MONROE COUNTY CRIME LABORATORY
85 W. Broad Street, Rochester NY, 14614
(585) 753-3535

John R. Clark
Laboratory Administrator

Appendix to Biology Reports

Body Fluid Identification

Presumptive - A non-confirmatory test used for detecting the possible presence of biological fluids. Presumptive tests make use of a target chemical to establish the possibility that a particular body fluid is present.

- Phenolphthalein is used as a presumptive test for blood.
- Hematrace is used as a presumptive test for human blood.
- Detection of acid phosphatase is used as a presumptive test for seminal protein.
- Detection of amylase is used as a presumptive test for saliva.
- Amylase may also be found in lower levels in urine, feces, perspiration, and vaginal secretions.
- Prostate Specific Antigen (PSA) is a protein (also known as P30) produced by the prostate gland and found in semen. PSA concentration in semen is typically in levels far in excess of those found in other fluids.

Spermatozoa - The male reproductive cell that can be found in semen.

Semen is comprised of two components: the seminal plasma and spermatozoa. Seminal plasma contains PSA and acid phosphatase, typically in levels far in excess of those found in other fluids.

Y-screening for Male DNA

The Y-screen assay is a rapid and reliable test to screen for male DNA in the processing of sexual assault kit samples. Utilizing a simplified extraction step which rapidly lyses cells (including sperm) and a quantification kit, the Y-screening rapidly detects the presence of male DNA in sexual assault kit swab evidence. The assay may be used in conjunction with, or independent from, other screening methods to assist in deciding whether to proceed with DNA analysis procedures.

DNA Analysis

DNA (**D**eoxyribo**n**ucleic **A**cid), the inherited genetic material found in most cells, contains markers that can differ from person to person. DNA analysis can determine these genetic markers and compare biological samples from different individuals.

DNA Analysis is comprised of several steps, including DNA extraction, DNA quantification, PCR/DNA amplification, and analysis of the resulting DNA alleles.

Alleles are an alternative form of DNA markers. Alleles are found at specific areas, or locations, of the DNA called **loci** (singular, **locus**).

DNA extraction recovers DNA from biological samples such as blood, saliva, semen, bone, hair, tissue, and skin cells.

Differential Extraction – A procedure in which sperm cells are separated from all other cells in a sample, resulting in a fraction which is enriched for sperm DNA (Fraction 2 or F2) and a fraction which contains DNA from other cell types (Fraction 1 or F1). Incomplete separation can occur and fractions may contain both sperm DNA and non-sperm DNA. Microscopic examination is performed on a portion of Fraction 2 which may or may not contain sperm cells.

Appendix to Biology Reports

DNA quantification provides an estimate of the amount of DNA recovered from samples by using a technique called real time polymerase chain reaction (qPCR). The quantification testing uses the **Applied Biosystems Quantifiler® Trio DNA Quantification Kit**. If sufficient DNA is detected, DNA amplification and analysis can be attempted.

The **PCR** (polymerase chain reaction) is a technique that copies specific areas of DNA. PCR generates large amounts of DNA from small starting amounts of DNA by repeated cycles of copying the DNA loci (**DNA amplification**); after amplification, the alleles present in the sample are identified.

PCR DNA testing for STRs uses a **DNA amplification kit**, a commercial product used to generate a DNA profile.

The MCCL uses the **Applied Biosystems AmpFlSTR Globalfiler® PCR Amplification Kit** using 29 amplification cycles. Each STR locus tested in the Globalfiler® kit contains between 9 and 34 identifiable alleles. The loci tested are D3S1358, vWA, D16S539, CSF1PO, TPOX, D8S1179, D21S11, D18S51, D2S441, D19S433, TH01, FGA, D22S1045, D5S818, D13S317, D7S820, SE33, D10S1248, D1S1656, D12S391, D2S1338. The kit also tests the Amelogenin locus, Y-indel, and DYS391 which are used to determine the sex origin of a sample.

The MCCL uses the **Applied Biosystems AmpFlSTR YFiler™ Plus PCR Amplification Kit** using 30 amplification cycles. Each Y-STR locus tested in the YFiler® Plus kit contains between 8 and 23 identifiable alleles. The loci tested are DYS576, DYS389I, DYS635, DYS389II, DYS627, DYS460, DYS458, DYS19, GATAH4, DYS448, DYS391, DYS456, DYS390, DYS438, DYS392, DYS518, DYS570, DYS437, DYS385, DYS449, DYS393, DYS439, DYS481, DYF387S1, and DYS533.

Stochastic effects are defined as unequal sampling of the two alleles present from a heterozygous individual that result when only a few DNA copies are used to initiate PCR. Such samples may exhibit significantly different heterozygous peak heights or allelic dropout.

