Department of Forensic Science

FORENSIC BIOLOGY PROCEDURES MANUAL

TRUEALLELE® CASEWORK SYSTEM

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1   ANALYZING SAMPLES USING THE TRUEALLELE® CASEWORK SYSTEM

1.1   Background

The TrueAllele® (TA) Casework System is a probabilistic genotyping approach validated for its effectiveness with complex mixtures while preserving profile information which may be ignored or discarded with other traditional statistical approaches and 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. .

Routinely, the TA Casework system will be used for non-kinship statistical analyses involving relatives and 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 on which a major or minor random match statistic can be applied to all probative portions will not be forwarded for TA Casework statistical calculations unless the traditional calculation discards too much information (e.g., minor calculation lacks many loci). 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 designee(s) will serve as notification of the analysis request. The e-mail will contain the probabilistic genotyping case referral form. TA examiners will complete TA analysis on their own cases as necessary.

1.2.3   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:

- A .pdf or paper copy of both the applicable RFLE(s) and the applicable technically reviewed/released Certificate(s) of Analysis and Appendices
- The .fsa or .hid file(s) of the evidence sample(s) and a .pdf or paper copy of each e-gram
- The .fsa or .hid file(s) of the included reference sample(s)
- 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
- A copy of the landscape with both evidence and reference profiles
- 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 and negative control e-grams associated with the evidence extraction(s) and amplification(s), preferably showing bins.

1.2.4   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.

1.2.5   Update the TA worksheet.

1.3   Importing the Evidence Profile into the TrueAllele® Casework System Using the Analysis Module

Evidence sample(s), a selected positive control and the ladder(s) are uploaded to the Analysis Module (in either .fsa or .hid format) following the procedures described in the TrueAllele® Analysis Module manual. Associated reference samples (either assumed or for comparison) can be uploaded at this time or later in either .fsa, .hid, or text format.

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NOTE: Because reference .hid files are evaluated as one (1) unknown, uncertainty may be assigned due to uneven peak heights or stochastic data. Uploading such files in text format will eliminate this unnecessary uncertainty.

1.3.1 The analysis (parent) folder can be labeled with the case number and requesting 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 Analysis Module.

1.3.2.1 Ensure the "Init preferences" are set to PP16 or PPFusion Data Disk (_DD) template, as applicable, with the appropriate Panel, Format and Size Standard selected.

NOTE: To display the data in the order corresponding to the amplification kit used to develop the evidence profile, the 'locus_order.txt' file found in the preferences folder can be set to the appropriate amplification kit (PP16 or PPFusion), if desired.

1.3.2.2 Specify allelic ladder and control abbreviations.

NOTE: Using 'al' for allelic ladder can cause unintended consequences when samples with 'al' within the name are included (e.g., s**al**iva)

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' 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® Analysis Module.

1.3.5 Update the TA worksheet.

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.

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 and spot check them for accuracy prior to deconvolution.

NOTE: Allele calls shown in the Data Module are approximates that could be slightly modified during deconvolution (e.g., a 12.1 could be modified to a 12). A spot check for accuracy of final allele calls should be conducted in the Report Module.

1.4.5 If any of the items of evidence contain artifact peaks such as spikes or dye blobs, activate the allele labeling by clicking ⌷N⌷ . 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.

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Peaks associated with increased stutter should not be turned off. Pull up and/or raised baseline should be turned off if excessive. Examiner discretion should be used in determining when to leave pull up or raised baseline on for deconvolution. Use the arrow key ⬆ to upload the .gel file to the server.

NOTE: Because GMID-X uses smoothing algorithms different from TA's, artifacts not seen in GMID-X may be observed in the TA raw data display.

1.4.6 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. Under the Database drop-down window, select Find and then Data. Using the .hid/.fsa folder name for 'gel' will limit returned data to that case. Select the case samples and positive control to create a request.

NOTE: The allelic ladder, reagent blank(s), and negative control(s)are 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. If a locus is deemed inconclusive by the original examiner (due to a quality control issue, for example), deselect all data at that locus.

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: If a mixture is referred for TA analysis based on an assumption of a number of contributors by the original referring examiner, but the data suggest to the TA examiner the possibility that there is actually one fewer or one more than originally assumed, the TA examiner has the option to analyze the mixture under both assumptions. Therefore, although four contributor mixtures are not routinely analyzed, it is possible that a mixture referred as a three contributor mixture may also be run as a four contributor mixture.

