Quality Assurance Standards For Forensic DNA Testing Laboratories: Section 8. Validation

TrueAllele® Casework System

Cybergenetics, Pittsburgh, PA

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Introduction

This document describes how Cybergenetics TrueAllele® Casework system complies with section 8 (Validation) of the FBI's Quality Assurance Standards (QAS) for DNA testing laboratories, as promulgated in their July 1, 2020 document.

The document embeds the QAS section 8 standards, and gives a paragraph-by-paragraph description of system compliance. Separate appendices list the many TrueAllele validation studies that establish the system's reliability. There is also an appendix on the availability of the supporting documents referred to herein.

The FBI QAS document is downloadable from:
https://www.fbi.gov/file-repository/quality-assurance-standards-for-forensic-dna-testing-laboratories.pdf/view

Glossary

• *Cybergenetics* is a Pittsburgh-based company founded in 1994 that specializes in computer interpretation of DNA evidence data.
• *Peer review* is an assessment scientific research by a journal that has two (or more) independent workers review a manuscript before accepting it for publication.
• *Probabilistic genotyping* is any method that interprets DNA data and produces more than one genotype, assigning probabilities to the possibilities.
• *SWGDAM* is the Scientific Working Group on DNA Analysis Methods, a standing committee that helps establish guidelines of interest to the FBI.
• *TrueAllele* Casework is a computer system that accurately and automatically interprets DNA evidence data, producing reliable match statistics.
• *Validation* is a testing procedure for establishing the reliability of a method.
• *Validation study* is a scientific study that documents validation testing.

FBI Quality Assurance Standards, Section 8 - Validation (effective July 1, 2020)

8. VALIDATION

STANDARD 8.1 The laboratory shall use validated methods for DNA analyses.

The TrueAllele Casework system has been extensively validated on both laboratory and casework DNA samples, with over 40 studies completed. Eight of these validation studies have been published in peer-reviewed journals. Currently, TrueAllele validation studies have been completed on samples containing up to 10 unknown contributors with both high and low template samples tested across a range of conditions. Sensitivity, specificity, and reproducibility of the TrueAllele system have been thoroughly established, with other measures studied as well. Performance checks are done when software updates are made.

Appendix 1 (*TrueAllele Validation Summary*) lists all TrueAllele validation studies and describes the metrics tested in each validation study based on the 2015 SWGDAM Guidelines for Validation of Probabilistic Genotyping Systems.

STANDARD 8.2 Developmental validation shall precede the implementation of any new methods used for forensic DNA analysis.

There are 8 TrueAllele developmental validation studies. Appendix 2 (*TrueAllele Developmental Validations*) lists these studies.

8.2.1 Developmental validation studies shall include, where applicable, characterization of the genetic marker, species specificity, sensitivity studies, stability studies, case-type samples, population studies, mixture studies, precision and accuracy studies, and PCR-based studies. PCR-based studies include reaction conditions, assessment of differential and preferential amplification, effects of multiplexing, assessment of appropriate controls, and product detection studies. All validation studies shall be documented.

Appendix 1 (*TrueAllele Validation Summary*) lists all TrueAllele validation studies and describes the metrics tested in each developmental validation study based on the 2015 SWGDAM Guidelines for Validation of

Probabilistic Genotyping Systems. All studies are documented and available upon request.

8.2.2 Peer-reviewed publication of the underlying scientific principle(s) of a method shall be required.

TrueAllele's underlying scientific principles, methods of analysis, and statistical formulae are described in various peer-reviewed and other publications. The *TrueAllele Methods: Statistical Model* document summarizes those methods and citations. Appendix 3 (*TrueAllele Peer-reviewed Papers*) lists TrueAllele related peer-reviewed papers.

STANDARD 8.3 Except as provided in Standard 8.3.1.1, internal validation of all manual and robotic methods shall be conducted by each laboratory with the appropriate sample number and type to demonstrate the reliability and potential limitations of the method.