STR (short tandem repeat) loci contain alleles with a variable number of short repeating segments. Each STR allele can be described using a number that represents its number of repeats. A **DNA profile** is the collection of these numbers describing the DNA alleles found at an individual's STR DNA loci.

STRmix™ In forensic DNA testing, mixed DNA samples are often obtained. The MCCL uses the STRmix™ software to assist with interpretation of DNA profiles and mixture deconvolution. STRmix™ is a fully continuous probabilistic genotyping software that uses a mathematical process of Markov Chain Monte Carlo modeling. The process assigns a weight to the possible genotype combinations of each contributor which indicates how well it fits the observed DNA profile.

Y-STR loci contain alleles with a variable number of short repeating segments on the **Y chromosome**. Y-STRs are polymorphic among *unrelated* males and are inherited through the paternal line with little change through generations. Barring a mutation event, a person of interest's Y-STR haplotype will be the same in all paternal male relatives. Y-STRs differ from autosomal STRs in other ways. First, only male samples have a Y-STR haplotype because females do not possess a Y chromosome. Secondly, because the Y chromosome is inherited from the father, there is only a single allele at each locus, with

the exception of a duplication at DYS385 and/or DYF387S1. Lastly, the 27 loci amplified with the AmpFlSTR Yfiler Plus® PCR Amplification kit are not independent of each other and represent a single haplotype.

Statistics

Statistical calculations are performed on evidentiary DNA profiles that are deemed as relevant in the context of the case to assist in the assessment of the significance of an inclusion. STRmix™ can calculate a likelihood ratio on a DNA profile after deconvolution.

A likelihood ratio is the ratio of two probabilities of the same event under different hypotheses: the probability of the genetic evidence being observed given one hypothesis compared to the probability of the genetic evidence being observed given the alternative hypothesis. Typically one hypothesis would represent the prosecution position in the form of the probability of observing the DNA results given that the person of interest is a donor. The other hypothesis would be set to represent the potential defense position in the form of the probability of observing the DNA results given that an unknown person (not the person of interest) is a donor. The convention is to set the order of the propositions indicating the proposition with the higher probability first. These hypotheses are modified to include additional known and unknown contributors depending on the total number of contributors to the profile.

The MCCL reports a unified likelihood ratio which accounts for the fact that the pool of unknown contributors within both hypotheses is made up of both related and unrelated individuals. The MCCL reports the most common of the 98% lower bound of the highest posterior density value of the unified likelihood ratio of the four more common populations in the US: African-American, Caucasian, Hispanic, and Asian.

Y-STR haplotype - All Y-STR loci analyzed are physically linked on the Y-chromosome. Due to the lack of recombination, the entire Y-chromosome haplotype must be treated as a single locus. Haplotype frequencies are estimated using the **counting method**. The counting method involves searching a given haplotype against a database to determine the number of times the haplotype was observed in that database. The frequency of the haplotype in the database is then estimated by dividing the count by the number of haplotypes searched. The profile probability is estimated by applying a 95% confidence upper bound to the haplotype frequency, using the method described by Clopper and Pearson (1934) as per the SWGDAM Interpretation Guidelines for Y-Chromosome STR Typing by Forensic DNA Laboratories, 2014: Section 10.2.3.

The statistic reported is a count of the number of times that the haplotype in question was observed when searched against the database. The statistical strength of Y-STR testing is affected by the size of the population database searched. Profiles in the population database may not have results for all of the Y-STR loci. Therefore, the total number of observations in the database will vary dependent upon which loci are searched. Population databases may contain profiles typed with different multiplexes, containing different numbers and/or sets of loci, such that only a subset of the database may have been typed at all of the loci present in the evidence profile.

Appendix to Biology Reports

The MCCL uses the Y-Chromosome Haplotype Reference Database (https://yhrd.org/) for calculating profile probability. The Release Number of the Y-Chromosome Haplotype Reference Database is updated periodically. The statistical values may change based upon database size and composition. If prosecution of a case is anticipated, an updated calculation may be performed upon request.

Conclusions for DNA Comparisons

The DNA profile obtained from Item X is # times more likely to be observed if the DNA came from Name than if the DNA came from an unknown individual. When making a direct comparison between two specimens, the likelihood ratio will indicate which hypothesis has the higher probability.

The mixed DNA profile obtained from Item Y is # times more likely to be observed if the DNA came from Name 1 and Name 2 than if the DNA came from Name 1 and an unknown individual. For locations where comparisons could be made, all or most of the DNA alleles seen in an individual's DNA profile were represented in the mixture. The allele(s) that are not represented could be explained by any of several factors. The likelihood ratio will indicate which hypothesis has the higher probability.

