



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 for presumptive testing of 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) - 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.

Background to DNA Testing

DNA (Deoxyribo-Nucleic Acid), 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.

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

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.

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 the duplication at DYS385. Lastly, the 17 loci amplified with the AmpFISTR Yfiler® PCR Amplification kit are not independent of each other and represent a single haplotype.



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DNA Analysis

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

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 Sperm Fraction which is enriched for sperm DNA and a Non-Sperm/Epithelial Fraction which contains DNA from other cell types. Incomplete separation can occur and fractions may contain both sperm DNA and non-sperm DNA.

DNA quantification provides an estimate of the amount of DNA recovered from samples by using a technique called real time polymerase chain reaction (q-PCR). The quantification testing uses the **Applied Biosystems Quantifiler® Duo 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 AmpFISTR Identifiler® PCR Amplification Kit** using 28 amplification cycles. Each STR locus tested in the Identifiler® kit contains between 8 and 32 identifiable alleles. The loci tested are D8S1179, D21S11, D7S820, CSF1PO, D3S1358, TH01, D13S317, D16S539, D2S1338, D19S433, vWA, TPOX, D18S51, D5S818, and FGA. The kit also tests the Amelogenin locus, which is used to determine the sex origin of a sample.

The MCCL uses the **Applied Biosystems AmpFISTR 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.

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The MCCL uses the **Applied Biosystems AmpFISTR YFiler® PCR Amplification Kit** using 30 amplification cycles. Each STR locus tested in the YFiler® kit contains between 5 and 19 identifiable alleles. The loci tested are DYS456, DYS389-I, DYS390, DYS389-II, DYS458, DYS19, DYS385 a/b, DYS393, DYS391, DYS439, DYS635, DYS392, Y GATA H4, DYS437, DYS438, and DYS448.

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.

The MCCL uses a **stochastic threshold** of 130 RFUs for Identifiler data and 300 RFUs for Globalfiler data as a means to ascertain if data detected is complete in mixtures and low-level samples. When a sample containing low-level amount of DNA template (0.10ng for single source samples) or a minor component(s) of a mixture exhibits peaks below **stochastic threshold**, it is possible that complete amplification has not occurred and not all alleles have been detected.

Statistics

The rarity of a DNA profile can be expressed as an STR population frequency estimate, how often one would expect to see the DNA profile derived from the evidentiary item(s). The frequency estimate is expressed as a probability.

Autosomal STR population frequency estimates are based on the NIST population database (2013) and the National Research Council (1996) The Evaluation of Forensic DNA Evidence, Natl. Acad. Press, Washington DC.

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.

For Y-STR mixtures, haplotype profile probabilities are not reported. The statistic reported is a count of the number of times that those haplotypes that would be included as possible contributors to the evidence were observed when those haplotypes are searched against the database.

MCCL uses the consolidated U.S. Y-STR database <https://www.usystrdatabase.org>

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The statistical values reported reflect the approximate frequency of the occurrence of a given DNA profile in a population of unrelated individuals. Therefore, these values are not appropriate for relatives.

The Monroe County Crime Laboratory reports the most common frequency estimate of the three more common populations in the US: African-American, Caucasian, and Hispanic.

Conclusions for DNA Comparisons

The DNA profile obtained from Item X matches the DNA profile of Item Y: When making a direct comparison between two specimens, a match may be reported if the DNA profile of the reference specimen is the same as the DNA profile of the questioned specimen(s).

Item Y and Item Z cannot be excluded as a contributor(s) to the mixture: 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. Therefore, an individual cannot be ruled out as a possible contributor to the mixture.

Item Y and Item Z are excluded as a contributor(s) 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.

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. 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.

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Conclusions for 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) 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 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.