

# FORENSIC BIOLOGY PROTOCOLS FOR FORENSIC STR ANALYSIS

SAMPLE COMPARISONS		
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## Sample Comparisons

### Autosomal STR Results

The purpose of these guidelines is to provide a framework for sample comparisons in STR casework. (Refer to the Evidence and Case Management Manual for further details on reporting.) These guidelines are based on validation studies, literature references, some standard rules and experience. However, not every situation can be covered by a pre-set rule or proposed report wording. Equipped with these guidelines, analysts should rely on professional judgment and expertise.

Report templates are available and should be used. These report templates have many pre-written statements which are applicable to most cases and save valuable time by eliminating the need to write the same sentences repeatedly. There are different report templates depending on case type and testing performed (Serology, DNA, suspect, missing persons, etc.); make sure the correct template is used for the type of case analyzed. Pre-written statements cannot cover every possible case scenario and should be modified as necessary for accuracy.

Any documentation developed outside of the LIMS (e.g., statistical calculations) must be scanned to a PDF document and attached to the appropriate electronic case record.

### Statistics

In general:

- A. Instances where an individual's DNA on an item is reasonably expected may not require a statistic when making a positive association. In those instances the positive association may be reported using a qualitative statement. Examples include:
- Victim on intimate samples that originate directly from the individual's body: body cavity swabs, swabbing from any skin surface, or samples from fingernails
  - Elimination/victim profile on their own clothing (single-source or mixtures)
  - Elimination homeowner on any item from their house (single-source or mixtures)
  - Person on any mixture on an item on which that person has already been demonstrated to be present elsewhere on that same item (Male Donor A on a

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mixture from cuffs scrapings of a shirt where Male Donor A was single-source or deconvoluted major from the collar scrapings on the same shirt)

- Person on any mixture from an item where that person has already been demonstrated to be present from a different item at the same location
  - i. Male Donor A in mixture on gear shift when Male Donor A was major or single source on steering wheel
  - ii. Male Donor A and Male Donor B on two different cigarette butts, third and fourth cigarette butts are mixtures of the two Males.
  - iii. Mixtures on sexual assault items/swabs/fractions where Male Donor A was already identified on one of the items/swabs/fractions

- B. Statistical calculations made must be clearly and properly qualified in the test report. Statistical calculations for more than one test can be reported together if the results of those calculations are identical or, where applicable, are above the source attribution threshold.
- C. Statistical information can be reported in the evidence report if appropriate. For example, where a probative sample matches a relevant victim or elimination sample, the statistic is reported in the evidence report.
- D. When using Random Match Probability, report the lowest statistic amongst the ethnic groups.
- E. When using the US Y-STR Database (<http://www.usystrdatabase.org>), report the 95% upper-bound confidence statistic from all ethnic groups.
- F. When using the Forensic Statistical Tool (FST), perform the calculation using the appropriate scenario(s) and report the lowest likelihood ratio amongst the ethnic groups for each scenario.

## Comparison of samples based on Autosomal STR results, Statistical Treatment, and Reporting

- A. State the type of testing that was performed and, when needed, include the minimum number of contributors to the sample.
- B. For each available comparison sample, the following conclusions can be made.

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1. Comparison to a single source profile or to a deconvoluted profile from a mixed sample.
    - a. The comparison sample is a match.
    - b. The comparison sample is not a match.
  2. Comparison to a mixed sample that was not deconvoluted.
    - a. The comparison sample is included as a possible contributor to the mixture.
    - b. No conclusions can be drawn regarding whether the comparison sample could be a possible contributor to the mixture.
    - c. The comparison sample is excluded as a possible contributor to the mixture.
  3. Statistics
    - a. For single source profiles, or profiles deconvoluted from a mixed sample, the Random Match Probability (RMP) will be used. Refer to the “Population Frequencies for STR’s” procedure.
    - b. For mixed samples not deconvoluted in their entirety, a likelihood ratio can be calculated; refer to the “Forensic Statistical Tool (FST)” procedure.
- C. Single source profiles or deconvoluted profiles from mixed samples where a positive association is stated.**
1. **The random match probability (RMP) will be used for statistical analysis of these profiles.** Refer to the “Population Frequencies for STR’s” procedure for details on calculating this value.
  2. **Source Attribution Threshold:**
    - a. If the RMP of an evidentiary profile is at least as rare as the source attribution threshold, 1 in greater than 6.80 trillion for all ethnic groups, then the profile may be attributed to the donor of a comparison sample. This threshold was calculated by applying a 99% confidence interval on the probability of not observing that profile in the world population as estimated by The US Census Bureau World Population Clock as of July 2010.
    - b. If the RMP does not meet the threshold, source attribution may not be used.
- D. Mixed samples that are not deconvoluted in their entirety**
1. **These samples may include the following:**

