**Department of Forensic Science** 

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# FORENSIC BIOLOGY SECTION PROCEDURES MANUAL OF TRUEALLEEE<sup>®</sup> CASEWORK SYSTEM

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VIRGINIA DEPARTMENT OF **FORENSIC SCIENCE** 

### 1 ANALYZING SAMPLES USING THE TRUEALLELE® CASEWORK SYSTEM

### 1.1 Background

The TrueAllele<sup>®</sup> (TA) Casework system can be used to aid the casework examiner in mixture analysis and likelihood ratio calculations of 2, 3 and 4 person DNA mixture evidence samples. TA Casework is not intended to replace the random match probability, traditional likelihood ratio, or the combined probability of inclusion (CPI) statistics. Rather, it is a probabilistic modeling approach validated for its effectiveness with complex mixtures while preserving profile information which may be ignored with the former statistical approaches.

Routinely, the TA Casework system will be used with 'persons' cases (e.g., homicides, rapes, malicious wounding) in which traditional statistical approaches either cannot be applied, or discard too much information.

### 1.2 Identification and Initiation of a TrueAllele Casework Analysis Request

- 1.2.1 All DNA evidence profiles must first be analyzed using Gene Mapper ID (GMID) or other current CE analysis software. If a major or minor profile can be extrapolated, or the mixture profile is above the stochastic threshold, then these cases will not be forwarded for TA Casework statistical calculations. Exceptions may be made at the discretion of the Biology Program Manager or designee.
- 1.2.2 An e-mail from the originating examiner to the TA examiner will serve as notification of the analysis request. The e-mail will contain the TA case referral form.
- 1.2.3 Prior to submitting a case for TA Casework analysis, the originating examiner will re-evaluate the associated negative controls (reagent blank control for the evidence and negative amplification control) using appropriate data analysis software. All channels for these samples will be scaled to 50 rfu and print-outs of these samples, preferably with the bins turned "on", will be supplied to the TA examiner.
- 1.2.4 For the evidence sample(s) being submitted for TA Casework analysis, the following files will be uploaded to the secure Department server to the TA examiner:
  - 1.2.4.1 The evidence sample(s) .fsa and .pdf files
  - 1.2.4.2 The included reference(s) .fsa file and landscape profile
  - 1.2.4.3 Assumed reference .fsa file (if applicable)
  - 1.2.4.4 The associated allelic ladder(s) .fsa file
  - 1.2.4.5 The .ser project file
  - 1.2.4.6 The positive control(s) .fsa file(s) associated with the evidence amplification(s)
  - 1.2.4.7 .pdf (or other copy) of negative controls and reagent blanks (preferably binned) scaled to 50 rfu (see above)
- 1.2.5 Review the evidence sample electropherogram for details such as the number of possible contributors (using allele counts, peak height ratio information and any assumed knowns) and other pertinent information such as the level of stochastic effects, potential differential degradation, and possible artifacts such as polymer spikes.
- 1.2.6 Update the TrueAllele Notes worksheet and describe actions taken.

### 1.3 Importing the Evidence Profile into the TrueAllele Casework System Using the Analyze Module

Evidence sample electropherogram files (.fsa files), assumed reference (if applicable), a selected positive control

and the ladders are uploaded to the Analysis module following the procedures described in the TrueAllele Technology Analyze Module. Associated reference samples can be uploaded at this time or later.

- 1.3.1 The analysis (parent) folder can be labeled with the case number and examiner's initials (e.g., C13-4321.BCJ). A sub folder must be created within that folder which will contain the .fsa files of the case data. The sub folder can be labeled with the case number and .fsa (eg. C13-4321.fsa).
- 1.3.2 Using one of the TA workstations, open the Analyze module using the VUIer interface.
- 1.3.3 Follow the steps outlined in the TA Casework Analysis manual for creation of the .gel file and \_DD folder.
- 1.3.4 Process the data using Image Call, Marker Call and Allele Call. Use the 'capillary view' and 'display grid' view with accompanying 'control check' to ensure the appropriate capillaries are captured, the fragments of the internal lane standard (ILS) in all of the capillaries are accurately assigned, the marker bands are properly labeled and the allele calls are made.
  - 1.3.4.1 If any edits need to be made to the ILS or other data associated with the electropherogram data, follow the directions in the TrueAllele Technology Analyze Module.
- 1.3.5 Update the TrueAllele Notes worksheet, date, and describe actions taken.
- 1.3.6 Exit the Analysis module.