TrueAllele Casework has been extensively validated to show reliability and potential limitations. Appendix 1 (*TrueAllele Validation Summary*) describes the TrueAllele validations.

8.3.1 Internal validation studies shall include as applicable: known and non-probative evidence samples or mock evidence samples, precision and accuracy studies, sensitivity and stochastic studies, mixture studies, and contamination assessment studies.

These studies have been conducted. Appendix 1 (*TrueAllele Validation Summary*) describes the metrics tested in each internal TrueAllele validation study based on the 2015 SWGDAM Guidelines for Validation of Probabilistic Genotyping Systems.

8.3.1.1 Internal validation data may be shared by all locations in a multi-laboratory system. The summary of the shared validation data shall be available at each site. Each laboratory in a multi-laboratory system shall complete, document and maintain applicable site-specific precision, sensitivity, and contamination assessment studies.

Appendix 1 (*TrueAllele Validation Summary*) lists all TrueAllele validation studies. All studies are documented and available upon request.

8.3.2 Internal validation shall define quality assurance parameters and interpretation guidelines, including, as applicable, guidelines for mixture interpretation and the application of appropriate statistical calculations.

Cybergenetics TrueAllele workflow and interpretation guidelines are described in the *TrueAllele® Casework Process: Standard Operating Procedures* document.

8.3.2.1 Mixture interpretation validation studies shall include samples with a range of the number of contributors, template amounts, and mixture ratios expected to be interpreted in casework.

These studies have been conducted. Appendix 1 (*TrueAllele Validation Summary*) describes the metrics tested in each internal TrueAllele validation study based on the 2015 SWGDAM Guidelines for Validation of Probabilistic Genotyping Systems.

8.3.3 Internal validation studies shall be conducted prior to implementing a change in platform instrument model or typing test kit.

When server code updates affect interpretation, internal validation is done before the new version is distributed and used in routine processing.

8.3.4 Internal validation studies shall be documented and summarized. Internal validation shall be reviewed and approved by the technical leader prior to implementing a procedure for forensic applications.

Appendix 1 (*TrueAllele Validation Summary*) lists all TrueAllele validation studies, including over 30 internal validations. All studies are documented and available upon request.

STANDARD 8.4 Newly validated DNA methods (from amplification through characterization), typing test kit, or platform instrument model shall be checked against an appropriate and available certified reference material (or sample made traceable to

the certified reference material) prior to the implementation of the method for forensic analysis.

Validation studies numbered 25, 37, and 39 in Appendix 1 (*TrueAllele Validation Summary*) show TrueAllele's performance and testing on certified reference material.

STANDARD 8.5 The performance of a modified procedure shall be evaluated by comparison to the original procedure using similar DNA samples and the evaluation documented. The evaluation shall be reviewed and approved by the technical leader prior to the implementation of the modified procedure into casework applications.

TrueAllele's workflow, operation, and system inputs and outputs are described in the *TrueAllele® Visual User Interface (VUIer™)* user manuals and Cybergenetics *TrueAllele® Casework Process: Standard Operating Procedures* document. Any procedural changes are documented and distributed accordingly.

STANDARD 8.6 A Rapid DNA instrument used for modified Rapid DNA analysis on casework reference samples shall be validated in accordance with Standard 8.

Standard 8.6 is applicable when using a Rapid DNA instrument with TrueAllele Casework.

STANDARD 8.7 An NDIS approved Rapid DNA System shall require a performance check prior to use on casework reference samples.

Standard 8.7 is applicable when using a Rapid DNA instrument with TrueAllele Casework.

STANDARD 8.8 New software or new modules of existing software and modifications to software shall be evaluated to assess the suitability of the software for its intended use in the laboratory and to determine the necessity of validation studies or software testing. This evaluation shall include the determination of which studies will and will not be conducted and shall be documented.

When server code updates affect interpretation, validation is done before the new version is distributed and used in routine processing.