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- a. The DNA profiles of the individual contributors could not be deconvoluted, but the sample may be used for comparison. For example, a two-person mixture where the peak height ratio of the contributors are approximately 1:1 and the individual contributors could not be determined.
  - b. The DNA profiles of the individual contributors were not deconvoluted, but the sample may be used for comparison. For example, a two-person mixture where the major and minor contributors could be deconvoluted, but was not done so at the time of report writing.
  - c. The DNA profile of the major contributor was determined, and there are sufficient labeled peaks that cannot be attributed to the major contributor that may be used for comparison.
2. **Comparisons to these samples within a case are done as appropriate.** This decision is made on a case by case basis.
  3. **Comparisons are based on previously determined allele calls at conclusive loci.** Loci that are designated as “NEG” for negative or “INC” for inconclusive cannot be used. For LT-DNA samples, conclusive loci must have repeating alleles.
  4. **All results for the same sample are evaluated and may be used for comparison.**
  5. **The source of a comparison sample is included as a possible contributor to the mixture if:**
    - a. For samples amplified with 28 or 31 cycles, all of the alleles seen in the comparison sample are also labeled in the evidence sample.
    - b. If most of the labeled peaks seen in the comparison sample were also seen in the mixture, and the absent (or unlabeled) peak(s) can be explained. Explanations for absent or unlabeled peaks may include any of the following:
      - i. Amount of DNA amplified
      - ii. Artifacts such as stutter
      - iii. Degradation
      - iv. Empirically defined locus characteristics – (In-house validation studies of Identifier® demonstrated that the large and/or less

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- efficient loci are: CSF1PO, D2S1338, D18S51, FGA, TH01, D16S539, and in mixed samples also TPOX.)
- v. Length of the STR repeat
  - vi. Minimum number of contributors to the sample
  - vii. For mixed HT-DNA samples, no more than two alleles can be completely absent or not visible that cannot be explained as above.
  - viii. For mixed LT-DNA samples, no more than two alleles can be unlabeled or absent.
  - ix. For all samples, if less than 10 loci are detected and two alleles are absent, the comparison may be inconclusive depending upon the characteristics of the sample and the loci from which the alleles are absent (refer to section D7).
- c. The likelihood ratio (LR) can be calculated (if appropriate) using the Forensic Statistical tool (FST) if there is a positive association (is included) between the comparison sample(s) and the evidence sample. For further details on performing this calculation, refer to the “Forensic Statistical Tool (FST)” procedures of the manual.
6. **The source of a comparison sample is excluded as a possible contributor to the mixture if:**
- a. One or more alleles seen in the DNA profile of the comparison sample are not seen in the mixture, and the absence cannot be explained. Explanations for absent or unlabeled alleles may include any of the following:
    - i. Amount of DNA amplified
    - ii. Artifacts such as stutter
    - iii. Degradation
    - iv. Empirically defined locus characteristics – (In-house validation studies of Identifier<sup>®</sup> demonstrated that the large and/or less efficient loci are: CSF1PO, D2S1338, D18S51, FGA, TH01, D16S539, and in mixed samples also TPOX.)
    - v. Length of the STR repeat
    - vi. Minimum number of contributors to the sample
  - b. The phrase **is excluded** is used when:
    - i. For HT-DNA samples,
      - 1) If a sample shows no unlabeled peaks, the unexplained absence of one peak may be indicative of an exclusion.

