



**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 which 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 which 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.

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

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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. MCCL uses 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 kit also tests the Amelogenin locus, which is used to determine the sex origin of a sample.

**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 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 130 RFUs, 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. 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.

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.

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**Appendix to Biology Reports**

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.

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.

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