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### Original Citation

Coult, Natalie, Uchimoto, Mari and Williams, Graham (2012) Forensic MicroRNA (miRNA) Analysis of Skin Specific Markers. In: Forensic Science Society Inaugural Postgraduate Research Symposium, 10th November 2012, Coventry, UK.

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# Forensic MicroRNA (miRNA) Analysis of Skin Specific Markers



Natalie Coult, Mari L. Uchimoto, Graham A. Williams

## Introduction

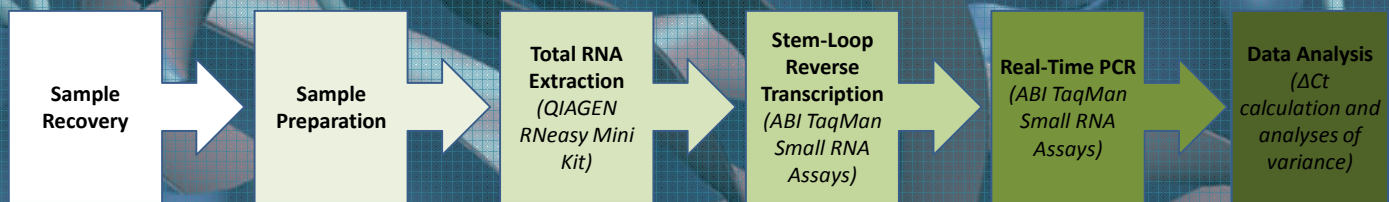
Conventional body fluid identification methods are typically presumptive tests. These tests have a large number of false positives and because of this they are not confirmatory. Messenger RNA (mRNA) profiling was developed as a body fluid identification method as it was determined that mRNAs are expressed in a tissue-specific manner. Unfortunately, given mRNA's size (~200-300 nucleotides), using this technique may not be ideal for compromised or degraded samples often encountered in forensic casework<sup>1</sup>.

MicroRNAs (miRNA) are small, non-protein coding RNA molecules approximately 22 nucleotides in length. The first miRNA was discovered by Rosalind Lee et al. in 1993, whilst studying the *lin-14* gene in *C. elegans* development<sup>2</sup>.

miRNAs are believed to control gene expression involved in metastasis, proliferation, apoptosis and differentiation<sup>3</sup>. A large number of cellular pathways are affected by the regulatory function of miRNAs; the most notable of these pathways control oncogenic and developmental processes. It has also been found that miRNA processing defects can enhance tumorigenesis<sup>4</sup>.

Many people argue that there is no need to test for the presence of a specific body fluid as the analysis of any DNA present can confirm a body fluid is present. This is true, DNA won't be present if a biological material isn't present, but the confirmation of the body fluid could prove crucial in the investigation of a case. There is a need to identify skin alongside other body fluids within forensic investigations, especially sexual offences. For example, it is not currently possible to distinguish between vaginal material and skin cells from, for example, fingernail scrapings. Thus, in order to develop a technique that is capable of doing so, it is necessary to be able to identify skin cells. The aim of this project was to determine whether skin, saliva and vaginal material (VM) could be correctly differentiated from one another using microRNA analysis.

## Method & Materials



## Results

### Skin Cell Recovery Methods

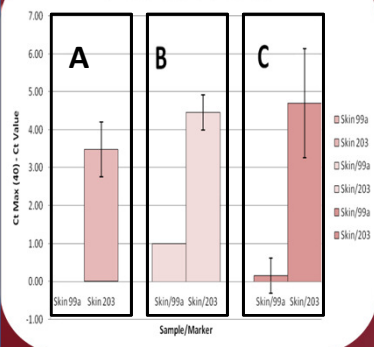
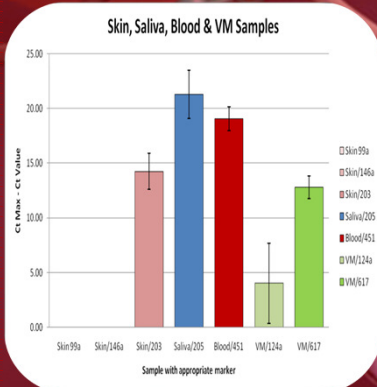


Figure 1. A comparison between three different sample recovery techniques. Method A is a dry cotton swab. Method B is a wet cotton swab. Method C is using a blunt edge and a wet cotton swab. Method C was found to be the optimal method to use.