Additionally, when a new module is added, a performance check is done to test the new software. Once sufficient testing has been done, the software or server version is deployed for use in casework.

8.8.1 New software or new modules of existing software that are used as a component of instrumentation, for the analysis and/or interpretation of DNA data, or for statistical calculations, shall be subject to developmental validation prior to implementation in forensic DNA analysis.

When server code updates affect interpretation, validation is done before the new version is distributed and used in routine processing. Additionally, new modules added to the VUIer software are thoroughly tested before use.

8.8.1.1 With the exception of legally protected information, the underlying scientific principle(s) utilized by software with an impact on the analytical process, interpretation, or statistical calculations shall be publicly available for review or published in a peer-reviewed scientific journal.

TrueAllele's underlying scientific principles, methods of analysis, and statistical formulae are described in various peer-reviewed and other publications. The *TrueAllele Methods: Statistical Model* document summarizes those methods and citations. Appendix 3 (*TrueAllele Peer-reviewed Papers*) lists TrueAllele related peer-reviewed papers.

8.8.1.2 Developmental software validation studies for new software or new modules of existing software used as a component of instrumentation shall include at a minimum, functional testing and reliability testing.

When server code updates affect interpretation, validation is done before the new version is distributed and used in routine processing. Additionally, new modules added to the VUIer software are thoroughly tested before use. This includes functional and reliability testing.

8.8.1.3 Developmental software validation studies for new software or new modules of existing software for the analysis and/or interpretation of DNA data shall include at a minimum, functional testing, reliability testing, and as applicable, accuracy, precision, sensitivity, and specificity studies.

These studies have been conducted. Appendix 1 (*TrueAllele Validation Summary*) describes the metrics tested in each developmental TrueAllele validation study based on the 2015 SWGDAM Guidelines for Validation of Probabilistic Genotyping Systems.

8.8.1.4 Developmental software validation studies for new software or new modules of existing software for statistical calculations shall include at a minimum, functional testing, reliability testing, and as applicable, accuracy, and precision studies.

These studies have been conducted. Appendix 1 (*TrueAllele Validation Summary*) describes the metrics tested in each developmental TrueAllele validation study based on the 2015 SWGDAM Guidelines for Validation of Probabilistic Genotyping Systems.

8.8.2 New software or new modules of existing software that are used as a component of instrumentation, for the analysis and/or interpretation of DNA data, or for statistical calculations shall be subject to internal validation specific to the laboratory's intended use prior to implementation in forensic DNA analysis.

Appendix 1 (*TrueAllele Validation Summary*) lists all TrueAllele validation studies and describes the metrics tested in each internal validation study based on the 2015 SWGDAM Guidelines for Validation of Probabilistic Genotyping Systems. These studies encompass the processes and procedures Cybergenetics follows when analyzing casework data.

8.8.2.1 Internal software validation studies for new software or new modules of existing software used as a component of instrumentation shall include functional testing and reliability testing.

When server code updates affect interpretation, validation is done before the new version is distributed and used in routine processing. Additionally, new modules added to the VUIer software are thoroughly tested before use. This includes functional and reliability testing.

8.8.2.2 Internal software validation studies for new software or new modules of existing software for the analysis and/or interpretation of DNA data shall include functional testing, reliability testing, and, as applicable, precision and accuracy studies, sensitivity, and specificity studies.

These studies have been conducted. Appendix 1 (*TrueAllele Validation Summary*) describes the metrics tested in each developmental TrueAllele validation study based on the 2015 SWGDAM Guidelines for Validation of Probabilistic Genotyping Systems.

8.8.2.3 Internal software validation studies for new software or new modules of existing software for statistical calculations shall include functional testing, reliability testing, and, as applicable, precision and accuracy studies.

These studies have been conducted. Appendix 1 (*TrueAllele Validation Summary*) describes the metrics tested in each developmental TrueAllele validation study based on the 2015 SWGDAM Guidelines for Validation of Probabilistic Genotyping Systems.

