



Practical Aspects of the Implementation of TrueAllele Casework

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Outline

- History of mixture statistics in VA
- Collaboration with Cybergenetics
- Training to use TrueAllele
- Implementation in VA casework



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Mixture Statistics in Virginia


- Pre-SWGDAM 2010 Guidelines:
 - Capillary electrophoresis platform was implemented in 2008
- VA was using the following statistical calculations on mixtures:
 - Likelihood Ratio
 - Combined Probability of Inclusion



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**2010 SWGDAM
Mixture Interpretation Guidelines**


- Primary impact on VA DFS:
 - Introduced the idea of a stochastic threshold and that only data above should be included in the CPI
 - DFS Forensic Science Board and DFS Scientific Advisory Committee approved stochastic threshold validation plan
- Allows for the use of a validated probabilistic modeling program as another approach



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Post-SWGDAM


- Stochastic threshold validation was completed
- Stochastic thresholds were implemented for CPIs
 - 2, 5 and 10 second injections
 - 210, 320 and 460 rfu, respectively
- Loci with alleles below STH were used for interpretation, but not for statistical calculations



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DFS modified the approach to the modified CPI

- In an undifferentiated mixture, if some loci have alleles in the stochastic range, is it possible remaining non-breakout loci have total drop-out of a contributor?
 - DFS limited use of CPI to profiles which have ALL alleles above stochastic threshold



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Mixture statistics

- DFS did not have a way to calculate statistics on undifferentiated mixtures with alleles below the stochastic threshold
 - Began looking for vendors who would calculate mixture statistics, write reports and testify in court
 - Simultaneously, DFS would be researching and validating statistical approaches to mixtures



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Growing Pains

- Mixture profiles needing statistics continued to be generated
 - Interim solution was to validate, train, and implement restricted random match probabilities



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Casework Contract with Cybergenetics

- In 2011, DFS entered a contract with Cybergenetics:
 - Build a 'library' of ~150 mixture profile analyses
 - Current cases without stats and previous cases with CPIs
 - Reports were generated on an as-needed basis
 - Mixtures would be used for initial validation



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Cybergenetics' Reports

- Files were transferred on a secure server
 - Mixture and reference .fsa's
 - Information worksheet
- Format of report was determined
- Cybergenetics was notified of the court date
 - Cybergenetics wrote the report
 - DFS conducted an admin review
- Report was forwarded to DFS
 - DFS released the report with a cover letter



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DFS Casework Library

- ~80 case reports were issued by Cybergenetics
 - Dr. Perlin testified ~10 times
 - DFS testified 2 times
- Cybergenetics, in conjunction with VA DFS, published TrueAllele Casework on Virginia DNA Mixture Evidence: Computer and Manual Interpretation in 72 Reported Criminal Cases. PLoS ONE 9(3): e92837, 2014



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Pre-training Materials

- Prior to orientation training, pre-training materials were recommended:
 - Understanding Uncertainty by Lindley
 - Literature review list
 - Lectures on Cybergenetics' website
 - User's Manual



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Initial Orientation TrueAllele Training

- TrueAllele training and in-house validation were pursued
 - TL and one supervisor attended initial 3 day training (now called Science and Software)
- Software and hardware were procured
 - Two TA stations and server with 8 processors
 - Training held via internet for remaining 3 scientists



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TrueAllele Team at DFS

- Team comprised of:
 - Research scientist
 - Section supervisor
 - Group supervisor
 - Senior scientist
- Three levels of experience add dimension to collaboration and trouble shooting
- Team would eventually support casework of 55 scientists statewide



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TrueAllele Operator 1 Training 2012

- After orientation training with TrueAllele, individuals completed the Operator 1 course comprised of 6 lessons covering simple DNA mixture problems and their TA solutions
 - Each lesson included
 - Uploading data and processing mixtures
 - Uploading and evaluating additional processing requests
 - Submitting results along with written answers to homework questions from Cybergenetics



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TrueAllele Operator 2 Training 2013

- Operator 2 course consisted of 6 lessons covering complex DNA mixtures and problem solving strategies in TA



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Cybergenetics-sponsored Training