The option to analyze a mixture under two different assumptions with regard to contributor number is not to be utilized as a routine course of action. This option is available for situations in which true ambiguity in the estimated number of contributors (two vs. three and/or three vs. four) exists.

1.5.5.1 Each request for analysis of a mixture, 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 should be named to include the FS Lab #, TA examiner initials, item # and description, # unknowns + assumed known (if applicable), # cycles, , differential degradation (if applicable) and unique request number.

EXAMPLES: C13-4321. BCJ.13handle.3uk+35JS.100k.r5
C23-4321.BCJ.14VCsp.1uk+AC.50k.r3

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 or decreasing the number of cycles in a request:

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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.

> **NOTE**: If a mixture might be better resolved using a four unknown person mixture deconvolution process, then duplicate analyses at a minimum of 150K cycles 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. In general, requests should first be attempted without the 'differential degradation' setting selected. When the 'differential degradation' setting is selected, a minimum of 100K cycles should be employed.

1.5.5.7   The use of an assumed reference in a request will be limited to those known references for which a conclusion of not eliminated can be reached when following the interpretation guidance found in the applicable FB Interpretation Procedures Manual (i.e., FB PM Interpretation of Fusion Data or FB PM Interpretation of PP16 Data). Review of the data and/or the use of attribution to the owner in the Original Certificate of Analysis will also be considered when determining whether to use an assumed known.

1.5.5.7.1   Duplicate TA analyses may be performed assuming no known reference to demonstrate statistical support for the owner/potential assumed reference being a contributor prior to conducting additional analyses using the owner as an assumed known.

1.5.5.7.1.1   The same approach may be used with an elimination sample (e.g., 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 or based

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upon the use of attribution to the victim in the
Original Certificate of Analysis.

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.
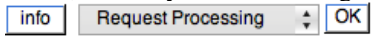
EXAMPLE: C13-4321.BCJ.051113.req

1.5.7 Verify the request(s) and upload the request file to the TA world for processing.

1.5.8 Update the TA worksheet.

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. info | Request Processing ⬍ | OK .

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.

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

1.6.5.1 Other messages such as "no data" or "null" indicate a problem such as a power interruption to the server and may require that a new request be made or that a previous request be reuploaded or reactivated.

1.6.6 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 can also be deactivated but will then need to be reactivated.

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.

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

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

1.6.6.3 Requests can be re-activated in a similar manner to begin processing.

1.6.6.4 Update the TA worksheet, if applicable.

1.6.7 Exit the Tools module.

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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, it is not necessary to turn those loci 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 arranged such that they will appear in the following order: Caucasian, African American, Hispanic.

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 on the TA Worksheet. Similarly, the inferred genotype of each reference sample will be checked against its known profile determined by the originating examiner and should 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' (i.e., significant stalling of $\geq 95\%$) with very little to no exploration (i.e., variance standard deviation < approximately 2%).

        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, differential degradation, if applicable, and resulting mixture weight histogram is not necessary.

    1.7.6.4   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.

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1.7.7   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 and/or probabilities demonstrate moderate differences.

- Poor: Inferred genotypes and/or probabilities differ substantially.

1.7.7.1   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 generally 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.

1.7.8   Complete the TA worksheet with the requested information for all but 'Failed' analyses (i.e., unacceptable convergence values and/or mixture weight histograms). Genotype concordance observations will only be addressed among acceptable analyses.

1.7.9   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.

1.7.9.1   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.

1.7.9.2   In order to keep loci in PPFusion order when creating projects with both PPFusion and PP16 data, the 'VADFS$placeholder.txt' file may be uploaded to either the evidence or reference tab which contains the PP16 data, or .txt files in the proper locus order for the reference(s) may be used.

1.7.9.3   The match score (log(LR)) for a reported contributor 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, the match statistic derived from the match score (log(LR)) closest to zero (0) 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 or eliminated contributor are not within the 2 ban range. This is not unexpected with a minor contribution at or below 10%. Reporting either the minor contributor(s)' match statistics in these situations will be handled on a case-by-case basis in consultation with the Biology Program Manager or designee.

1.7.9.4   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.

1.7.9.4.1   A maximum of six (6) analyses for a two-contributor mixture and eight (8) analyses for a mixture with more than two-contributors will routinely be allowed. Mixtures requiring more analyses may be approved for reporting by the Biology Program Manager or designee.

> **NOTE:** Analyses used to establish assumed known contributor(s) or failed analyses will not count toward this maximum number of analyses.