8.8.2.4 Software that does not impact the analytical process, interpretation, or statistical calculations shall require at a minimum, a functional test.

Established data sets are used during performance checks. Once sufficient testing has been done, the software or server version is deployed for use in casework.

8.8.3 Modifications to software as described in Standards 8.8.1 and 8.8.2 shall be evaluated to determine if the modifications result in major or minor revisions to the software.

When server code updates affect interpretation, validation is done before the new version is distributed and used in routine processing.

Additionally, when a new module is added, a performance check is done to test the new software. Once sufficient testing has been done, the software or server version is deployed for use in casework.

8.8.3.1 A major revision to software used as a component of instrumentation shall require validation prior to implementation. Software validation studies shall include functional testing, reliability testing, and regression testing.

When server code updates affect interpretation, validation is done before the new version is distributed and used in routine processing. This includes functional, reliability, and regression testing.

8.8.3.2 A major revision to software used for the analysis and/or interpretation of DNA data shall require validation prior to implementation. Software validation studies shall include functional testing, reliability testing, regression testing, and, as applicable, precision and accuracy studies, sensitivity, and specificity studies.

When server code updates affect interpretation, validation is done before the new version is distributed and used in routine processing. Additionally, new modules added to the VUIer software are thoroughly tested before use. This includes functional, reliability, and regression testing as well as precision, accuracy, sensitivity, and specificity studies.

8.8.3.3 A major revision to software used for statistical calculations shall require validation prior to implementation. Software validation studies shall include functional testing, reliability testing, regression testing, and, as applicable, precision and accuracy studies.

When server code updates affect interpretation, validation is done before the new version is distributed and used in routine processing. Additionally, new modules added to the VUIer software are thoroughly tested before use. This includes functional, reliability, and regression testing as well as precision and accuracy studies.

8.8.3.4 A minor revision to software that does not impact the analytical process, interpretation, or statistical calculations shall require at a minimum, a functional test.

Established data sets are used during performance checks. Once sufficient testing has been done, the software or server version is deployed for use in casework.

The validation and testing done for software changes are described in the studies and application notes listed below:

Software change: LR interface theta calculation change (VUIer version 3.3.5227.1b (13-Feb-2014) and greater)
Validation and testing: Cybergenetics. “TrueAllele® VUIer™: Likelihood Ratio Calculation Application Note.” 2019.

Software change: D22S1045 update (TrueAllele server code version 3.25.5682.1 and greater)
Validation and testing: J.M. Hornyak, T. Hebert, W.P. Allan, M.W. Perlin. "Baltimore Police Department TrueAllele® Validation." Cybergenetics (Pittsburgh, PA) and Baltimore City Police Department Laboratory Section (Baltimore, MD), August 2015.

8.8.4 Software validation studies and software testing may be shared by all locations in a multi-laboratory system. The summary of the shared validation data shall be available at each site. Each laboratory in a multi-laboratory system shall complete, document and maintain applicable site-specific reliability testing.

Appendix 1 (*TrueAllele Validation Summary*) lists all TrueAllele validation studies. All studies are documented and available upon request.

8.8.5 Software validation and testing shall be documented. Software validation and testing shall be reviewed and approved by the technical leader prior to implementation.

Appendix 1 (*TrueAllele Validation Summary*) lists all TrueAllele validation studies. All studies are documented and available upon request. Regular testing is done before any new software is released.

STANDARD 8.9 *Developmental validation studies, internal validation studies, modified procedure evaluations, and software testing, including the approval of the technical leader, shall be retained and available for review.*

Appendix 1 (*TrueAllele Validation Summary*) lists all TrueAllele validation studies. All studies are documented and available upon request.

Appendix 1: TrueAllele Validation Summary

Introduction

The TrueAllele Casework system has been thoroughly validated across a range of conditions. Cybergenetics and other groups have conducted over 40 validation studies. These studies have been presented either as peer-reviewed papers, or as written reports or presentations. Additional validation studies are currently being conducted.

