

Department of Forensic Science

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**FORENSIC BIOLOGY
PROCEDURES MANUAL
OF
TRUEALLELE® CASEWORK SYSTEM**

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1 ANALYZING SAMPLES USING THE TRUEALLELE® CASEWORK SYSTEM

1.1 Background

The TrueAllele® (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 for ‘persons’ cases (e.g., homicides, rapes, malicious woundings) on evidential profiles generated with PowerPlex® 16 (PP16) or PowerPlex® Fusion (PPFusion) on 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 current capillary electrophoresis (CE) analysis software. Mixture profiles with all alleles above the stochastic threshold or mixture profiles from which a major or minor profile can be extrapolated 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. TA examiners will complete TA analysis on their own cases as necessary.
- 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, with the exception of the internal lane standard (ILS) channel, 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 provided to the TA examiner either through upload to the secure Department server or other secure manner:
 - The .fsa or .hid file(s) of the evidence sample(s) and a .pdf or paper copy of each
 - The .fsa or .hid file(s) of the included reference sample(s) and the profile (s) in landscape form
 - The .fsa or .hid file(s) of the assumed reference(s) (if applicable)
 - The .fsa or .hid file(s) of the associated allelic ladder(s)
 - The project file
 - The .fsa or .hid file(s) of the positive control(s) associated with the evidence amplification(s)
 - A .pdf or paper copy of reagent blank(s) and negative control(s) associated with the evidence extraction(s) and amplification(s).
 - These are to be scaled to 50 rfu for each channel not containing the ILS and preferably show the bins.
- 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 or .hid files), assumed reference(s) (if applicable), a selected positive control and the ladders are uploaded to the Analyze Module following the procedures described in the TrueAllele® Analyze Module manual. Associated reference samples can be uploaded at this time or later in either .fsa, .hid, or text format.

- 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 or .hid files of the case data. The sub folder can be labeled with the case number and .fsa or .hid (eg., C13-4321.fsa or C13-4321.hid).
- 1.3.2 Using one of the TA workstations, open the Analyze Module using the VUIer interface.
 - 1.3.2.1 Ensure the "Init preferences" are set to PP16 or PPFusion Data Disk template, as applicable, with the appropriate Panel, Format and Size Standard selected.
- 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' views with accompanying 'control check' to ensure the appropriate capillaries are captured, the fragments of the 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® 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 Log into the appropriate server world and ensure the 'locus_order.txt' file is set to the appropriate amplification kit (PP16 or PPFusion).
- 1.4.3 In the Data Module, import the .gel file previously created following the directions in the TA Casework Analysis Data Module manual.
- 1.4.4 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 or dye blobs, activate the allele labeling by clicking . Right click on each peak label associated with an artifact and 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, pull-up or raised baseline should not be turned off. Use the arrow key  to upload the .gel file to the server.
- 1.4.5 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 Log into the appropriate server world and ensure the 'locus_order.txt' file is set to the appropriate amplification kit (PP16 or PPFusion). Under the Database drop-down window, select Find and then Data. Leave the default settings in place. Select the case samples and positive control to create a request.

NOTE: 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 or dye blobs not edited previously, edit these out as described in 1.4.4 above.

- 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.

NOTE: 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. +35JS.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, low mixture weights, and server availability 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 25-50K 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 50-100K cycles may be the starting point with additional analyses for longer cycles when necessary.

NOTE: If a mixture might be better resolved using a four unknown person mixture deconvolution process, then a minimum of duplicate analyses at 150K cycles or greater is 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 Assumed reference sample(s) (if applicable) must be analyzed simultaneously with the corresponding evidence sample(s) and may be uploaded in .fsa, .hid, or text format.

- 1.5.5.5 Included reference sample(s) may be included in the same group of requests as the evidence sample(s) or at another time and may also be uploaded in .fsa, .hid, or text format.
- 1.5.5.6 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 type of sample (e.g., trace, wearer), the nature of the sample (e.g., exposure to elements, DNA deposition at different times), or the appearance of the electropherogram should be considered when determining whether to use this setting in a request or not.
- 1.5.5.7 For intimate items, the owner reference profile (individual from whom the intimate item was collected) will be used as an assumed reference in the creation of the request when the evidence supports that the owner is present in the data.
- 1.5.5.8 For non-intimate ownership type items (e.g., steering wheel of a car, victim’s clothing recovered at a crime scene), the owner’s reference profile may be used as an assumed reference only after it has been demonstrated by the data and supported by investigative information that the owner’s DNA is present. Duplicate TA Casework analyses shall be performed assuming no known reference to demonstrate statistical support of the owner being a contributor to the non-intimate ownership type item of evidence resulting in a conclusion of ‘not elimination.’ Once this has been demonstrated, additional analyses may be performed using the owner’s profile as an assumed reference profile.
 - 1.5.5.8.1 The same approach may be used with an elimination sample (boyfriend, husband) in a sexual assault case or with a victim sample compared to a bloodstain mixture developed from crime scene evidence, if applicable.