The statistical analysis does not provide sufficient support for whether Name 1 is a contributor to this DNA profile. The likelihood ratio value noted for an individual within the uninformative range does not provide sufficient support for whether they are included or excluded as a contributor to this sample. Internal validations and published studies help inform the limits to where a false positive or false negative result may possibly arise. Likelihood ratios with exponents between 10^{-3} and 10^{3} have the potential to support a false inclusion or exclusion based on internal validation studies.

Item Z is excluded as a contributor to the mixture. For locations where comparisons could be made, the DNA alleles seen in an individual's DNA profile were not represented in the mixture. The allele(s) that are not represented could not be explained. Therefore, an individual can be ruled out as a possible contributor to the mixture.

The mixture is uninterpretable or inconclusive. The DNA results from the evidence are either limited or too complex and are not suitable for comparison. Therefore, it cannot be determined whether an individual is a contributor to this mixture.

Not suitable for comparison. When a sample is amplified, DNA results may be inconclusive due to insufficient data or genetic complexity. Comparisons to known items and/or statistical calculations cannot be made. No further conclusions can be drawn regarding the source or sources of the resulting data.

Appendix to Biology Reports

Disposition of Evidence

In order to obtain the best results, items may be consumed in analysis.

DNA extracts will be retained at the laboratory.

Items that have been tested will be returned to the submitting agency.

Items that have not been tested based on case information and/or results from other items will be returned to the submitting agency at the discretion of the laboratory. Items that were not tested may be re-submitted to the laboratory for DNA analysis with an explanation of how the additional item(s) are beneficial to the investigation.

CODIS

The main function of the **Combined DNA Index System (CODIS)** is to provide associations between cases and offenders or between two cases. All eligible DNA profiles that are suitable are entered into CODIS into the Local DNA Index System (LDIS). If a sample meets the completeness requirements for the state level, the profile is uploaded to the State DNA Index System (SDIS). If the DNA profile also meets the completeness requirements for the national level, the profile is uploaded to the National DNA Index System (NDIS).

SDIS has an Index for DNA profiles developed from a firearm collected for a criminal possession-related offense. These profiles are not eligible for upload to NDIS.

LDIS is searched prior to performing an upload to the State DNA Index System (SDIS). If an association is made at the LDIS level, one of the DNA profiles may be unmarked. Unmarking a DNA profile means that it will not be uploaded to SDIS or NDIS. SDIS and NDIS searches are performed daily, Monday through Friday. NDIS may also perform additional searches. In the event of a new, positive association, a letter is generated by the MCCL notifying the investigating agency and their legal representative of any pertinent CODIS match information.

The notification letter will indicate the stringency of the association. A high stringency match indicates that all the alleles in the forensic specimen are the same as the candidate specimen. A moderate stringency match indicates that at least one locus has a different number of alleles between the forensic specimen and the candidate specimen, but all of the allelic values in the specimen with the fewest number of alleles are represented in the other specimen. A moderate stringency match may occur when one or both of the specimen profiles is a partial profile or a DNA mixture.

Conclusions for CODIS

A DNA profile from the following item(s) was entered into the Local DNA Index System (LDIS) for comparison to forensic profiles. LDIS does not contain offender profiles. If these statements are not followed by an indication that the profile was also uploaded, the DNA profile does not meet the completeness requirements to be uploaded to the state or national level. The DNA profile will be searched at the local level only.

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Appendix to Biology Reports

A DNA profile from the following item(s) will also be uploaded to the State DNA Index System (SDIS) for comparison to forensic and offender profiles. The DNA profile does not meet the completeness requirements to be uploaded to the national level or the DNA profile is from a firearm collected for a criminal possession-related case and is not eligible for the upload to the national level. The DNA profile will be searched at the local and state levels.

A DNA profile from the following item(s) will be uploaded to the State DNA Index System (SDIS) and National DNA Index System (NDIS) for comparison to forensic and offender profiles. The DNA profile will be searched at the local, state, and national levels of CODIS.

***The DNA profile(s) obtained from Item(s) is/are not currently eligible for entry into the CODIS database for comparison purposes*:** Based on the current documentation in the laboratory case file, the documentation is lacking for one or more of the following general requirements:

1) That the item is related to a crime.
2) How the item is connected to the crime scene.
3) How the item (or DNA profile) is connected to the perpetrator of the crime.
4) That the item is ***not*** from a place where the perpetrator's profile can reasonably be expected to be found.

The laboratory can re-evaluate CODIS eligibility if more case documentation is submitted.

The DNA profile obtained from Item X is not suitable for entry into the CODIS database for comparison purposes: The profile is too complex or not complete enough to be entered into the CODIS database.

Reference samples will not be entered into CODIS: The Monroe County Crime Laboratory does not enter reference samples into CODIS unless submitted for the purpose of identifying a missing person.