This section contains a table describing the validation studies that fulfill the various developmental and internal validation guidelines presented in sections 3 and 4 of the 2015 SWGDAM Guidelines for Validation of Probabilistic Genotyping Systems. The table contains the SWGDAM *Guideline* number, a *Description* of the guideline, and a *Study* number that corresponds to the study fulfilling the guideline. These *Study* numbers correspond to both the *TrueAllele Validation Citations* section in this document as well as the study information contained in the *TrueAllele Validation Reports and Papers (ReadMe)* document. Many of these guidelines appear in other standards and guideline documents. Thus, this appendix can be used to show how TrueAllele complies with those standards and recommendations as well.

A Dropbox link to all of the papers and reports can be provided upon request. It should be noted that this table may not list every topic covered in a study but is representative of the major points covered in each study.

Note: SWGDAM guideline 4.1.12 (establishing in-house parameters) is not applicable to TrueAllele analysis.

TrueAllele Studies and SWGDAM Guidelines

Guideline	Description	Study
3.2.1, 4.1.13	Sensitivity	4, 5, 7, 8, 9, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 27, 28, 29, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43
3.2.1.1	Type I errors (False exclusions)	16, 21, 22, 23, 24, 27, 28, 32, 34, 36, 37, 39, 40, 42, 43
3.2.1.2	Sensitivity range of LR values expected for contributors	4, 5, 7, 8, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 27, 28, 31, 32, 33, 34, 35, 36, 37, 39, 40, 43
3.2.2, 4.1.13	Specificity	7, 8, 12, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 27, 28, 29, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 43
3.2.2.1	Type II errors (False inclusions)	16, 18, 19, 20, 21, 22, 23, 24, 27, 28, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 43
3.2.2.2	Specificity range of LR values expected for non-contributors	12, 15, 16, 18, 19, 20, 21, 22, 23, 24, 25, 27, 28, 31, 32, 33, 34, 35, 36, 37, 39, 40, 43
3.2.3, 4.1.13	Precision	2, 5, 7, 8, 9, 11, 12, 13, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 27, 28, 29, 31, 32, 33, 34, 35, 36, 37, 39, 40, 43
3.2.3.1	Range of LR values expected between multiple analyses (σ_w)	5, 7, 8, 13, 15, 16, 17, 19, 20, 21, 22, 23, 24, 25, 27, 28, 31, 32, 33, 34, 35, 36, 37, 39, 40, 43
3.2.3.2	Reducing the variability of LR variation (e.g., increasing MCMC iterations)	15, 16, 18, 28, 29, 31, 33, 34, 37, 39, 42
3.2.4, 3.2.4.1, 4.1.1	Case-type samples (reliable evaluation)	5, 6, 7, 9, 10, 13, 17, 19, 25, 27, 31, 33, 37, 38, 40, 43
3.2.5	Control samples	1, 9, 25
3.2.6	Accuracy	2, 4, 5, 6, 8, 9, 13, 15, 17, 19, 21, 24, 26, 27, 29, 31, 34, 35, 38, 39, 40, 43
3.2.6.1, 4.2	Comparison with manual review	1, 2, 4, 5, 6, 7, 8, 9, 10, 11, 13, 15, 17, 19, 25, 29, 31, 33, 35
3.2.6.2	Comparison of allele calling of raw data (.fsa) files	1, 17
4.1	Data from kits, instruments, and analysis software used in casework	1, 3, 4, 5, 7, 8, 9, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 43
4.1.1	Known contributor samples	4, 8, 9, 12, 14, 15, 16, 18, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43
4.1.2, 4.1.2.1	Hypothesis testing with contributors and non-contributors	4, 5, 9, 11, 12, 13, 17, 18, 19, 25, 26, 28, 29, 31, 32, 37, 38, 39, 40, 42, 43
4.1.3	Variable DNA typing conditions	9, 16, 18, 19, 22, 24, 28, 31, 32, 36, 37, 40, 43
4.1.4	Allelic peak height	3, 9, 16, 18, 19, 22, 24, 28, 30