EXAMPLE: A homicide victim is found at a scene. A bloodstain from near the body yields a DNA mixture profile from which the victim is not eliminated. The victim’s reference profile may be used as an assumed reference after it has been demonstrated by the data that the victim’s DNA is present.

- 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 Verify the requests and upload the request file(s) for processing.
- 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 or Prioritizing a Rush Request Using the Tools Module

- 1.6.1 Checking on the progress of a request is not required for casework analysis; however, doing so can provide the examiner with information regarding a failed request due to a power outage, an estimation as to when a request will be completed and how busy the server is with other requests.
- 1.6.2 Open the Tools Module using the VUIer interface.
- 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.

EXAMPLE: A 100K burn-in and read-out analysis was setup. Under Request Processing, it is shown that the sample analysis cycle number is at 150,000. This means the analysis is in the read-out phase and there are 50,000 cycles remaining.

1.6.4.1 Other messages such as “no data” indicate a problem and require that a new request be made.

1.6.4.2 The Review Module can also be used to show requests completed requests, requests still in process, and requests waiting to be processed.

1.6.5 To prioritize and accommodate a rush case request, other requests which have not yet started processing may be deactivated to free processors. Requests in process cannot be deactivated.

NOTE: Within the same TrueAllele® world, requests marked with the ‘high priority’ flag will be solved on the next open processor before other non-priority requests, but not necessarily before other non-priority requests in other worlds.

1.6.5.1 Under the Tools drop-down menu, select Interpretation Requests; then, select Deactivate and click OK in the pop-up window.

1.6.5.2 Select the case and request(s) to be deactivated and click OK.

1.6.5.3 Requests can be re-activated in a similar 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 Log into the appropriate server world and ensure the ‘locus_order.txt’ file is set to the appropriate amplification kit (PP16 or PPFusion).

1.7.3 When assessing the match statistics, ensure the co-ancestry coefficient is set to 1% as the default.

1.7.3.1 If the data analyzed is PPFusion data, ensure that the D12S391 locus is turned off or that the database used does not include D12S391 frequency data.

NOTE: TrueAllele® does not use Amelogenin or DYS391; therefore, those loci do not have to be turned off.

1.7.4 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.

1.7.5 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 the Evidence window, the inferred genotypes for the positive control will 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 will be checked against the known profile determined by the originating examiner and can be similarly documented.

1.7.6 For each TA Casework evidence sample, the following must be evaluated:

1.7.6.1 Ideally, the GR convergence value should be ≤ 1.2 ; however, analyses with values above 1.2 but less than 1.5 may still be acceptable if they show genotype concordance with other

analyses. Analyses with convergence values ≥ 1.5 should generally not be used, but exceptions may be made by the Biology Program Manager or designee on an individual basis.

1.7.6.2 Provided the GR convergence value is < 1.5 , the Markov chain Monte Carlo (MCMC) analysis and resulting mixture weight histogram will be evaluated to ensure sufficient sampling (exploration) has occurred given the complexity of the mixture and that no significant stalling of the read-out cycles has occurred 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.

1.7.6.3 If the GR convergence value is ≥ 1.5 , suggesting the chains have not settled, the run will not be used without an exception granted by the Biology Program Manager or designee. Unless the run is used, the evaluation of the MCMC analysis and resulting mixture weight histogram is not necessary.

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%) may 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.

1.7.6.1 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, while the minor contributor may be inconclusive unless the match scores fall within two ban.

1.7.7 Complete the TrueAllele® Data Review worksheet with the requested information for all but ‘Failed’ analyses (i.e., unacceptable convergence values and mixture weight histograms). 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 will be evaluated.