1.7.9.4.2   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. Consultation with the Biology Program Manager or designee may be appropriate.

1.7.9.5   Update the TA worksheet.

1.7.10   Once two or more fully concordant analyses have been identified, the following steps must be taken:

1.7.10.1   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. Within each analysis (e.g., r1), the match statistic associated with the closet fit to the inferred genotypes (most discriminating) will be identified. Across those analyses, the race-specific match statistics derived from the match scores (log(LR))s closest to zero (0) will be reported.

> **NOTE:** In order to fully explore a mixture, it may be necessary to vary the number of contributors which can affect the reported match statistics. It is possible that reproducibility is achieved for both contributor numbers attempted with reported match statistics varying between contributors. Exceptions to the determination of which analysis is to be 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 statistics derived from the match scores with r1 contributor 2 would be reported.

> **NOTE:** Match statistics derived from match scores associated with analyses to support the use of an assumed known will be reported as the basis for the assumed known, if applicable. These match scores will not routinely be considered in determination of which statistics to report for the person of interest when an assumed known is used.

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1.7.10.1.1   If the reported match statistics are derived from match scores among reproducible and fully concordant analyses that 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.

1.7.10.1.2   Based on validation data, if the reported match statistics are derived from match scores among reproducible and fully concordant analyses that fall between -2 and +2 ban for PP16 analyses and between -3 and +3 ban for PPFusion analyses, the analyses will be independently confirmed by a human review of the data. The statistical support will be considered uninformative and will be reported in addition to the results of the statistical analysis.

1.7.10.1.3   If the reported match statistics are derived from match scores among reproducible and fully concordant analyses that are less than -3 ban, indicating more support for a match to a random individual, the analyses will be independently confirmed by a human review of the data. This conclusion (i.e., elimination) will be reported without statistics.

NOTE: Match scores for low level eliminated contributors may not always be within 2 ban. See the Biology Program Manager or designee for guidance in this instance.

1.7.10.1.4   PP16 match scores falling between -3 and -2 ban or +2 and +3 ban will be handled on a case by case basis in consultation with the Program Manager or designee.

1.7.10.1.5   If the race specific match scores of the reported contributor among reproducible and fully concordant analyses have both positive and negative values, the analyses will be independently confirmed by a human review of the data. The statistical support will be considered uninformative and will be reported accordingly.

1.7.10.2   For all but eliminations 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).

NOTE: 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 separately.

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

EXAMPLE: C12-4321.all matches

1.7.10.3.1   The .xls table should be formatted for ease of review (analyses in chronological order, etc.).

1.7.10.4   Save the TA Match table which contains only the fully concordant analyses as an .xls file and name accordingly:

EXAMPLE: C12-4321.final matches

1.7.10.4.1   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.

1.7.11   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 (.tas) for storage and peer review.

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, and date of compilation:

- .fsa or .hid files supplied by the examiner (evidence, references, positive controls, ladders)
- _DD folder (data disk)
- .req file(s) (request file(s))
- report file of all TA analyses (.tas)
- .xls or .xlsm file of Match locus detail table(s)
- .xls or .xlsm file of TA Match table – all matches
- .xls or .xlsm file of TA Match table – final matches (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.

3   REPORTING RESULTS

TrueAllele® Casework System Analysis Certificates of Analysis will follow the FB PM Report Writing with regard to the requirements listed in accordance with the FBI's Quality Assurance Standards for Forensic DNA Testing Laboratories and the Department Quality Manual.

The reported likelihood ratio values (match statistics) will be truncated to two (2) significant figures.

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 uninformative.

3.2   General Reporting Format

The Certificate of Analysis will contain a METHODS section and RESULTS AND INTERPRETATIONS 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 AND INTERPRETATIONS 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:

STATISTICAL ANALYSIS 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 (SWGDAM) 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).
- The D12S391, DYS391 and Amelogenin loci are not used for statistical purposes.

    3.2.2.2   Methods to be used for PP16 cases:

STATISTICAL ANALYSIS METHODS

- The DNA PowerPlex® 16 profiles referenced in this report were previously developed and addressed in a Certificate of Analysis dated _____.

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- 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 (SWGDAM) 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.

3.2.2.3   Methods to be used for cases referred based upon STRmix™ Analysis:

For cases referred by the Central Laboratory:

STATISTICAL ANALYSIS METHODS

- The DNA PowerPlex® Fusion profiles referenced in this report were previously developed and addressed in a Certificate of Analysis dated _____.
- A STRmix™ System analysis to estimate mixture proportions and weights was conducted, and the data were then referred for analysis using the TrueAllele® Casework System.
- 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 (SWGDAM) 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).
- The D12S391, DYS391 and Amelogenin loci are not used for statistical purposes.