4.1.5	Single-source samples	1, 5, 6, 8, 9, 12, 15, 25, 28, 29, 31, 35, 37, 38, 40, 43
4.1.6	Mixture samples	2, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43
4.1.6.1	Various contributor ratios	4, 7, 8, 9, 11, 12, 13, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 34, 35, 36, 37, 39, 40, 41, 42, 43
4.1.6.2	Various total DNA template quantities	4, 7, 8, 9, 11, 12, 15, 17, 18, 19, 20, 21, 27, 28, 32, 35, 36, 37, 40, 41, 43
4.1.6.3	Various numbers of contributors in samples	7, 10, 11, 12, 15, 16, 17, 18, 19, 21, 23, 24, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43
4.1.6.4	Over- and under- estimating of number of contributors input	8, 27, 28, 30, 32, 34, 39
4.1.6.5	Allele sharing among contributors	8, 11, 12, 18, 20, 26, 29, 38, 40
4.1.7	Partial profiles	5, 8, 9, 14, 15, 18, 28, 29, 35
4.1.7.1	Allele and locus drop-out	5, 8, 15, 18, 29, 34, 35, 39
4.1.7.2	DNA degradation	8, 12, 28, 29, 30, 32, 36, 37, 40, 43
4.1.7.3	Inhibition	30, 32, 36, 43
4.1.8	Allele drop-in	14
4.1.9	Forward and reverse stutter	1, 8, 13
4.1.10	Intra-locus peak height variation	1, 3, 29, 41
4.1.11	Inter-locus peak height variation (mixture weight modeling)	4, 5, 13, 14, 15, 17, 27, 41
4.1.14	Additional challenge testing (spikes, etc.)	1, 29
4.2.1	Determination if results produced are intuitive and consistent with expectations	1, 2, 4, 5, 6, 7, 8, 9, 10, 11, 13, 15, 17, 18, 19, 25, 29, 31, 33, 35
4.2.1.1	If included manually, also included with probabilistic genotyping	1, 2, 4, 5, 6, 7, 8, 9, 10, 13, 15, 17, 19, 25, 29, 31, 33, 35
4.2.1.2	Single-source concordance between manual and probabilistic genotyping methods	1, 5, 6, 8, 9, 15, 17, 25, 31, 35
4.2.1.3	Weightings given to individual genotypes decrease with increasing mixture complexity	5, 8, 11, 15, 16, 17, 18, 21, 22, 23, 24, 26, 27, 28, 31, 32, 33, 34, 35, 36, 37, 39, 42, 43

TrueAllele Validation Citations

This section lists the citations for all TrueAllele validation studies.

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21. J. Hornyak, W.P. Allan, and M.W. Perlin. “TrueAllele® Casework Validation on PowerPlex® 21 Mixture Data.” *Cybergenetics (Pittsburgh, PA),* March 2014.

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38. G. Sugimoto. “Validation of the TrueAllele® Casework VUIer™ Kinship Application.” Kern Regional Crime Laboratory (Bakersfield, CA), August 2019.

39. Bauer DW, Butt N, Hornyak JM, Perlin MW. Validating TrueAllele® interpretation of DNA mixtures containing up to ten unknown contributors. *J Forensic Sci*, 2020; 65(2):380-398.

40. B.A. Pujols, B.M. Browning, J.M. Bracamontes, M.M. Legler, D.W. Bauer, and M.W. Perlin. “TrueAllele® Casework Validation on Greenville County DNA Lab GlobalFiler™ Data.” Cybergenetics (Pittsburgh, PA) and Greenville County Department of Public Safety Forensic DNA Laboratory (Greenville, SC), March, 2020.

41. S. Antillon. “Deconvolution of DNA mixtures using replicate sampling and TrueAllele® mixture interpretation [master’s thesis].” George Mason University (Fairfax, VA), 2020.

