and
- ✓**degradation/inhibition** (including varying degrees of degradation between contributors) of template DNA

“FACTOR SPACE”

Validation study design → limits of analysis

Four-person mixtures	Three-person mixtures	Two-person mixtures	Total target template (pg)
1:1:1:1, 4:4:1:1, 6:3:1:1, 13:3:3:1	1:1:1, 3:3:1, 6:3:1, 8:1:1	N/A	1000
1:1:1:1, 4:4:1:1, 6:3:1:1, 13:3:3:1	1:1:1, 3:3:1, 6:3:1, 8:1:1	1:1, 5:1, 10:1, 19:1	500
1:1:1:1, 4:4:1:1, 6:3:1:1, 13:3:3:1	1:1:1, 3:3:1, 6:3:1, 8:1:1	1:1, 5:1, 10:1, 19:1	250
1:1:1:1, 4:4:1:1, 6:3:1:1, 13:3:3:1	1:1:1, 3:3:1, 6:3:1, 8:1:1	1:1, 5:1, 10:1, 19:1	100

The lowest percentage/ratio for any of these contributors, no matter the number of contributors, is 1:19 (1/20) or 5%. Mixtures where the lowest level contributor comprises less than 5% of the sample are beyond the bounds of validation.

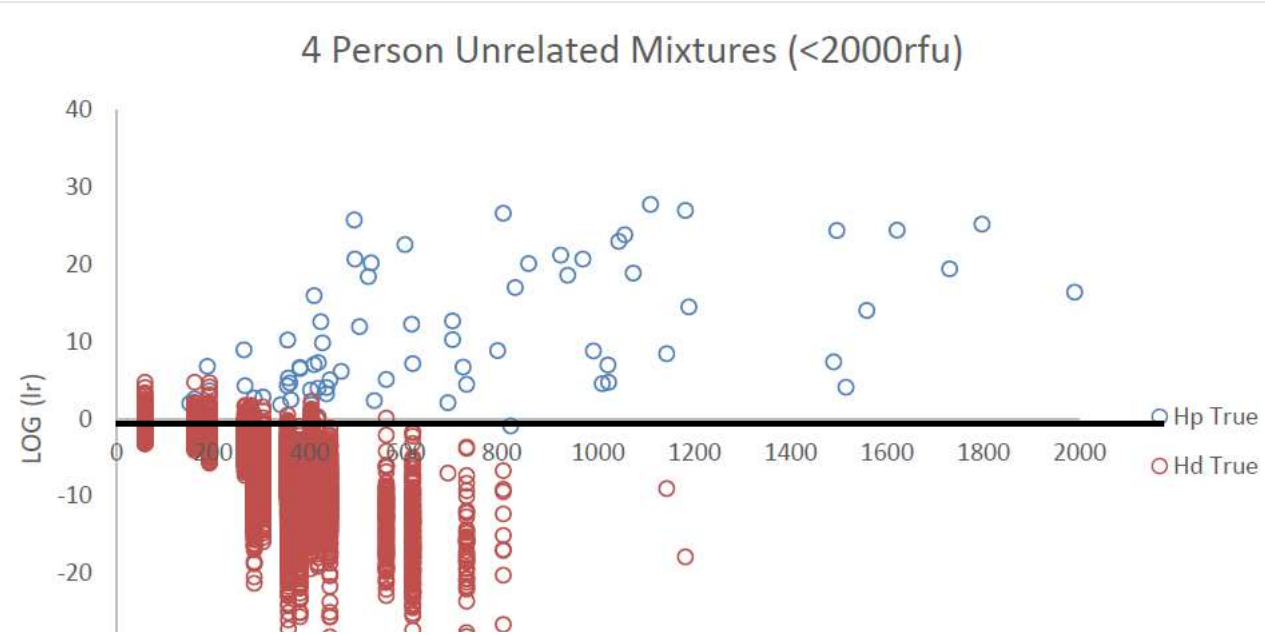
WARNING!