For cases referred by the Eastern Laboratory:

STATISTICAL ANALYSIS METHODS

- The DNA PowerPlex® Fusion profiles referenced in this report were previously developed and addressed in a Certificate of Analysis dated _____.
- A STRmix™ System analysis to estimate mixture proportions and weights was conducted at the Eastern Laboratory, 830 Southampton Avenue, #400, Norfolk, VA 23510, and the data were then referred for analysis using the TrueAllele® Casework System.
- 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 (SWGDAM) 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).
- The D12S391, DYS391 and Amelogenin loci are not used for statistical purposes.

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For cases referred by the Northern Laboratory:

STATISTICAL ANALYSIS METHODS

- The DNA PowerPlex® Fusion profiles referenced in this report were previously developed and addressed in a Certificate of Analysis dated _____.
- A STRmix™ System analysis to estimate mixture proportions and weights was conducted at the Northern Laboratory, 10850 Pyramid Place, Manassas, VA 20110, and the data were then referred for analysis using the TrueAllele® Casework System.
- 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 (SWGDAM) 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).
- The D12S391, DYS391 and Amelogenin loci are not used for statistical purposes.

For cases referred by the Western Laboratory:

STATISTICAL ANALYSIS METHODS

- The DNA PowerPlex® Fusion profiles referenced in this report were previously developed and addressed in a Certificate of Analysis dated _____.
- A STRmix™ System analysis to estimate mixture proportions and weights was conducted at the Western Laboratory, 6600 Northside High School Road, Roanoke, VA 24019, and the data were then referred for analysis using the TrueAllele® Casework System.
- 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 (SWGDAM) 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).
- The D12S391, DYS391 and Amelogenin loci are not used for statistical purposes.

3.2.3   The RESULTS AND INTERPRETATIONS 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 as well as any locus-specific comments, as needed.

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 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 generally be one narrative statement and multiple statistical statements unless different assumptions are made (e.g., sequential assumptions made in cases involving related individuals).

3.2.3.3   The narrative statement will address the following:

- Evidence sample being analyzed
- The number of unknown contributors tested
- Assumed reference(s) (if applicable)
- The reference sample(s) to which comparisons were made

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(s) of PERSON(s) OF INTEREST, relative to reference populations, to compute likelihood ratio (LR) DNA match statistics.

EXAMPLE (assumed reference on intimate evidence):

Assuming the DNA profile data previously developed from the evidence is a mixture of two unknown contributors and ASSUMED KNOWN, 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(s) of PERSON(s) OF INTEREST, 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 ASSUMED PERSON 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(s) of PERSON(s) OF INTEREST, relative to reference populations, to compute likelihood ratio (LR) DNA match statistics.

EXAMPLE (new reference sample, previous TrueAllele® analysis):

As previously reported in the Certificate of Analysis dated [DATE], the DNA profile data developed from the evidence is assumed to be 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(s) of PERSON(s) OF INTEREST, 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 will be edited based on whether or not a conclusion was previously reported and/or to report the appropriate assumption of number of contributors, for grammatical reasons, etc., as applicable.

3.2.3.4.1   Match score favors the person of interest (no assumed known reference)

EXAMPLE:

Based on these results, PERSON OF INTEREST cannot be eliminated as a contributor to this DNA mixture profile.

The DNA profile developed from the EVIDENCE at the PowerPlex® [16/Fusion] loci is approximately:

_____ times more likely to be observed if it originated from PERSON OF INTEREST and two unrelated unknown individuals in the Caucasian population than if it originated from three unrelated unknown individuals in the Caucasian population.

_____ times more likely to be observed if it originated from PERSON OF INTEREST and two unrelated unknown individuals in the African American population than if it originated from three unrelated unknown individuals in the African American population.

_____ times more likely to be observed if it originated from PERSON OF INTEREST and two unrelated unknown individuals in the Hispanic population than if it originated from three unrelated unknown individuals in the Hispanic population.

3.2.3.4.2   Match score favors the person of interest (assumed known reference used)

EXAMPLE:

Based on these results, PERSON OF INTEREST cannot be eliminated as a contributor to this DNA mixture profile.