- 1.7.8.1 The revised NIST 2017 population frequency database will be used with PPFusion evidential profiles. The Virginia population frequency database will be used with PP16 evidential profiles.
- 1.7.8.2 In order to keep loci in PPFusion order when creating projects with both PPFusion and PP16 data, the 'VADFS\$placeholder.txt' file should be uploaded to either the evidence or reference tab which contains the PP16 data.
- 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 for which 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 either the major or minor contributor(s)' match scores in these situations 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 produce match score values that are not within 2 ban among the reported contributors, then additional analyses will be performed to attempt to attain more concordant results that 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 this maximum number of analyses.

1.7.8.4.2 After thoroughly exhausting appropriate analysis conditions, if at least two (2) independent acceptable analyses cannot be obtained, the results will be reported as inconclusive based on the 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 closest 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 (non-intimate items) will be reported as the basis for the assumed known, if applicable. These match scores will not routinely 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 analyses are greater than +3 ban, 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 Based on validation data, if the reported match scores among reproducible and fully concordant analyses fall between -2 and +2 ban for PP16 analyses and between -3 and +3 for PPFusion analyses, the analysis will be independently confirmed by a human review of the data. The statistical support will be considered inconclusive and will be reported accordingly.
- 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 without statistics.
- 1.7.9.1.4 PP16 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 statistical support will be considered inconclusive and will be reported accordingly.
- 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 do not need to be documented.

1 Analyzing Samples Using the TrueAllele® Casework System

- 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

- 1.7.9.4.1 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.

NOTE: 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 populations utilized (e.g., NIST 2017, all three major population groups) to the parent case folder as a report file for storage and peer review.

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2 RETENTION OF ELECTRONIC DATA

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

- .fsa or .hid files supplied by the examiner (evidence, references, positive controls, ladders)
- _DD folder
- .req file(s)
- report file of all TA analyses
- .xls or .xlsx file of Match locus detail table
- .xls or .xlsx file of TA Match table – all matches
- .xls or .xlsx 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 may 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:

- The match score favors the person of interest.
- The match score favors a coincidental match.
- The meaning of 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 will be located prior to the RESULTS section in the Certificate of Analysis and will include a description of the methodology used.

3.2.2.1 Methods to be used for PPFusion cases:

METHODS

- The DNA PowerPlex® Fusion profiles referenced in this report were previously developed and addressed in a Certificate of Analysis dated _____.
- The TrueAllele® 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 calculation addressed below has been applied in accordance with the Scientific Working Group on DNA Analysis Methods (SWGDM) 2017 Interpretation Guidelines and Departmental Procedures.
- The DNA match statistics calculated herein used the 2017 revised population allele frequencies provided by the National Institute of Standards and Technology (NIST) with a theta co-ancestry coefficient of 1%.
- The D12S391, DYS391 and Amelogenin loci are not used for statistical purposes.

3.2.2.2 Methods to be used for PP16 cases:

METHODS

- The DNA PowerPlex® 16 profiles referenced in this report were previously developed and addressed in a Certificate of Analysis dated _____.
- The TrueAllele® 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 calculation addressed below has been applied in accordance with the Scientific Working Group on DNA Analysis Methods (SWGDM) 2017 Interpretation Guidelines and Departmental Procedures.
- The DNA match statistics calculated herein used the population allele frequencies generated by the Virginia Department of Forensic Science with 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 non-elimination (inclusion) will be included, if applicable. If a previous report issued a conclusion regarding the person of interest as a contributor to the evidence profile, the 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:

- Evidence sample being analyzed
- The number of unknown contributors tested
- Degradation option (if applicable)
- Assumed reference(s) (if applicable)
- The reference sample(s) to which comparisons were made
- 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® 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 reference 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® 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® Casework System objectively inferred genotypes solely from these data. Use of [NAME] as an assumed contributor 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):

The locus Penta E did not meet the quality control standard during the original analysis; therefore, this locus was not used in the statistical calculation.

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 [EVIDENCE] and [NAME] is:

10 billion times more probable than a coincidental match to an unrelated African American 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

EXAMPLE:

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 populations 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 fall into 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 [EVIDENCE] and [NAME] is:

10 billion times more probable than a coincidental match to an unrelated African American 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.

Based on these results, assuming the DNA profile data previously developed from the evidence is a mixture of two unknown contributors and [NAME], the TrueAllele® 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 use of [NAME] as an assumed contributor was also considered.