- Each DFS scientist worked independently at their own pace and turned in their own homework
- Both courses culminated with a comprehensive exam which included a mixture profile to analyze and questions to answer



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TA Supplemental Training

- Attendance at some of Dr. Perlin's testimonies in Virginia
 - Including attendance at the 4.5 day Spencer (Frye) hearing in Colonial Heights, VA
- Reanalysis of mixture profiles previously reported by Cybergenetics
- Culmination of TA training:
 - Analysis of a mock case
 - Oral technical competency



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DFS Validation of TrueAllele

- Validation studies were conducted simultaneously with training
 - Majority of validation was conducted by DFS's research scientist and reviewed by one of the remaining team members using DFS's server and processors



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DFS Validation Studies

- Used mock casework samples which included the following:
 - 17 single source profiles (degraded and stochastic)
 - 18 two person mixtures
 - 14 three person mixtures
 - 7 four person mixtures



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DFS Validations (continued)

- Reproducibility
- Accuracy
 - Mixture weight assessment for two-person mixtures
 - LR's compared to 1/RMP for single source profiles
- Sensitivity
 - Inclusion of true contributors
 - Minor contributor contribution level below which results in false exclusion
- Specificity
 - Exclusion of non-contributors
 - Exclusion of relatives of the contributors



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DFS Validations (continued)

- Assuming an incorrect # of contributors
- Assuming a wrong assumed known contributor
- Dropout and non-ideal profiles
- Differential degradation function



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DFS Validation Studies

- Once completed, validation studies were
 - Reviewed by the VA DFS Scientific Advisory Committee
 - Provided and testified to during the Spencer (Frye) hearing in Virginia
- Accepted for publication by JFS: Establishing the Limits of TrueAllele® Casework: A Validation Study



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TrueAllele Affects DFS Mixture Interpretation

- Observations of TA results led to modification of DFS mixture interpretation guidelines:
 - Allelic dropout is tolerated at a maximum of 3 loci for 'non-elimination'
- Now have an alternative to method to use for calculating statistics involving positive associations of related individuals



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DFS TrueAllele Protocols

- TrueAllele procedure manual based on:
 - TA user manual
 - Validation data
 - Very conservative approach to reporting match scores
- Worksheets were created to document pre-analysis profile impressions, analysis steps, and locus-specific LRs of the reported match
- TrueAllele was officially put on-line for casework in January, 2014



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TA Analysis Quality Control

- The positive control associated with the evidence is processed through TA
- The genotype produced by TA for known references is compared to that reported by the scientist.



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Virginia's caseload

- TrueAllele team made up of 4 scientists in the Central (Richmond) laboratory
- Approx. 300 cases completed per month among 4 regional laboratories



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Triaging Mixtures at DFS

- Mixture deconvolutions are done prior to looking at reference samples (except for references and intimate items)
- Deconvoluted profiles are then compared to references and conclusions are made.
- If comparison results in positive association, then type of statistic to be applied is determined.



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DFS and uRMs

- Statewide staff was trained to use unrestricted RMPs as a means to provide mixture statistics for undifferentiated mixtures for certain samples.
 - uRMs are only applied at break-out loci to minimize the possibility of including a locus demonstrating dropout



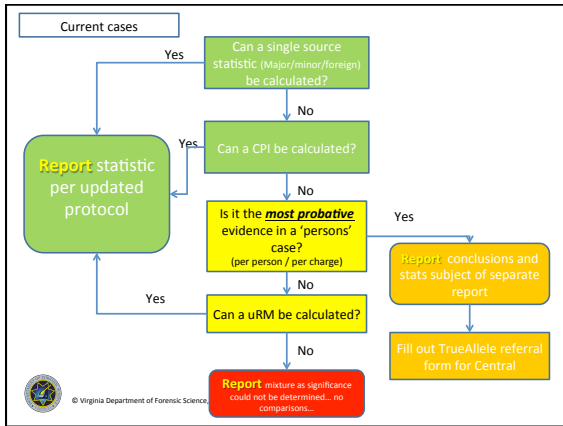
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Use of TrueAllele in Virginia

- For all profiles, the original scientist will try first to generate a “traditional” statistic (single source, CPI)
- If a traditional statistic cannot be calculated, and the profile is from a ‘persons’ case, it is transferred to the TA team
 - Due to the resource-intensive nature of TA, it is not used for every mixture