### 1.4 Uploading the .gel File to the Server Using the Data Module

- 1.4.1 Open the Data module using the VUIer interface.
- 1.4.2 In the Data module, import the .gel file previously created following the TA Casework analysis Data manual directions.

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- 1.4.3 Once the appropriate .gel file is located and the samples have populated the Data module window, ensure all peaks have designated allele calls. If any of the items of evidence contain artifact peaks, such as spikes, pull-up peaks, or dye blobs not edited previously, activate the allele labeling by clicking spikes, pull-up peaks, or dye blobs not edited previously, activate the allele labeling by clicking spikes, will now be grayed out and will not be used during the deconvolution process. Peaks associated with increased stutter or raised baseline should not be turned off. Use the arrow key to upload the .gel file to the server.
- 1.4.4 Exit the Data module.

### 1.5 Creating Requests from Sample Files Uploaded to the Server in the Request Module

- 1.5.1 Open the Request module using the VUIer interface.
- 1.5.2 Under the Database dropdown window, select Find and then Data. Leave the default settings in place. Select the case samples and positive control to create a request. The allelic ladder is not required for the request.
- 1.5.3 If not done previously, verify that each sample (item) is present. If any of the items of evidence contain artifact peaks, such as spikes, pull-up peaks, or dye blobs not edited previously, activate the allele labeling by clicking  $\mathbb{N}$ . Right click on the peak label(s) associated with the artifact and then select "peak off". The peak label(s) will now be grayed out and will not be used during the deconvolution process. Peaks associated with increased stutter or raised baseline should not be turned off.

- 1.5.4 Create a TA item for each lane of data (positive control, evidence or known reference sample). The name may include the item with the FS lab # and the item # (e.g., C13-4321.13handle or C13-4321.35TS for a reference), and will be classified as individual (reference) or DNA template (evidence or QC).
- 1.5.5 Create a request from the TA item. For an evidence item, create the request based on the number of contributors estimated previously. Since the number of contributors may not always be clear, the TA operator has the discretion to enter multiple requests using slightly different numbers of unknown contributors as deemed necessary.
  - 1.5.5.1 Each evidential request, regardless of the parameters selected, will be labeled with a unique (and consecutive) request number. (i.e., r1, r2, r3....)
  - 1.5.5.2 Requests for evidence samples can be named using the FS lab #, TA examiner initials, item #, # unknowns, # cycles, + assumed known (if applicable), differential degradation (if applicable) and request number.

EXAMPLE: C13-4321. BCJ.13handle. 3uk.100k. +35.r5

- 1.5.5.3 The following guidelines will be used for evidence samples when determining the initial number of TA Casework cycles to employ (for burn-in and read-out). Low peak rfus and low mixture weights are considerations for increasing the number of cycles in a request:
  - 1.5.5.3.1 For two unknown person mixtures, duplicate analyses of the mixture at 50K cycles may be the starting point with additional analyses for longer cycles when necessary.
  - 1.5.5.3.2 For three unknown person mixtures, duplicate analyses of the mixture at 100K cycles may be the starting point with additional analyses for longer cycles when
    - necessary. SIC SCIENCE NOTE: If a mixture might be better resolved using a four unknown person mixture deconvolution process, then a minimum of duplicate analyses at 100K cycles would be recommended.
  - 1.5.5.3.3 For the positive control and known reference samples, the standard procedure as described in the TA Casework Request manual of a single analysis of the sample at 500 cycles (reference default setting) will be performed. Analysis of the positive control is a check of the TA casework system; it is not necessary to analyze multiple positive controls.
- 1.5.5.4 Included reference samples associated with the case also need to be analyzed and can be included in the same group of requests or at another time; however, reference samples used as assumed knowns must be analyzed simultaneously with the corresponding evidence sample. Reference sample genotypes may also be uploaded manually to the server at this or a later time.
- 1.5.5.5 The 'differential degradation' setting in TA Casework deconvolution will assess possible degradation of each contributor to a mixture independently as compared to the mixture as a whole. The use of this setting in a Request should be considered with the type of sample (touch or wearer\_DNA), the nature of the sample (exposed to elements, DNA deposition at different times), or the appearance of the electropherogram.
- 1.5.5.6 For intimate items, use the owner reference profile (individual from whom the intimate item was collected) as an assumed known in the creation of the request when the evidence supports that the owner is present in the data.

