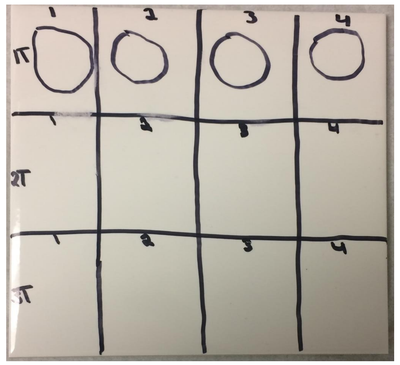
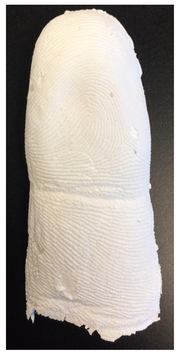
DNA Transfer Assessment

By Mark Verrilli, and David San Pietro PhD





Introduction

DNA holds a wealth of information on its own in a criminal investigation, but a major aspect that most investigators have little knowledge of is the actual transfers of DNA. Transfer of DNA can be influenced by a number of different factors, but understanding when the DNA was deposited relative to the other stains found at a crime scene can help the reconstruction process tremendously. This is only possible, however, if it is better understood the nature of how DNA is lost between transfers.

Materials and Methods

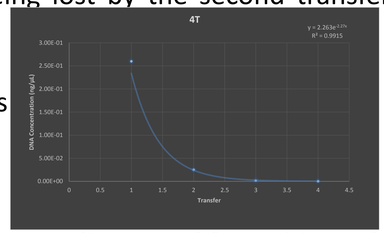
Buccal samples were collected via cotton tipped swab and DNA was extracted from them using the Buccal Swab Spin Protocol. A blood sample was also collected and kept in a purple top tube containing EDTA. The DNA solution from the buccal samples were diluted to known concentrations with deionized water after quantitation to act as working standards. The stock blood extract was quantitated and dilutions were prepared in the same manner. Concentrations were determined using both the NanoDrop One^C (ThermoFisher), as well as 7500 RT-PCR systems (Applied Biosystem). Real Time PCR quantitation was run using the Plexor HY system (Promega). Utilizing dental stone a negative impression of a finger was created from which a positive mock finger was prepared using Mikrosil to fill the mold. A white tile, 6 in x 6 in was used as the substrate for the transfers.

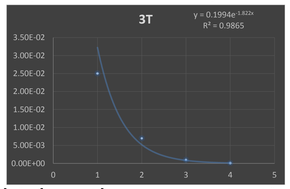
DNA Transfers: Epithelial Cells: The mock finger and tile were cleaned with a sterile alcohol pad before being placed in the SpectroLinker XL-1500 UV Crosslinker (Spectronics Corporation) for 10 minutes to ensure sterility before every series of transfers. Prior to UV exposure the tile was marked to designate spotting areas. A volume of 12.5 µL of solution containing various concentrations of DNA (0.12, 0.24, 1.5, 13 ng/µL) was placed on the mock finger and then immediately pressed onto the surface of a tile for approximately three seconds. After this time, the finger was moved to a new spot on the tile, directly to the right of the first transfer, and pressed again for approximately three seconds. This was repeated two more times for a total of four transfers. This procedure was then repeated for each dilution amount of DNA. Transfers were left to air dry and then collected via moistened cotton tip swab. Deionized water was used to moisten the swab. Swabs were allowed to air dry before extraction. The Qiagen buccal swab extraction protocol was followed for these extractions.

DNA Transfers: Leukocytes: Working standard solutions were prepared and quantitated via the NanoDrop One^C via standard protocol found in the NanoDrop One user guide. As done previously, a 12.5 µL aliquot was pipetted onto the mock finger and transfers were conducted. The Qiagen Investigator kit was used for extraction purposes for both buccal and surface swabs.

Results and Discussion

A consistent trend was observed with 56%-95% (mean 81.98%) of DNA collected on the first transfer being lost by the second transfer while only an average of 9.00% of DNA was lost between the second and third transfer across all of the DNA quantities spotted on the finger. This shows the large drop off point characteristic of an exponential decay function.