The DNA profile developed from the EVIDENCE at the PowerPlex® [16/Fusion] loci is approximately:

_____ times more likely to be observed if it originated from ASSUMED KNOWN, PERSON OF INTEREST, and one unrelated unknown individual in the Caucasian population than if it originated from ASSUMED KNOWN and two unrelated unknown individuals in the Caucasian population.

_____ times more likely to be observed if it originated from ASSUMED KNOWN, PERSON OF INTEREST, and one unrelated unknown individual in the African American population than if it originated from ASSUMED KNOWN and two unrelated unknown individuals in the African American population.

_____ times more likely to be observed if it originated from ASSUMED KNOWN, PERSON OF INTEREST, and one unrelated unknown individual in the Hispanic population than if it originated from ASSUMED KNOWN and two unrelated unknown individuals in the Hispanic population.

EXAMPLE:

Based on these results, PERSON OF INTEREST cannot be eliminated as a contributor to this DNA mixture profile.

The DNA profile developed from the EVIDENCE at the PowerPlex® [16/Fusion] loci is approximately:

_____ times more likely to be observed if it originated from ASSUMED KNOWN and PERSON OF INTEREST than if it originated from ASSUMED KNOWN and an unrelated unknown individual in the Caucasian population.

_____ times more likely to be observed if it originated from ASSUMED KNOWN and PERSON OF INTEREST than if it originated from ASSUMED KNOWN and an unrelated unknown individual in the African American population.

_____ times more likely to be observed if it originated from ASSUMED KNOWN and PERSON OF INTEREST than if it originated from ASSUMED KNOWN and an unrelated unknown individual in the Hispanic population.

3.2.3.4.3   Match score favors a coincidental match

EXAMPLE:

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

3.2.3.4.4   Match scores are not reproducible between analyses

EXAMPLE:

Based on these results and the inability to achieve reproducible match scores, no conclusions will be offered regarding PERSON OF INTEREST 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 PERSON OF INTEREST as a possible contributor to this mixture.

3.2.3.4.5   Reproducible match scores fall into the uninformative range

EXAMPLE:

Based on these results, the DNA profile developed from the EVIDENCE at the PowerPlex® [16/Fusion] loci is approximately:

_____ times more likely/equally likely to be observed if it originated from PERSON OF INTEREST and two unrelated unknown individuals in the Caucasian population than/as if it originated from three unrelated unknown individuals in the Caucasian population.

_____ times more likely/equally likely to be observed if it originated from PERSON OF INTEREST and two unrelated unknown individuals in the African

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American population than/as if it originated from three unrelated unknown individuals in the African American population.

_____ times more likely/equally likely to be observed if it originated from PERSON OF INTEREST and two unrelated unknown individuals in the Hispanic population than/as if it originated from three unrelated unknown individuals in the Hispanic population.

As a result of internal validation testing performed at the Virginia Department of Forensic Science, the foregoing match statistics are considered uninformative. Therefore, no conclusions can be made regarding PERSON OF INTEREST as a possible contributor to this DNA mixture profile.

3.2.3.4.6   Match scores are the basis for using an assumed known

EXAMPLE:

Based on these results, the DNA profile developed from the EVIDENCE at the PowerPlex® [16/Fusion] loci is approximately:

_____ times more likely to be observed if ASSUMED KNOWN is a contributor to this DNA mixture profile than if the mixture originated from three unrelated unknown individuals in the Caucasian population.

_____ times more likely to be observed if ASSUMED KNOWN is a contributor to this DNA mixture profile than if the mixture originated from three unrelated unknown individuals in the African American population.

_____ times more likely to be observed if ASSUMED KNOWN is a contributor to this DNA mixture profile than if the mixture originated from three unrelated unknown individuals in the Hispanic population.

Using the foregoing analysis to assume the DNA profile data previously developed from the evidence is a mixture of ASSUMED KNOWN and two unrelated 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 of PERSON OF INTEREST, relative to reference populations, to compute likelihood ratio (LR) DNA match statistics.

3.2.3.4.7   Match scores are the basis for NOT using an assumed known

EXAMPLE:

The use of [NAME] as an assumed contributor was also considered.

3.2.3.5   Locus-specific comment

EXAMPLES:

To be used in instances for which a locus is deemed inconclusive due to quality control (e.g., the original evidence reagent blank had a peak observed, etc.):

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

<u>To be used in instances for which a locus is not used for reasons other than quality control (e.g., no typing results at that locus for the evidence, no typing results or an inconclusive result at that locus for the reference compared, etc.)</u>

The Penta E locus was not used in the statistical calculation.

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