- 1.5.5.7 When the owner reference profile is available in a non-intimate item of owner evidence, such as the steering wheel of the car owned by the individual or a stain on a victim's outer clothing, the owner reference profile may be used as an assumed known only after it has been demonstrated by the data. Duplicate TA Casework analyses must be performed assuming no known reference to demonstrate statistical support for the owner being a contributor to the non-intimate item of evidence. After that has been confirmed, then additional analyses may be performed using the owner profile as an assumed known.
  - 1.5.5.7.1 The same approach would be used with an elimination sample (boyfriend, husband) in a sexual assault case.
- 1.5.6 Export the request (.req file containing all of the requests shown in the Request window) into the parent folder named for the case. The TA examiner initials and date can be added to the request file name.

EXAMPLE: C13-4321.BCJ.051113

1.5.7 Upload the request files for processing. Use this opportunity to double check the requests prior to finalizing the upload.

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- 1.5.8 Update the TrueAllele Notes worksheet and describe actions taken.
- 1.5.9 Exit the Request module.

### 1.6 Checking on the Progress of a Request Using the Tools Module

- 1.6.1 Checking on the progress of a request is not required for casework analysis, however, it can helpful in providing the examiner with an idea of when the analysis might be completed and how busy the server is with other requests.
- 1.6.2 Open the Tools module using the VUIer interface. CIENCE
- 1.6.3 Under the Tools drop-down menu, select System Processing and then click OK for information about current request processing.
- 1.6.4 All currently processing items ("solving") will be displayed along with the status of the cycle number (e.g., A 100K burn-in and read-out analysis was setup. Under Request Processing it shows the sample analysis cycle number is at 150,000. That means it is in the read-out phase of the analysis and it has 50,000 cycles remaining). Other messages such as "no data" indicate a problem and a new request should be made.
- 1.6.5 If it is necessary to accommodate a rush case, requests which haven't started processing may be 'deactivated' to free processors. Under the Tools drop-down menu, select Interpretation Requests and then select Deactivate and OK from the pop-up window. Select the case and requests to be deactivated and OK. Requests can be 'activated' in the same manner to begin processing.
- 1.6.6 Exit the Tools module.

### **1.7** Assessing the Results Using the Report Module

- 1.7.1 Open the Report module using the VUIer interface.
- 1.7.2 When assessing the match statistics, be certain the co-ancestry coefficient is set to 1% as the default.
- 1.7.3 Following the TA Casework Report manual, populate the Genotype Selector window with the inferred contributors from the analysis in the Evidence window, with the reference profiles pertaining to the case in the References window, and with the appropriate population databases in the Population window.

- 1.7.4 The positive control utilized as a quality control check provides evidence for the accuracy with which TA Casework infers the genotypes of a profile and must be evaluated. Incorporated into either the References or the Evidence window, the inferred genotypes for the positive control can be checked against the expected known profile. This quality control check will be documented in the corresponding location on the TrueAllele Match and Report Data\_Worksheet. Similarly, the inferred genotype of each known sample can be checked against the known profile determined by the originating examiner and can be similarly documented.
- 1.7.5 For each TA Casework evidence sample, the following must be evaluated:
  - 1.7.5.1 Ideally the GR convergence value should be  $\leq$  1.2; however, analyses with values above 1.2 but less than 1.5 may still be acceptable and show genotype concordance with other ideal analyses. Analyses with convergence values above 1.5 should generally not be used, but exceptions may be made by the Biology Program Manager or designee on an individual basis.
  - 1.7.5.2 The Markov chain Monte Carlo (MCMC) analysis and resulting mixture weight histogram must show sufficient sampling (exploration) given the complexity of the mixture and no significant stalling of the read-out cycles. If the GR score is > 1.5 suggesting the chains haven't settled, generally the run won't be used and evaluation of the MCMC analysis is not necessary. Exceptions may be made by the Biology Program Manager or designee.
    - 1.7.5.2.1 Provided the GR score is < 1.5, the mixture weight MCMC chains can be evaluated as follows:

Pass ("acceptable"): Chains demonstrate exploration of mixture weight space and may have some stalling

Fail: Chains appear to be 'stuck' with very little to no exploration

**NOTE:** Data associated with a failed analysis will not be recorded on the worksheets but will be preserved with the electronic data.

- 1.7.5.3 The mixture weights assigned to each contributor should be consistent among acceptable analyses. While larger mixture weights are expected to be closer in value, lower mixture weights (especially below 10%) will have a wider range due to uncertainty expected with a low-level contributor.
- 1.7.6 Once a minimum of two independent acceptable analyses have been produced, genotype concordance should be evaluated as follows:

Good: Inferred genotypes and probabilities are consistent or demonstrate slight differences

Fair: Inferred genotypes or probabilities demonstrate moderate differences

Poor: Inferred genotypes and probabilities differ substantially

- 1.7.6.3 As with mixture weights, genotype concordance between larger contributors may be "good" while concordance among lower level contributors may only be "fair". If any of the contributors' replicate analyses result in "poor" genotype concordance, that analysis should not be used.
  - **NOTE**: Exceptions to the above will be handled on a case-by-case basis. Analyses with an inferred minor contributor with a mixture weight <10% may result in repeated "poor" genotype concordance with good/fair concordance among the remaining contributors. Therefore, the data produced for the more major contributors may be used, but the minor contributor would be inconclusive.

- 1.7.7 Complete the TrueAllele Data Review worksheet with the requested information for all but 'Failed' analyses (i.e., convergence values and mixture weights). Genotype concordance observations will only be addressed among acceptable analyses.
- 1.7.8 Once a minimum of two independent acceptable analyses with concordant genotypes have been produced ("fully concordant"), the match score (log(LR)) values created by comparison to the reference profile should be evaluated.
  - 1.7.8.3 The match scores among reported contributors should be within two (2) ban for independent analyses to be considered fully concordant and reportable. It is not necessary for conditions (contributor number, use of differential degradation, etc.) to be identical among analyses to be fully concordant.
    - **NOTE**: In cases with more than two fully concordant analyses, it is possible that the match scores span more than 2 ban. As long as the reported match score is within two ban of at least one additional analysis, it will be reported.
    - **NOTE:** There may be samples where the genotype concordance is good or fair and major match scores are within 2 ban, but the match scores for a minor contributor are not within the 2 ban range. This is not unexpected with a minor contribution at or below 10%. Reporting of the most minor contributor match scores in situations like this will be handled on a case-by-case basis.
  - 1.7.8.4 If two independent analyses which were deemed acceptable by evaluation of the MCMC chains, the convergence values, mixture weights and genotype concordance produced match score values that were not within 2 ban among the reported contributors, then additional analyses should be performed to produce more concordant results to meet the reporting requirements.
    - 1.7.8.4.1 A maximum of six (6) analyses for a non-complex mixture and eight (8) analyses for a complex mixture will routinely be allowed. Mixtures requiring more analyses will be reviewed by the Biology Program Manager or designee.
      - NOTE: Analyses used to establish assumed known contributor(s) will not count toward the maximum number of analyses listed above.
    - 1.7.8.4.2 After thoroughly exhausting appropriate analysis conditions, if at least two independent acceptable analyses cannot be obtained, the results will be reported as inconclusive based on inability to achieve reproducible results.
  - 1.7.8.5 Begin completing the TrueAllele Match and Report Data worksheet.
- 1.7.9 Once two or more fully concordant analyses have been identified, the following steps must be taken:
  - 1.7.9.1 Complete the TrueAllele Match and Report Data worksheet. Within an analysis (e.g., r1), there may be multiple positive match scores (cross-matches) among the different mixture genotypes (contributor 1, contributor 2, and contributor 3) when compared to a reference sample. Multiple negative match scores and/or a combination of positive and negative match scores may also be encountered. For each analysis, the match statistic associated with the closet fit to the inferred genotypes will be identified. Across those scores, the race-specific match scores associated with the most conservative match score across those analyses will be reported using 2 significant figures
    - NOTE: In order to fully explore a mixture, it may be necessary to vary the number of contributors which can affect the reported match scores. Exceptions to the determination of which analysis is reported can be made by the Biology Program Manager or designee.

