Invisible Evidence

What more can your cells reveal to forensic scientists?

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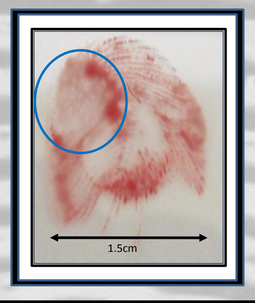
Background

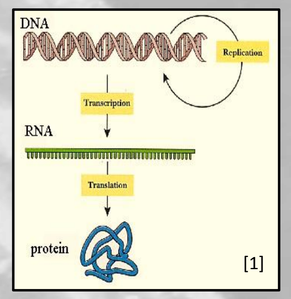
Homicide. A man is discovered dead in his house, dark red staining on the kitchen bench. Is it blood? Forensic scientists are called to the scene, but when faint finger-marks are found, how do they decide whether the fingerprints, or the blood, or the DNA, will be more valuable for identifying an offender and solving the case? Often only a tiny section of a finger-mark, as shown here, is collected to obtain cellular information, to preserve any patterns for fingerprint analysis.

Not all blood staining is visible to the naked eye. Strong dyes enhance fine details in finger-marks, but alter the visual appearance of the blood staining, and may have adverse effects on cellular identification methods.



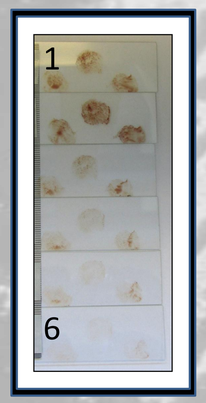
Cells use temporary molecules called RNA to copy DNA sequences and make proteins for all cellular functions. [1] ESR has developed a multiplex system named CellTyper, which targets tissue-specific RNA markers to confirm the presence of body fluids such as blood.[2] The RNA can be co-extracted with the DNA so that all genetic information comes from the same source sample.[3]

Many studies have investigated chemical effects on DNA profiling, but as yet little work has been published about factors influencing successful RNA profiling. This project contributes to a new, rapidly developing field in forensic science. The results obtained may also assist forensic experts to make critical choices without compromising the value of either cellular or fingerprint evidence in the case.



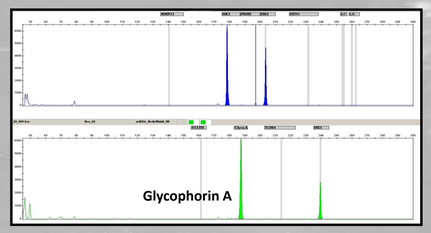
What we did

Finger-marks were made by spreading 5µL of fresh blood onto each of three fingertips and pressing them down simultaneously onto glass slides, six successive times. This created a number of depletion series sets such as the one pictured, of which the first (visible) and sixth (latent) slides were used.



These depletion sets were fixed and treated using one of four common blood-enhancing reagents used in New Zealand – aqueous Amido Black, methanol-based Amido Black, Acid Yellow 7 or Leucocrystal Violet.

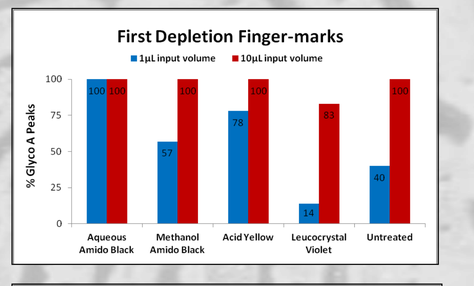
The treated finger-marks were collected and the RNA extracted [2]. This was purified, reverse-transcribed into cDNA, (complementary DNA), then amplified using CellTyper. Either a minimum (1µL) or a maximum (10µL) volume of cDNA was added to the CellTyper reaction, and the resulting profiles examined for the presence of a Glycophorin A peak, which is the blood-specific marker in this multiplex.

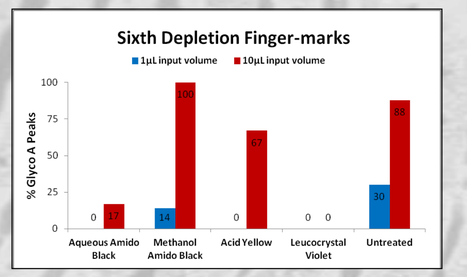


RNA profile from a blood sample after CellTyper – the blood-specific marker Glycophorin A and 3 control markers are shown

What we found

Glycophorin A marker peak presence was recorded for each sample, including untreated finger-marks as a baseline for comparison. Actual percentages for successful RNA detection, out of the total number treated, are provided.



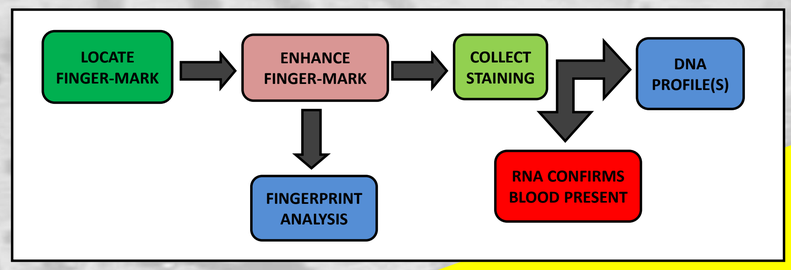


What this means

RNA levels vary between and within individuals, but obtaining a Glycophorin A marker peak in CellTyper can confirm the presence of blood in a sample as small as a latent finger-mark. We now know that RNA profiling can often be successful even after the fine detail has been enhanced for fingerprint analysis.

Consistent with previously documented impacts of blood enhancement procedures on genetic profiling, it appears that even with visible finger-marks, Leucocrystal Violet affects subsequent RNA profiling. It may still be necessary to choose between evidence types if this method will be used to enhance important detail.

Aqueous Amido Black and Acid Yellow 7 may also have adverse effects on RNA profiling of very low levels of cellular material.



To conclude, although further investigation into these and other enhancement methods is necessary, this work suggests good support for the sequence of analysis shown above, which would maximise evidence recovery from a single finger-mark in blood.

References

1. Childs, G.V. (1996) *Role of the Ribosome* University of Arkansas for Medical Sciences, http://www.cytochemistry.net/cell-biology/ribosome.htm
2. Fleming, R. and Harbison, SA (2010) *The development of a mRNA multiplex RT-PCR assay for the definitive identification of body fluids.* Forensic Science International: Genetics (4) p244 – 256.
3. Bowden, A., Fleming, R., and Harbison, SA (2011) *A method for DNA and RNA co-extraction for use on forensic samples using the Promega DNA IQ™ system.* Forensic Science International: Genetics (5) p64-68.

Acknowledgements

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