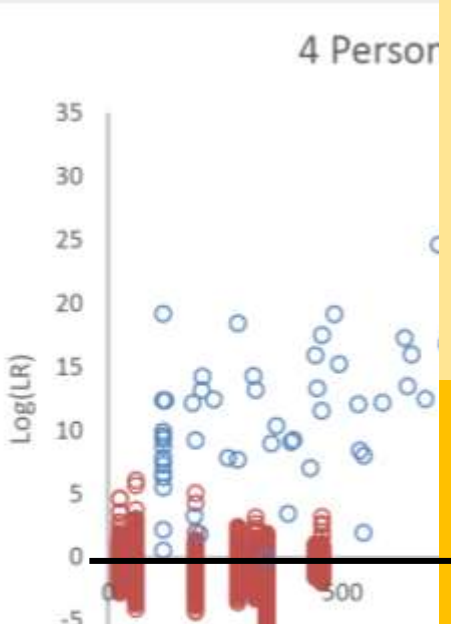
Mixtures with contributors donating as little as 5% and [X] pg of template DNA were tested during internal validation. If PGS analysis of a mixture associates a person of interest with a contributor whose estimated template DNA is lower (in terms of ratio or quantity), the sample is outside the scope of validation and should be deemed uninterpretable.

CAUTION!

Only two mixture samples tested during internal validation involved contributors donating as little as [X] pg of template DNA. If PGS analysis of a mixture associates a person of interest with a contributor donating similar amount of DNA, there are high levels of uncertainty associated with this analysis. Extreme caution should be exercised if interpretation is attempted.

Validation results → limits of reliable





E.g. *False inclusions can occur with low level contributors in DNA mixtures. In validation, false inclusions were observed for mixture components with average peak heights approaching 600 rfu. For mixtures with high levels of allele sharing (e.g. related individuals), false inclusions were observed for higher level components (~1250 rfu). Extreme caution should be exercised in interpretation when similar conditions are or may be present.*

E.g. *During validation, LRs associated with false inclusions were observed to be as high as 1,000,000 in mixtures with high levels of allele sharing and up to 100,000 for other mixture samples. Higher non-contributor LRs may occur in casework. This caveat shall be presented whenever an analyst is providing testimony that culminates in a LR.*

The plots in Figure D1 can help inform the limits of STRmix™, particularly the lower limit of DNA where an H_p true hypothesis results in a *LR* greater than 1 and the limit where false positives may arise (a *LR* greater than 1 where H_d is true).

false inclusions or exclusions were observed for single source samples. Because validation samples are specifically chosen to create mixtures with varying alleles, it is expected for casework samples to show a slightly larger range of false inclusions and exclusions.

Standard Operating Procedures

Interpretability

- If samples under comparison contain a partial profile, for example as a result of allele drop-out, stochastic effects, or an incomplete profile from locus dropout due to inhibition or degradation, or is a complex mixture, the DNA profile may or may not be interpretable and may be considered unsuitable for comparison.

- The following scenarios may be considered to determine if a DNA profile or portion of a DNA profile is unsuitable for comparison. (Note: this does not cover all possible scenarios):
    a. Data of limited or poor quality
    b. Mixture profiles or portions of a mixture profile where the presence of allelic drop-out is reasonable.
    c. Profiles or portions or profiles that exhibit excessive homozygosity
    d. Samples where the number of contributors cannot be determined
    e. Complex mixtures (e.g., >4 contributors, allele sharing between multiple contributors, drop-out…)

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4.2.4 The limitations of the interpretation methods used such as characterizing and defining the maximum number of contributors, and issues associated with low-level data, low-level contributors and potential contamination events.