Figure 2. Body fluid samples tested against their specific miRNA probe/marker. Blood, saliva, skin with hsa-mir-203 and VM with hsa-mir-617 all show high expression levels of their specific miRNA probe/marker.



### Blood vs Saliva markers

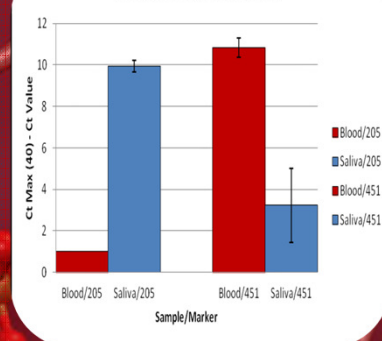
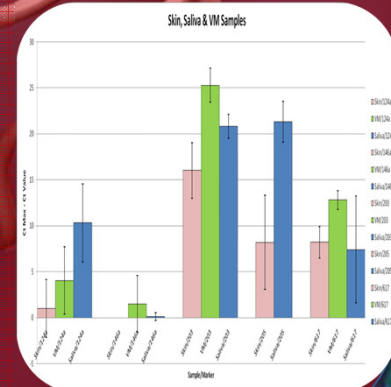


Figure 3. Blood and saliva samples with their respective miRNA probe/marker and with each other's miRNA probes/markers. A clear differentiation can be seen between blood and saliva with the two miRNA probes/markers. Thus showing the principle of the test is sound.

Figure 4. Skin, saliva and VM samples tested against each respective skin, saliva and VM miRNA probe/marker in order to determine a differentiation between the body fluid samples.



## Discussion

Skin cell recovery methods were optimised by comparing three different recovery methods. Method C, using a blunt edge to scrape skin cells off the back of a subject's hand proved to give the highest miRNA expression.

A number of miRNA probes/markers were obtained and were tested against their specific body fluid type, in order to determine which miRNA probes/markers would be used throughout experimentation. It was determined that hsa-mir-99a and hsa-mir-146a weren't suitable for skin samples and hsa-mir-124a wasn't suitable for VM samples.

As blood doesn't contain epithelial cells, it was decided this would be tested against saliva as a starting point for body fluid differentiation. Both body fluids gave strong expression levels of their own miRNA probe/marker, indicating a significant difference between them was possible.

Each sample gave high expression levels with their relevant miRNA probe/marker and gave much lower expression levels with the other samples miRNA probe/marker. Indicating a difference between the samples; paired sample T-tests confirmed a significant difference between each sample with p-values of 0.000 (below the 0.050 threshold).

Skin, saliva and VM samples were tested against each samples specific miRNA probe/marker. It was found that a differentiation could be determined between skin and saliva using hsa-mir-205 and a differentiation was possible between skin and VM using hsa-mir-617.

## Conclusion

- A significant difference between skin and saliva samples, and skin and VM samples has been determined using hsa-mir-205 and 617 respectively.
- Of the three skin specific miRNA probes/markers, none were able to determine a difference between skin and saliva or skin and VM.

## Future Work

Future research will involve identification of a skin specific miRNA probe/marker which can differentiate between skin, saliva and vaginal material, without the need for a second or third miRNA probe/marker to be used. Future research will also incorporate the differentiation of seminal fluid, sample mixtures and a single stream method for microRNA analysis and DNA analysis.

References: 1. J. Jusisko, J. Ballantyne, Messenger RNA profiling: a prototype method to supplant conventional methods for body fluid identification. *Forensic Science* 2003; 135:85-96. 2. R. C. Lee, R. L. Feihbaum, V. Ambros, The *C. elegans* heterochronic gene *lin-4* encodes small RNAs with antisense complementarity to *lin-14*. *Cell* 1993; 75:1035-52. 3. M. A. Almeida, R. M. Reis, E. A. Carril, MicroRNA history: discovery, recent applications, and next frontiers. *Mutation Research* 2013; 771:1-8. 4. M. S. Kumar, J. Lu, K. C. Mercer, T. R. Golub, T. Jacks, Impaired microRNA processing enhances cellular transformation and tumorigenesis. *Nature Genet* 2007; 39:673-677.