Evidence	Contributor	C13-ABC.BCJ.35TS
C13-4321.BCJ.1.3uk.100k.r1	1	10.7917
C13-4321.BCJ.1.3uk.100k.r1	2	12.6945**
C13-4321.BCJ.1.3uk.100k.r1	3	6.1549
C13-4321.BCJ.1.3uk.100k.r3	1	14.2631**
C13-4321.BCJ.1.3uk.100k.r3	2	7.7829
C13-4321.BCJ.1.3uk.100k.r3	3	9.9684

\*\* In the above example, r1 contributor 2 and r3 contributor 1 have the most discriminating scores. Among the two independent analyses, the race-specific match scores associated with r1 contributor 2 would be reported.

- NOTE: Match scores associated with analyses to support the use of an assumed known (nonintimate items) will only be reported as the basis for the assumed known, if applicable. These match scores will not be considered in determination of which scores to report for the person of interest when an assumed known is used.
- 1.7.9.1.1 If the reported match scores among reproducible and fully concordant analysis are greater than +3, supporting an inclusion, the analyses will be independently confirmed by a human review of the data. This conclusion (i.e., non-elimination) will be reported (if not previously reported by the referring examiner) in addition to the results of the statistical analysis.
- 1.7.9.1.2 If the reported match scores among reproducible and fully concordant analyses fall between -2 and +2, the analysis will be independently confirmed by a human review of the data. The statistics will be considered inconclusive and no conclusion will be reported.
- 1.7.9.1.3 If the reported match scores among reproducible and fully concordant analyses are less than -3, indicating more support for a match to a random individual, the analysis will be independently confirmed by a human review of the data. This conclusion (i.e., elimination) will be reported.
- 1.7.9.1.4 Match scores falling between -3 and -2 or +2 and +3 will be handled on a case by case basis in consultation with the Program Manager or designee.
- 1.7.9.1.5 If the race specific match scores of the reported contributor among reproducible and fully concordant analyses have both positive and negative values, the analysis will be independently confirmed by a human review of the data. The statistics will be considered inconclusive and no conclusion will be reported.
- 1.7.9.2 For all but inconclusive or non-reproducible conclusions, document the complete name of the reported match statistic (including contributor number) by cutting-and-pasting or copying from TA's Match table into the worksheet. Cut-and-paste or copy the locus-specific match scores for all 3 populations from TA's Locus Match table into the table on the worksheet. During the human review of the data, document any locus-specific comments (e.g., reason for significant negative match scores). Marginal negative match scores are understood to be a result of the probability assigned to the person of interest's allele pair being approximately equal to or less than the probability of the same allele pair found in the population and therefore does not need to be documented.

1.7.9.3 Save the TA Match table (with **ALL** analyses except positive control(s)) as an .xls file and name accordingly:

EXAMPLE: C12-4321.13handle vs 35TS all matches

- 1.7.9.3.1 Format the .xls table for ease of review (analyses in chronological order, etc.). Cut and paste the data from this table into the TrueAllele Match Table – All Matches worksheet.
- 1.7.9.4 Save the TA Match table which contains only the fully concordant analyses as an .xls file and name accordingly:

EXAMPLE: C12-4321.13handle vs 35TS final

Cut and paste the data from this table into the TrueAllele Final Matches worksheet. It is not necessary to save any negative cross-matches in this worksheet.

- 1.7.9.5 Only one match table is necessary if the information on the 'final matches' and 'all matches' is identical.
- 1.7.10 Export all of the evidence analyses for the case, the references associated with the case, the positive control and the population databases to the parent case folder as a report file for storage and peer review.



