Tears: the forgotten evidence type

Shirley Marshall[1], Ana Rodrigues[1] and Hez Hird[2]

[1]School of Science and Engineering, Teesside University, Borough Road, Middlesbrough TS1 3BA and
[2]Food and Environment Research Agency, Sand Hutton, York. YO41 1LZ

STUDY AIMS

The aim of this study was to investigate human tears as a source of DNA for forensic STR typing.

INTRODUCTION

- DNA is found in almost every cell of the body
- Forensic examination of body fluids for DNA is a routine procedure with methodology developed and validated for, amongst others, blood, semen and saliva.
- Human tears are often shed during the commissioning of a crime and have been shown to contain cellular material
- Tears found in cases of potential torture, rape, kidnap or murder can provide relevant information about the sequence of the events and the intention of the possible offender.
- Tears are not always considered as a potential evidence type and methods for their location, collection and analysis have yet to be developed
- This study investigated human tears as a source of evidence by collecting both internal and external tears, extracting the DNA using simple extraction methods, quantifying the amount of DNA present using Quantifiler® and then performing STR analysis.

EXPERIMENTAL WORK

- Tears were elicited from individuals using a variety of stimulants, including playing sad songs to them, blowing wind on the surface of the face, breathing in the vapour from cut onions and/or mint oil applied to the temples
- Individual external and internal (eyelid) tears for microscopy were collected in glass capillaries
- Microscope slides were then prepared and stained using standard Haematoxylin & Eosin staining methodology
- Individual external and internal (eyelid) tears for DNA extraction were collected with a sterile swab, which were then either frozen or briefly air dried.
- Swabs were incubated in 100µl of a 20% (w/v) Chelex® 100 suspension at 56^{0}C for 30 minutes
- The swabs were squeezed dry and the resultant liquid centrifuged at 13,000 g to pellet the Chelex® 100 resin.
- The supernatant was then transferred to a fresh microcentrifuge tube without disturbing the Chelex® 100 resin
- The quality and quantity of the DNA extracts was then assessed using the Quantifiler® human DNA quantification kit
- The DNA extracts were then typed using the AmpFlSTR® SGM Plus™ PCR Amplification Kit (28 and 34 cycle PCR) run on ABI 9700 thermocyclers followed by fragment analysis on an ABI 3130*xl*.
- Human profiles were analysed in Genemapper ID v3.2 using the SGM Plus™ _V1 panel set and analysis parameters: 50 rfu minimum peak height, 150 rfu minimum peak height for homozygotes and 50% peak balance.

RESULTS

- H&E staining revealed the presence of nucleated cells in human internal and external tears (Figure 1)
- The cells appeared to have an epithelial cell morphology, with evidence of the presence of lymphocytes in some slides
- STR typing was successful for a large proportion of the DNA extracts (Figure 2)
- Full profiles were obtained for some samples using 28 cycle PCR (Figure 3)
- There was heterozygous peaks imbalance at 34 cycles
- There was low stutter product even at 34 cycles (≥15 %)
- The STR typing success rate from frozen swabs was better than for air-dried swabs
- Most of the peaks over 50 amplitude threshold (minimum used for case work)
- Very low profiles at 28 cycles were successfully recovered at 34 cycles.

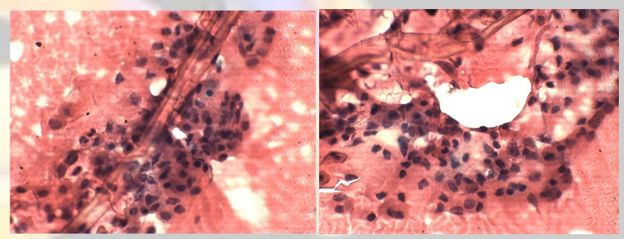

Figure 1. H&E staining of cells recovered from internal human tears. Nuclei are stained blue and cytoplasm stained pink

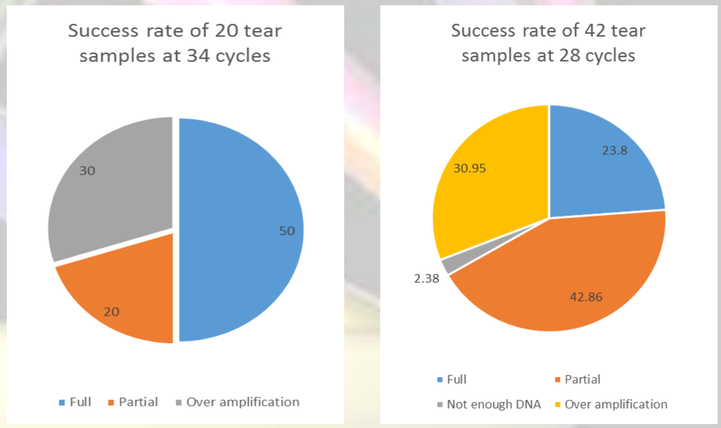

Figure 2. STR success for internal and external tears at 28 and 34 cycle PCR

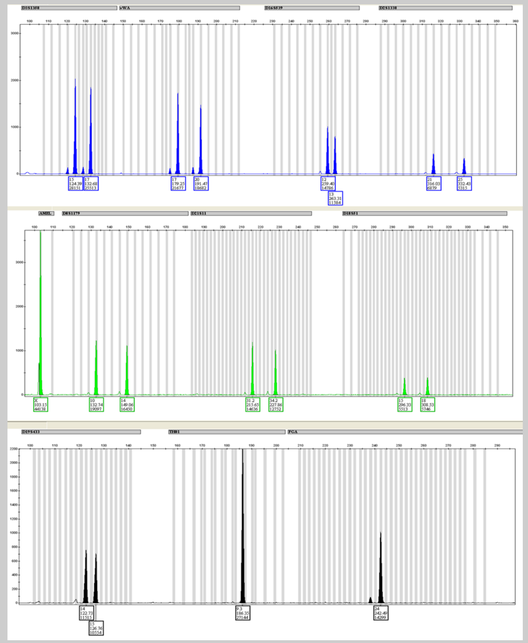

Figure 3. Example of a full profile obtained after 28 cycle PCR

CONCLUSION

This study has provided evidence that SGM Plus™ STR profiles can be generated from tears shed in a laboratory setting. The profiles were of sufficient quality to be used to search the UK National DNA Database, since the majority reached or exceeded the minimum load criteria. This study represents an important step forward since tears are not usually considered as a type of evidence, but are often shed during the commissioning of a crime.

PUBLICATION ARISING FROM THIS WORK

Rodrigues, A., Hird, H., Leadbeater, S., Chisholm, J., Holtom, L. and Marshall, S. Preliminary study of tears as a source of DNA for human profiling. Manuscript submitted for publication in Science and Justice.

ACKNOWLEDGEMENTS

The authors wish to thank the School of Science and Engineering at Teesside University and Fera for supporting this work.