42. H.S. Chaudhry. “Peeling away uncertainty: A probabilistic approach to DNA mixture deconvolution [master’s thesis].” George Mason University (Fairfax, VA), 2020.

43. E.E. Mole, J.M. Bracamontes, I. Fleming, M.M. Legler, and M.W. Perlin. "Metro Nashville Police Department Crime Laboratory TrueAllele® Casework Validation on PowerPlex® Fusion 6C data." Cybergenetics (Pittsburgh, PA) and Metro Nashville Police Department Crime Laboratory (Nashville, TN), June 2023.

Appendix 2: TrueAllele Developmental Validations

This section lists the citations for TrueAllele developmental validation studies.

1. Perlin MW, Sinelnikov A. An information gap in DNA evidence interpretation. *PLoS ONE.* 2009;4(12):e8327.

2. Perlin MW, Legler MM, Spencer CE, Smith JL, Allan WP, Belrose JL, Duceman BW. Validating TrueAllele® DNA mixture interpretation. *J Forensic Sci.* 2011;56(6):1430-1447.

3. Ballantyne J, Hanson EK, Perlin MW. DNA mixture genotyping by probabilistic computer interpretation of binomially-sampled laser captured cell populations: Combining quantitative data for greater identification information. *Sci Justice.* 2013;53(2):103-114.

4. Perlin MW, Belrose JL, Duceman BW. New York State TrueAllele® Casework validation study. *J Forensic Sci.* 2013;58(6):1458-1466.

5. Perlin MW, Dormer K, Hornyak J, Schiermeier-Wood L, Greenspoon S. TrueAllele® Casework on Virginia DNA mixture evidence: computer and manual interpretation in 72 reported criminal cases. *PLOS ONE*. 2014;9(3):e92837.

6. Perlin MW, Hornyak J, Sugimoto G, Miller K. TrueAllele® genotype identification on DNA mixtures containing up to five unknown contributors. *J Forensic Sci*. 2015; 60(4):857-868.

7. Greenspoon SA, Schiermeier-Wood L, Jenkins BA. Establishing the limits of TrueAllele® Casework: a validation study. *J Forensic Sci.* 2015;60(5):1263-1276.

8. Bauer DW, Butt N, Hornyak JM, Perlin MW. “Validating TrueAllele® interpretation of DNA mixtures containing up to ten unknown contributors.” *J Forensic Sci*, 2020; 65(2):380-398.

Appendix 3: TrueAllele Peer-reviewed Papers

This section lists citations for TrueAllele-related peer-reviewed papers.

1. Perlin MW. Transforming conjunctive match into RETE: a call-graph caching approach, *International Journal of Software Engineering and Knowledge Engineering*, 1991;1(4):373:408.

2. Perlin MW, Burks MB, Hoop RC, Hoffman EP. Toward fully automated genotyping: allele assignment, pedigree construction, phase determination, and recombination detection in Duchenne muscular dystrophy. *Am J Hum Genet*. 1994;55(4):777-87.

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4. Andrews C, Devlin B, Perlin M, Roeder K. Binning clones by hybridization with complex probes: statistical refinement of an inner product mapping method. *Genomics*, 1997;41(2):141-154.

5. Lancia G, Perlin M. Genotyping of pooled microsatellite markers by combinatorial optimization techniques. *Discrete Applied Math*. 1998;88(1-3):291-314.

6. Pálsson B, Pálsson F, Perlin M, Gubjartsson H, Stefánsson K, Gulcher J. Using quality measures to facilitate allele calling in high-throughput genotyping. *Genome Research*. 1999;9(10):1002-12.

7. Perlin MW, Szabady B. Linear mixture analysis: a mathematical approach to resolving mixed DNA samples. *J Forensic Sci*. 2001;46(6):1372-7.

8. Kadash K, Kozlowski BE, Biega LA, Duceman BW. Validation study of the TrueAllele® automated data review system. *J Forensic Sci.* 2004;49(4):1-8.