4.2.5 Criteria for defining what are interpretable data versus data that cannot be interpreted.

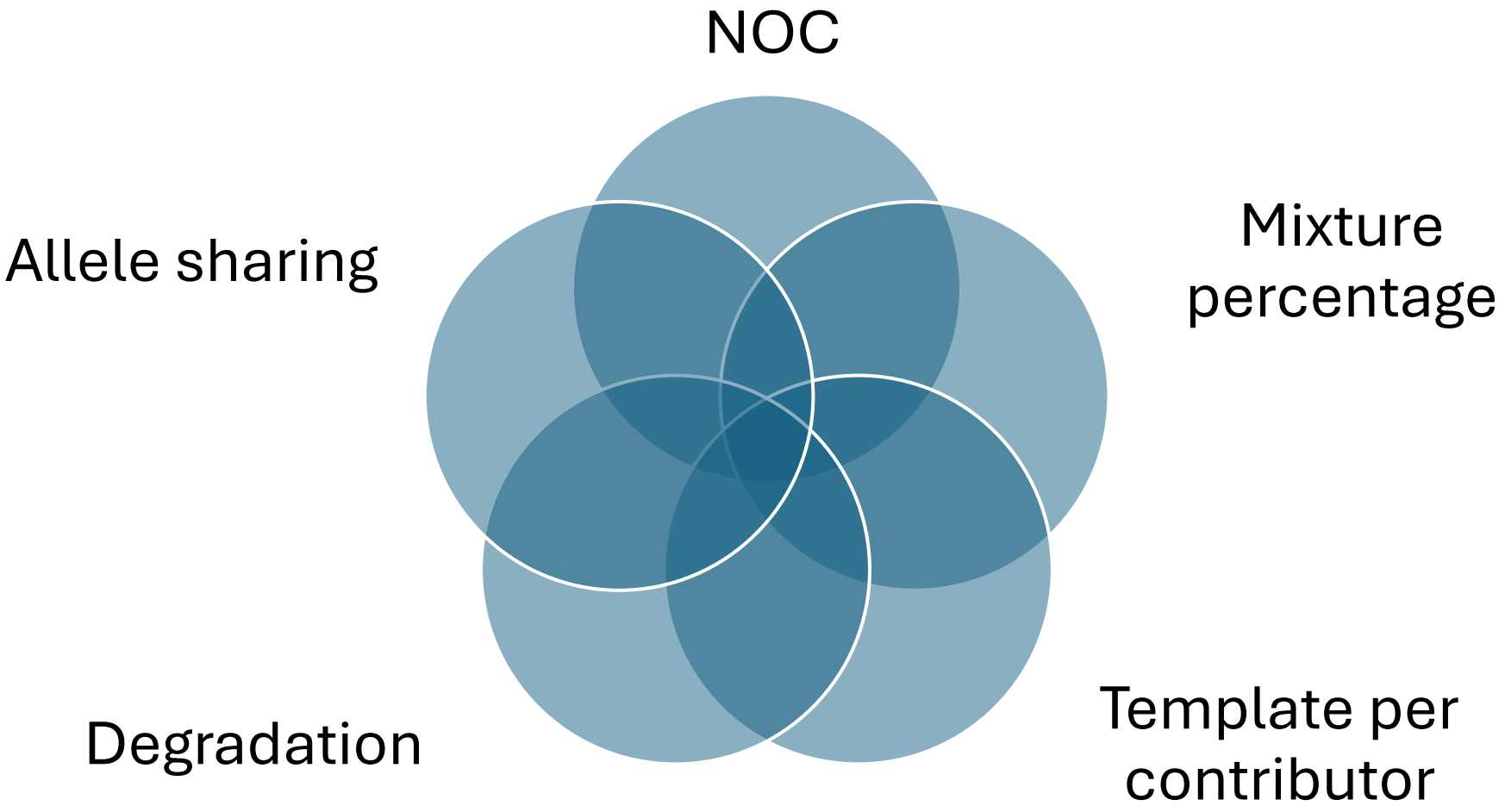
4.2.6 Criteria for defining data that are suitable for comparison versus data that are unsuitable for comparison.

Precedent for including boundaries and limitations in SOPs

2. When determining the number of contributors to a mixture, total allele number in the mixture and more discriminating loci should be considered.
3. The number of contributors to a mixed sample may be inferred based on the locus that exhibits the greatest number of allelic peaks. Counting the total number of alleles detected at the autosomal loci may provide guidance towards defining a minimum or finite number of contributors present in the mixture.
4. When using the total allele count to determine a finite number of contributors, the counting of the autosomal alleles assumes that the mixture profile has no alleles that are below the analytical threshold and therefore undetected. Also, the allele count does not take into consideration possible genotype combinations or modelling of the DNA profile (e.g. stutter or drop-in) with probabilistic genotyping.
5. If there is reason to believe that there may be undetected alleles (e.g., possibility of inhibition or degradation for one or more contributors, the possibility of stochastic effects or drop-out), counting of the autosomal alleles might only be useful in determining the minimum number of contributors.
6. Figure 7 illustrates the number of total alleles observed in two, three, and four person mixtures simulated from known samples typed with PowerPlex Fusion 5C. This data does not include allele counts for the SE33 locus. A mixture with a total of 68 autosomal alleles is more likely to consist of only two contributors than to consist of three or more contributors. A mixture with a total of 87 autosomal alleles is more likely to consist of only three contributors than consist of two or four contributors.

