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**DNA contamination from handled Sharpie markers used to outline bodily fluids in a forensic laboratory**

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**Introduction**

In a crime laboratory, Forensic Biologists outline bodily fluids with Sharpie markers to indicate where to swab or cut the piece of evidence. A single Sharpie marker is used on multiple pieces of evidence, even evidence from unrelated cases. This marker has the chance to increase contamination via DNA transfer. With this in mind, sensitivity and accuracy of new instrumentation and techniques, low quantities of DNA are more readily detected and observed in fragment analysis data. Current DNA transfer research has shown DNA is transferred easier depending on variables such as moisture, substrate type, and contact type. Additionally, research has shown the transfer of DNA can differ based on the laboratory environment. Identifying contributing factors of DNA contamination in a crime laboratory is imperative so that measures can be taken to prevent the contamination. This research will determine whether forensic biologists transfer DNA from evidence-to-evidence using Sharpie markers that outlined bodily fluids prior to DNA extraction.

**Proof of Concept**

A preliminary study was performed to analyze whether DNA can be extracted from the tip of Sharpie markers.

**Materials & Methods**

The mock scenario was set up to simulate practices in a crime laboratory. Differing from the proof of concept, the mock scenario used donated clothing as the substrate, blood and semen from six different donors, and reference samples. Additionally, the Sharpie markers were only used to outline the bodily fluids as a two-step DNA transfer event. The total sample size for the mock scenario, excluding the reference samples, was 110 samples.

**Mock Scenario Results**

Four human blood samples and five human seminal fluid samples contained DNA concentrations that were detectable. Figure 3 represents the qPCR results in a bar graph form for easier comparison. The DNA concentrations were low, however, the seminal fluid samples generally had higher DNA concentrations. Figure 4 shows an electropherogram of Sample 5B/5S (M), a semen fluid sample with a detectable quantity of DNA. Most of the alleles were flagged and had low relative fluorescence units (RFU), which is indicative of low DNA quantity. Figure 5 is the electropherogram of Sample 5B/6B (M). This is the only marker control with a detectable concentration of DNA.

**Discussion/Conclusion**

It is important to recognize all avenues of potential contamination in a crime laboratory so that the contamination can be prevented. Contamination can invalidate results, as well as cause the interpretation of data to be more difficult. Gloves, lab coats, and face masks are worn for this very reason. The Sharpie markers used to outline bodily fluids in a forensic laboratory may introduce detectable levels of contamination onto evidence. The preliminary experiment proved DNA can be extracted from the tips of Sharpie markers, and that certain variables, such as the Gagen extraction, will increase the chance of a higher DNA yield. The mock scenario experiment simulated a practical laboratory situation, using donated clothing and a two-step DNA transfer event. Fifteen DNA transfer events were implemented for both the human blood and human seminal fluid samples, using six different donors each. Reference samples were extracted for all the human blood and seminal fluid donors, as well as the clothing donors. Once the samples were extracted and quantified, the replicates with the highest DNA concentrations, as well as all the controls, were carried forward with amplification and genotyping.

Most of the experimental samples had undetectable, or undetermined, DNA quantities, however, 4/45 human blood samples and 5/45 human seminal fluid samples contained low DNA concentrations. These samples are shown in Figure 3.

In the mock blood samples, Sample 1B/3B (I) contained the highest DNA concentration of 0.000313415ng/µL. Among the seminal fluid samples, Sample 3B/4B (I) contained the highest DNA concentration of 0.000160025ng/µL. The human seminal fluid samples generally contained higher DNA quantities.

Figure 4 shows a representative electropherogram of Sample 2S/5S (I), which had a DNA concentration of 0.001230391ng/µL. Even though the DNA concentration was low, the software called alleles. However, 15/15 of the alleles were either Inconclusive Heterozygous (IH) or Inconclusive Homozygous (IH), meaning the peaks could not be confidently called via the software without a quality check. Figure 5 shows the electropherogram of Sample 5B/8B (M), which had a DNA concentration of 0.008551471ng/µL. This was the only marker control that contained a detectable quantity of DNA. 11/15 of the alleles were flagged for quality checks, such as IH, IHE, and Heterozygote Imbalance (IR1).

Although few samples had measurable DNA concentrations, and they were low in quantity, the chance of contamination is still present. It may not occur with every sample, but the markers themselves are capable of transferring DNA in high enough concentrations. Cleanings steps may need to be implemented before and after utilizing the marker for outlining to prevent the contamination. This research is ongoing and further analysis is necessary for concrete conclusions to be made. The reference samples need to be compared and statistical analysis needs to be conducted to determine the significance of the results.

**Future Directions**

1. Genotype reference samples again for better electropherograms.
2. Increase cycle number to 34 during PCR amplification for the DNA positive blood and semen samples using a Low Copy Number protocol
3. Compare reference samples to experimental samples
4. Perform statistical analysis on the results
5. Repeat the experiment with the addition of bleaching the Sharpie marker tips prior to outlining a new sample.
6. Repeat experiment using urine and saliva as the bodily fluids

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**References**