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Profiles referred to TrueAllele

- Examiner and technical reviewer agree a POI could be a possible contributor
- Report “conclusions and statistical estimates regarding item _____ will be the subject of a separate report”
- Conclusions and TA statistics are provided in a Supplemental Report

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TrueAllele processing in VA

- Currently 16 processors with 4 TA stations
- Amount of processing time is dependent on variables:
 - Number of contributors
 - Complexity of profile
 - Number of MCMC cycles
- Under VA’s conditions, 5 profiles (3 contributors) run in triplicate will tie up DFS processors for 2.5-3 days. Additional runs may be necessary

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TrueAllele Case Files

- Disc of all electronic data
- Original e-grams and interpretation documentation
- Manual documentation of
 - run conditions, MCMC chain appearance, Gelman-Rubin (GR) score and mixture weights
 - genotype concordance and match score reproducibility
- Printouts of all and final match score tables
- Locus specific printout of reported matches



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DFS's TrueAllele Reports

STATISTICAL ANALYSIS METHODS

- The DNA PowerPlex® 16 profiles referenced in this report were previously developed and addressed in a Certificate of Analysis dated May 13, 2014.
- 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 method addressed below has been applied in accordance with the Scientific Working Group on DNA Analysis Methods (SWGIDAM) 2010 Interpretation Guidelines and Departmental procedures.
- The DNA match statistics calculated herein used the population allele frequencies generated by the Virginia Department of Forensic Science, and a theta co-ancestry coefficient of 1%.

Assuming the DNA profile data previously developed from the sperm fraction of the lipa/lip area sample is a mixture of two unknown contributors and [REDACTED], 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 provided reference genotype ([REDACTED]), relative to reference populations, to compute likelihood ratio (LR) DNA match statistics.



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Reported Match Statement

Based on these results, a match between the sperm fraction of the lipa/lip area sample and [REDACTED] is:

210 billion times more probable than a coincidental match to an unrelated Black person,
9.7 billion times more probable than a coincidental match to an unrelated Caucasian person, and
100 billion times more probable than a coincidental match to an unrelated Hispanic person.



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TrueAllele Cases in Virginia

- Approximately 150 cases have been completed by DFS
- Results have been admitted into state, federal and military courts
- Approximately 20 court testimonies
- 1 admissibility hearing (2013)
 - Commonwealth vs. Matthew Brady (Colonial Heights)
 - Circuit court trial is still pending



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Court Prep and Testimony

- In Virginia, challenges to DNA are case-dependent
- The party subpoenaing the TA scientist is offered pre-trial assistance
 - Statistical set-up questions
 - Useful materials on Cybergenetics' website
 - Names of other attorneys who have used TA results



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Virginia Testimony Experience

- Other than Spencer hearing, challenges to TA have been limited:
 - DFS's database is limited to 200 individuals of each reported race
 - It's a black box
 - It's new and not widely used
 - The match scores for one item are very different than the match scores for another item.



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Admissibility Hearing, July, 2013

- Colonial Heights, Virginia
- Testified:
 - Bench scientist
 - Section supervisor
 - Technical leader (Program Manager)
 - DFS research scientist
 - Dr. Mark Perlin



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Admissibility Hearing: Defense Challenges to TA

- Source code hasn't been released
- TA doesn't use thresholds
- No controls are used
- Making assumptions isn't objective
- When using an assumed known, the known sample is processed first
- Use of the term 'match'
- Error rates
- Drop-out
- 'good' vs. 'bad' runs
- 'ground truth' samples



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Current work at DFS

- New version of VUIer software is being performance checked
 - The new calculation is more accurate
- As with other methodologies, we watch what new programs are released and will evaluate them as possible complements to TA



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For Future Consideration...

- VA DFS has validated TA for up to 4 contributors
 - TA can currently handle up to 6 contributors
- VA DFS has validated TA for individual evidence samples with assumed knowns (or to use as basis for ownership)
 - TA can do joint interpretation of multiple evidence samples
 - Operator can use TA results for 'nested' interpretation



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Questions?

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