How can we do better?

- SOPs that clearly define areas of out-of-bounds (i.e. clearly describe factor space)
- SOPs that clearly communicate limitations within tested factor space



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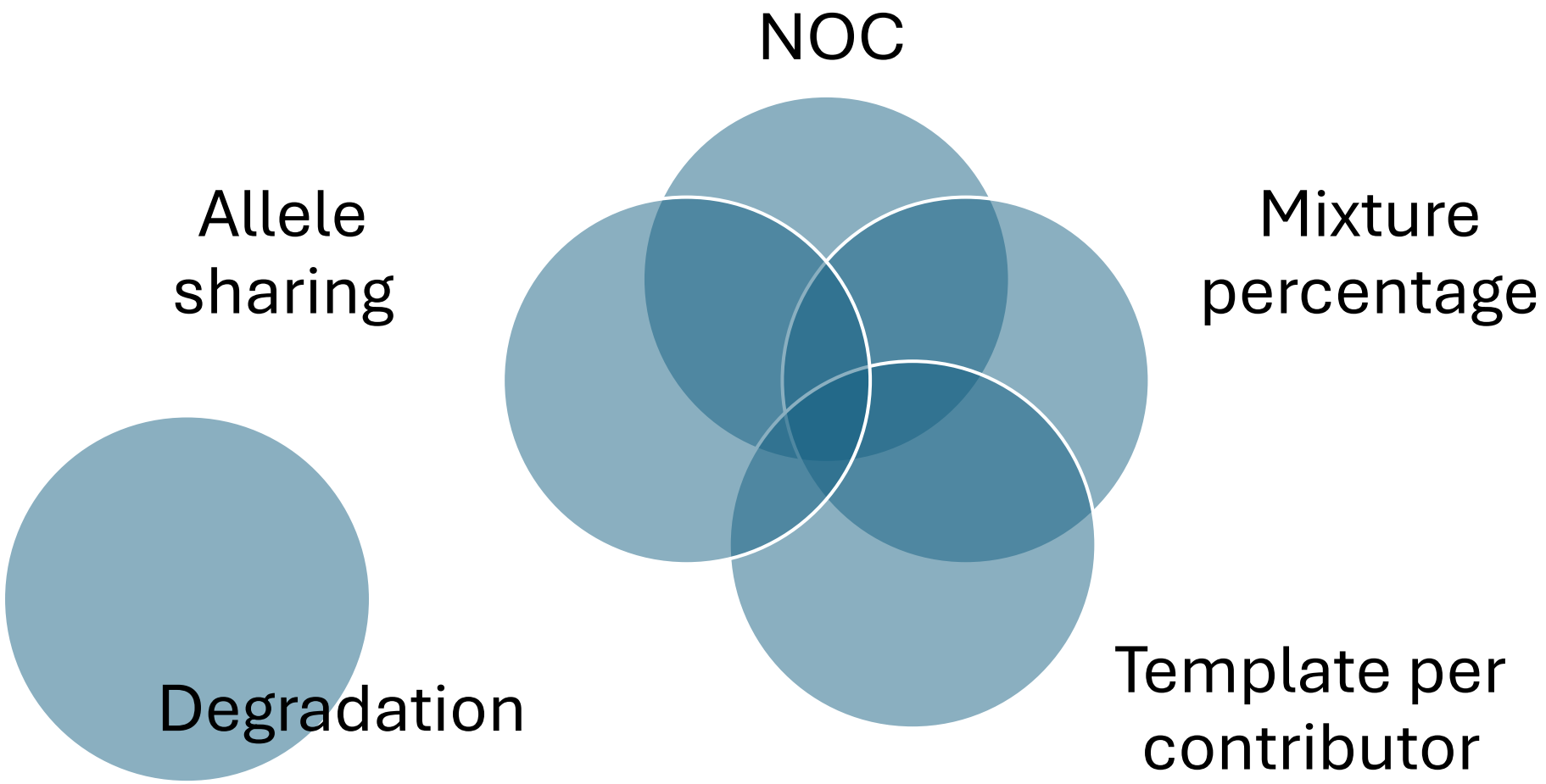

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