9. Hill SY, Shen S, Zezza N, Hoffman EK, Perlin M, Allan W. A genome wide search for alcoholism susceptibility genes. *American Journal of Medical Genetics Part B: Neuropsychiatric Genetics*. 2004;128B(1):102-13.

10. Perlin MW, Kadane JB, Cotton RW. Match likelihood ratio for uncertain genotypes. *Law, Probability and Risk*. 2009;8(3):289-302.

11. Perlin MW, Sinelnikov A. An information gap in DNA evidence interpretation. *PLoS ONE.* 2009;4(12):e8327.

12. Perlin MW, Legler MM, Spencer CE, Smith JL, Allan WP, Belrose JL, Duceman BW. Validating TrueAllele® DNA mixture interpretation. *J Forensic Sci.* 2011;56(6):1430-1447.

13. Ballantyne J, Hanson EK, Perlin MW. DNA mixture genotyping by probabilistic computer interpretation of binomially-sampled laser captured cell populations: Combining quantitative data for greater identification information. *Sci Justice.* 2013;53(2):103-114.

14. Perlin MW, Belrose JL, Duceman BW. New York State TrueAllele® Casework validation study. *J Forensic Sci.* 2013;58(6):1458-1466.

15. Perlin MW, Dormer K, Hornyak J, Schiermeier-Wood L, Greenspoon S. TrueAllele® Casework on Virginia DNA mixture evidence: computer and manual interpretation in 72 reported criminal cases. *PLOS ONE*. 2014;9(3):e92837.

16. Perlin MW. Inclusion probability for DNA mixtures is a subjective one-sided match statistic unrelated to identification information. *Journal of Pathology Informatics*, 6(1):59, 2015.

17. Perlin MW, Hornyak J, Sugimoto G, Miller K. TrueAllele® genotype identification on DNA mixtures containing up to five unknown contributors. *J Forensic Sci*. 2015; 60(4):857-868.

18. Greenspoon SA, Schiermeier-Wood L, Jenkins BA. Establishing the limits of TrueAllele® Casework: a validation study. *J Forensic Sci.* 2015;60(5):1263-1276.

19. Stokes NA, Stanciua CE, Brocatoa ER, Ehrhardta CR, Greenspoon SA. Simplification of complex DNA profiles using front end cell separation and probabilistic modeling. *Forensic Science International: Genetics*. 2018;36:205-212.

20. Perlin MW. Efficient construction of match strength distributions for uncertain multi-locus genotypes. *Heliyon*, 4(10):e00824, 2018.

21. Bauer DW, Butt N, Hornyak JM, Perlin MW. “Validating TrueAllele® interpretation of DNA mixtures containing up to ten unknown contributors.” *J Forensic Sci*, 2020; 65(2):380-398.

Appendix 4: Other Reports and Supporting Documentation

Several supporting reports and other materials are mentioned throughout this document. These materials give additional support for TrueAllele's compliance with various guidelines and standards. A Dropbox link to these documents can be provided upon request.

TrueAllele reports

Perlin MW. Scientific validation of mixture interpretation methods. Promega's Seventeenth International Symposium on Human Identification, 2006; Nashville, TN.

Perlin MW. Explaining the likelihood ratio in DNA mixture interpretation. Promega's Twenty First International Symposium on Human Identification, 2010; San Antonio, TX.

Other supporting documents:

- *TrueAllele® Methods: Statistical Model*
- *TrueAllele® VUIer™* user manuals:
    - *Workflow Introduction*
    - *Getting Started*
    - *Analyze Module*
    - *Data Module*
    - *Request Module*
    - *Review Module*
    - *Report Module*
    - *Tools Module*
    - *Tutorial*
    - *Database Application Note*
    - *Specificity Application Note*
    - *Likelihood Ratio Calculation Application Note*
- Cybergenetics' *TrueAllele® Casework Process: Standard Operating Procedures*
- *TrueAllele® Server Quality Assurance Checklist*