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- 2) If a sample shows an unlabeled peak(s) and/or dropout is suspected, do the following:
    - Evaluate the results at the efficient loci. The absence of even a single peak may be indicative of an exclusion.
    - Evaluate the results at the less efficient or large loci. If the absence of peaks cannot be explained, this may be indicative of an exclusion.
    - Regardless of the locus, for a mixture with only two contributors, if an allele seen in the comparison sample is not present at a locus with four peaks, this could be indicative of an exclusion.
  - ii. For LT-DNA samples,
    - 1) Three or more alleles seen in the DNA profile of the comparison sample are absent at the efficient loci.
    - 2) Many alleles seen in the DNA profile of the comparison sample are absent at any locus.
7. **No conclusions can be drawn regarding whether the source of a comparison sample is included or excluded as a possible contributor to the mixture.**
- a. When making a comparison, take into account the following:
    - i. Amount of DNA amplified
    - ii. Artifacts such as stutter
    - iii. Degradation
    - iv. Empirically defined locus characteristics – (In-house validation studies of Identifiler<sup>®</sup> demonstrated that the large and/or less efficient loci are: CSF1PO, D2S1338, D18S51, FGA, and TH01, D16S539, and in mixed samples TPOX.)
    - v. Length of the STR repeat
    - vi. Minimum number of contributors to the sample
  - b. The phrase **no conclusions can be drawn** is used if the criteria for “included” or “excluded” are not met. The factor(s) supporting this statement must be documented in the case file using the *Not Suitable for Comparison/Inconclusive Form*.

## E. Samples which are not suitable for comparison

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1. **Refer to the Guidelines for interpretation of results in the “STR Results Interpretation” procedure for details on this category of samples.**
2. **Documentation in the case record**

Factor(s) supporting this conclusion must be documented in the case record file using the *Not Suitable for Comparison/Inconclusive Form*. This includes mixtures which can be deconvoluted for the major contributor, but are not suitable for comparison to the minor contributor.

## Y-STR Results

### Comparison of samples based on Y STR results, Statistical Treatment, and Reporting

These guidelines address sample comparisons and reporting specific for Y STR analysis. Refer to the Autosomal STR Comparison section and the Evidence and Case Management Manual for further details on categorizing samples and reporting in general.

- A. **State the type of testing that was performed and, when appropriate, include the minimum number of contributors to the sample.**
- B. **Mixed samples with non-deconvoluted loci**
  1. To the extent possible, mixed samples must be deconvoluted for comparisons within a case, to other cases, or to known samples as needed.
  2. **Comparisons are based on deconvoluted allele calls only.** Loci that cannot be deconvoluted are designated as “INC” for inconclusive and cannot be used for comparison.
- C. **For each Y STR based comparison, the following conclusions can be made.**
  1. **Comparison to a single source profile or to a deconvoluted profile from a mixed sample.**
    - a. The comparison sample could be the source.
    - b. The comparison sample is not the source.
  2. **Statistics**

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The haplotype frequency is determined using the US Y-STR Database website at <http://www.usystrdatabase.org>.

**3. Exclusions**

**The donor of a comparison sample is excluded** if one or more alleles seen in the DNA profile of the comparison sample are not seen in the single-source or deconvoluted profile, and the absence cannot be explained.

**4. No conclusions can be drawn:**

The phrase **no conclusions can be drawn** is used if the criteria for “included” or “excluded” are not met. The factor(s) supporting this statement should be documented in the case file using the *Not Suitable for Comparison/Inconclusive Form*.

**D. Samples not suitable for comparison**

**1. Refer to the “STR Results Interpretation” procedure for details on categorizing samples as not suitable or comparison.**

**2. Documentation in file**

Factor(s) supporting this conclusion must be documented in the case record file using the *Not Suitable for Comparison/Inconclusive Form*. This includes mixtures which can be deconvoluted for the major contributor, but are not suitable for comparison to the minor contributor.