### 2 RETENTION OF ELECTRONIC DATA

The following electronic data will be retained on a case-specific CD which will be labeled with the case number, the examiner's initials, date of completion, and the peer-reviewer's initials:

- .fsa files supplied by the examiner (evidence, references, positive controls, ladders)
- \_DD folder
- .req file(s)
- report file of all TA analyses
- .xls file of Match locus detail table
- .xls file of TA Match table all matches
- .xls file of TA Match table final (fully concordant matches)
- Note: For cases in which analyses were done to establish the use of an assumed known, the 'all matches' table can be saved as two different tables: one with analyses establishing the assumed known, and the second with analyses including the assumed known.

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### **3 REPORTING RESULTS**

### 3.1 Categories of Results

The results will fall within one of the following categories:

- 3.1.1 The match score favors the person of interest
- 3.1.2 The match score favors a coincidental match
- 3.1.3 The match score is inconclusive

### **3.2 General Reporting Format**

The Certificate of Analysis will contain a METHODS section and RESULTS section.

- 3.2.1 In accordance with the Department's Quality Manual:
  - 3.2.1.1 The TrueAllele examiner will ensure identical description of the items of evidence as in previously released Certificates of Analysis.
  - 3.2.1.2 The TrueAllele Certificates of Analysis are considered "Supplemental" reports.
- 3.2.2 The METHODS section (as outlined below) will be located prior to the RESULTS section in the Certificate of Analysis and will include the description of methodology:

### METHODS

- The DNA PowerPlex<sup>®</sup> 16 profiles referenced in this report were previously developed and addressed in a Certificate of Analysis dated \_\_\_\_\_.
- The TrueAllele<sup>®</sup> Casework system processed each evidence item in independent replicate computer analyses in which possible DNA contributor genotypes were inferred from the evidence profiles.
  - The term "genotypes" used in this context refers to a probability distribution over allele pairs.
- The likelihood ratio statistical method addressed below has been applied in accordance with the Scientific Working Group on DNA Analysis Methods (SWGDAM) 2010 Interpretation Guidelines and Departmental procedures.
- The DNA match statistics calculated herein used the population allele frequencies generated by the Virginia Department of Forensic Science, and a theta co-ancestry coefficient of 1%.
- 3.2.3 The RESULTS section will contain the results of the TrueAllele analysis and will follow the METHODS section in the Certificate of Analysis. It is composed of a narrative statement and a statistical statement.
  - 3.2.3.1 Each evidence item analyzed will have a narrative and statistical statement.
    - 3.2.3.1.1 If not addressed in a previous report, a conclusion statement regarding nonelimination (inclusion) will be included. However, if a previous report issued a conclusion between the person of interest and the evidence, a conclusion will not be re-stated.
  - 3.2.3.2 If multiple known references are compared to a single evidence item, there will be one narrative statement and multiple statistical statements.
  - 3.2.3.3 The narrative statement will address the following:
    - 3.2.3.3.1 Evidence sample being analyzed

- 3.2.3.3.2 The number of unknown contributors tested
- 3.2.3.3.3 Degradation option (if applicable)
- 3.2.3.3.4 Assumed known(s) (if applicable)
- 3.2.3.3.5 The reference sample to which comparisons were made
- 3.2.3.3.6 Locus-specific comments (if necessary)

EXAMPLE (multiple contributor assumptions):

Assuming the DNA profile data previously developed from the evidence is a mixture of three or four unknown contributors, the TrueAllele<sup>®</sup> Casework system objectively inferred genotypes solely from these data.

Following duplicate/reproducible analyses, the computer system then compared each inferred evidence contributor genotype to the provided reference genotype, relative to reference populations, to compute likelihood ratio (LR) DNA match statistics.

EXAMPLE (assumed known on intimate evidence, degradation option used):

Assuming the DNA profile data previously developed from the evidence is a mixture of two unknown contributors and [NAME], the TrueAllele<sup>®</sup> Casework system objectively inferred genotypes solely from these data. DNA degradation was considered.

Following duplicate/reproducible analyses, the computer system then compared each inferred evidence contributor genotype to the provided reference genotype, relative to reference populations, to compute likelihood ratio (LR) DNA match statistics.