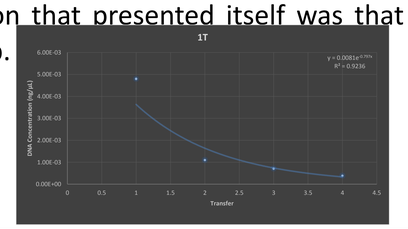


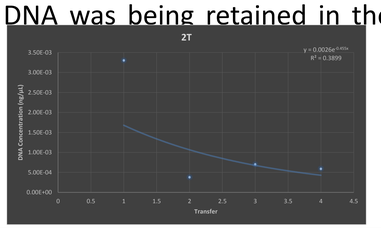


Essentially what was discovered from this study was that concentrations of DNA exponentially decrease as the number of transfers increase. The shape of the graph when concentration is plotted against transfer number is clearer the higher the concentration of DNA is, as shown by the low R^2 values of the low concentration transfer scenarios. Many of the lower concentration samples that were run had issues caused by the low amount of DNA causing stochastic effects during quantitation.

The trials that had a higher concentration of DNA to begin with showed a more defined trend as transfers increased. For example, transfer scenarios 1T and 2T had very low starting concentrations of DNA and their charts below show that the line of best fit derived from the points is not as well defined as transfer scenarios 3T or 4T (above), which both contain much higher concentrations of DNA in solution. The stochastic effects caused by such low concentrations of DNA lead to data that is difficult to interpret because some transfers show a higher concentration than the one preceding it, even thought the extraction negatives showed a concentration of zero, an indication that contamination was not a factor.

One of the more major issues that was observed during these series of experiments was determining the cause of the much lower DNA recovered after the first transfer compared to the original amount spotted on the finger. Ideally these two values would be very similar, but it is unreasonable to believe that 100% transfer could have been achieved due to multiple factors. With this in mind, it was hypothesized that values recovered after the first transfer would have been much higher than observed. The data collected shows that nearly all (around 91%) DNA was lost between the spotting of the DNA onto the finger and the first transfer. Some DNA could have been lost in the pipette tip while spotting, or possibly between the fingerprint ridges, but it is unlikely that this amount was lost by these means. The only other option that presented itself was that DNA was being retained in the swab.





Conclusions

From this study it is clear to see that the DNA concentration of a bodily fluid decreases exponentially as the number of transfers increases, on a nonporous substrate. The mean percentage lost between the initial spotting of DNA on the mock finger and the first transfer was 81.98% across all transfer scenarios, while the percentage lost between the first and second transfer was a much lower 9.00%, showing exponential loss. Even transfers that dealt with extremely low concentrations of DNA still showed a general exponential loss shape to some extent, indicating that even at very low levels this trend can be observed.

More testing is needed to observe the effect of DNA transfers on other substrates, such as porous materials including various fabrics and textiles. This study dealt exclusively with a nonporous tile, but it is important to gather data on other surfaces that can be found at the scene of a crime. Also further testing should be conducted in an attempt to find the most efficient way to collect and extract DNA from a nonporous substrate, such as the tile used in this experiment. Biological stains on porous substrates can often be cut away from the material and placed right in the tube for extraction, but nonporous surfaces must be swabbed, which can lead to useful information, in this scenario DNA, being potentially lost in the intermediate step of swabbing. Most samples collected from a crime scene will not be in an ideal condition due to the fact that they were exposed to the elements. Contamination and degradations render some samples useless, but if there is a way in which DNA collection can be optimized such that retrieval results in a high concentration of DNA more often than not, then many problems could be dealt with very easily.

Future Work

- Sample multiple substrates to determine if this trend can be observed for other materials.
- Test the collection method of bodily fluids in an attempt to increase the yield of DNA from cotton tipped swabs.
- Attempt to maximize usefulness of NanoDrop One^C for forensic work.

References

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Acknowledgements

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