NOTE: Only the conditions accompanying the assumed known analysis will be reported for the person of interest. Conditions accompanying the analysis to justify an assumed known are only reported with those results, if applicable.

EXAMPLE (analyses with and without assumed known):

Assuming the DNA profile data previously developed from the evidence is a mixture of three unknown contributors, the TrueAllele<sup>®</sup> Casework system objectively inferred genotypes solely from these data. Use of [NAME] as an assumed known was considered.

Following duplicate/reproducible analyses, the computer system then compared each inferred evidence contributor genotype to the provided reference genotype, relative to reference populations, to compute likelihood ratio (LR) DNA match statistics.

EXAMPLE (locus-specific comments - Reagent Blank RB contamination):

While the locus Penta E demonstrated allele peaks, this locus was not used in the statistical analysis as a result of not meeting the quality control criteria during the original analysis.

EXAMPLE (new reference sample, previous TrueAllele analysis):

As previously reported in the Certificate of Analysis dated [DATE], assuming the DNA profile data developed from the evidence is a mixture of three unknown

contributors, the TrueAllele Casework system objectively inferred genotypes solely from these data.

Following duplicate/reproducible analyses, the computer then compared each inferred evidence contributor genotype to the newly provided reference genotype (NAME), relative to reference populations, to compute likelihood ratio (LR) DNA match statistics.

- 3.2.3.4 The statistical statement(s) will be reported as follows:
  - NOTE: The examples below can be edited based on whether or not a conclusion was previously reported.
  - 3.2.3.4.1 Match score favors the person of interest

### EXAMPLE:

Based on these results, [Name] cannot be eliminated as a contributor to this DNA mixture profile. A match between the firearm and [Name] is:

10 billion times more probable than a coincidental match to an unrelated Black person,

780 billion times more probable than a coincidental match to an unrelated Caucasian person, and

- 1 trillion times more probable than a coincidental match to an unrelated Hispanic person.
- 3.2.3.4.2 Match score favors a coincidental match

### FOR EXAMPLE: C SCIENCE

Based on these results, [Name] is eliminated as a contributor to this DNA mixture profile.

3.2.3.4.3 Match scores are not reproducible between analyses or within the races of the reported analysis.

### EXAMPLE:

Based on these results and the inability to achieve reproducible match scores, there is no statistical support for a match between the "evidence" and [NAME]. Therefore, no conclusions will be offered regarding [NAME] as a possible contributor to this mixture.

#### Or

The significance of this DNA mixture profile cannot be determined using advanced statistical methods. Accordingly, no conclusions are being offered regarding [NAME] as a possible contributor to this mixture.

3.2.3.4.4 Reproducible match scores falling in the inconclusive range.

### EXAMPLE:

Based on these results and the inability to achieve meaningful match scores, there is no statistical support for a match between the

"evidence" and [NAME]. Therefore, no conclusions will be offered regarding [NAME] as a possible contributor to this mixture.

Or

The significance of this DNA mixture profile cannot be determined using advanced statistical methods. Accordingly, no conclusions are being offered regarding [NAME] as a possible contributor to this mixture.

3.2.3.4.5 Match scores are the basis for using an assumed known on a non-intimate item or an elimination known on an intimate item.

### EXAMPLE:

Based on these results, [Name] cannot be eliminated as a contributor to this DNA mixture profile. A match between the steering wheel and [Name] is:

10 billion times more probable than a coincidental match to an unrelated Black person,

780 billion times more probable than a coincidental match to an unrelated Caucasian person, and

1 trillion times more probable than a coincidental match to an unrelated Hispanic person.

This match strength implies that [Name] contributed DNA to his own steering wheel.

- **FORE** Based on that conclusion, assuming the DNA profile data previously developed from the evidence is a mixture of two unknown contributors and [Name], the TrueAllele<sup>®</sup> Casework system objectively inferred genotypes solely from these data. Following duplicate/reproducible analyses, the computer system then compared each inferred evidence contributor genotype to the provided reference genotype, relative to reference populations, to compute likelihood ratio (LR) DNA match statistics.
- 3.2.3.4.6 Match scores are the basis for NOT using an assumed known.

### EXAMPLE:

The reference genotype from [NAME] was not used in the analysis reported above.