*Body fluids are valuable evidence in a range of offences, with tests regularly being used to identify them. However, with the increasing sensitivity of DNA profiling, capability gaps are becoming increasingly obvious. DNA profiles can also be obtained from body fluids which cannot be identified. Efforts to improve the capability of BFID have focused on genetic strategies. However, such techniques could not be applied to the crime scene. Thus, there is also a need for improved BFID tests to be used at crime scenes or for the examination of items, which would also include the ability to locate vaginal fluids.*

*Keywords: Body fluid identification, blood, semen, RNA, DNA*

The terms “body fluids” and “bodily fluids” are not interchangeable as ‘bodily’ refers to within the body. Thus ‘bodily fluids’ refers to such samples taken directly from the body and its identification is not in dispute. Whereas ‘body fluids’ refers to suspected stains recovered from a surface outside of the originating body and a key part of the process is determining which body fluid it is. Thus, the term ‘bodily fluid identification’ is an illogical term and as such, the discipline is referred to as Body Fluid Identification or BFID.

Body fluids are a valuable evidence in many offences, with blood being commonly encountered in a wide variety of crimes, not just violent crimes, but where any sort of injury has occurred, such as cutting on broken glass, or even from nose bleeds where the offender has suffered from high blood pressure as a result of anxiety or stress. Semen is also encountered in many sexual assaults, as well as non-sexual crimes, such as product tampering. Typically blood and semen are the body fluids of most significance with saliva, urine and faeces being corroborative evidence or used to identify areas for DNA.

For example, saliva can be identified in sexual assaults where oral intercourse has taken place or more commonly it can be used to identify potential sources of DNA, such as from the wearer’s own garment. Urine and faeces are less utilised, again potentially within allegations of anal intercourse, or at crime scenes where someone has urinated or defecated either out of anxiety/stress or maliciously.

There are other body fluids which are forensically relevant, but currently, no routine test exists. One of the more commonly encountered such body fluid is vaginal fluid or vaginal material. Whilst the body fluid itself is referred to as vaginal fluid or vaginal secretions this tends to relate to a pure sample, whereas the term vaginal material refers to material that has been transferred from the vaginal cavity and will largely consist of vaginal fluids as well as other substances, such as menstrual blood and microflora. Whilst there have been some previous tests, such as the use of Lugol’s Iodine, these have been considered unreliable 1.

Other potential body fluids of interest include nasal mucus (could potentially be used to differentiate blood from nose bleeds or another injury or to differentiate between impact spatter and expirated blood) or sweat2. Skin has been touted as a potential marker of interest as it may be the source of touch DNA, however, skin cells would be ubiquitous and would, therefore, have very little probative value 3.

The initial body fluid tests for blood, semen and saliva are enzymatic based and presumptive in nature, although the chemical test for blood can be confirmative when used in conjunction with an organoleptic assessment (if it looks like blood and has a positive chemical reaction, then you could call it blood), and the test for semen can be followed up with a histological assessment. The histological assessment can be problematic when the semen donor is azoospermic of vasectomised4.

Both tests for blood and semen have been around for over 50 years and have not really been improved 5, 6. Thus, begs the question, if the tests have been around that long and works, then why do we need to research into this area?

The sensitivity of DNA profiling has exceeded the sensitivity of the enzymatic based body fluid identification, thus obtaining a DNA profile without being able to associate with a body fluid is an increasingly regular occurrence. The area of body fluid attribution is one that is subtle and often overlooked. It is necessary and important, especially in the eyes of the law, to be able to say which body fluid that the DNA profile was obtained from. In one example, if a full DNA profile was obtained from a high vaginal swab in a rape case, but you were unable to say that it came from the semen, then this should be inadmissible. If you cannot say that it came from the semen, then it could have come from any other body fluid, such as saliva, sweat, skin cells, and blood. In another example, following a physical assault, a speck of blood was found on a suspect’s jeans, and a DNA profile was obtained and again, it could not be said to come from the blood, then this would undermine the evidence in court, especially if the suspect and the victim were previously known to each other.

To use a more common example, the acquisition of DNA profiles from fingernail scrapings of the suspect following an allegation of sexual assault by digital penetration can be crucial evidence. However, if complainant and suspect are known to each other, then the DNA could be from other body fluids, such as epithelial cells. In such cases, being able to say that vaginal fluid was present under the suspect’s fingernail should result in improved criminal justice outcomes.

Even if the chemical test was positive, but if the bloodstain cannot be visualised, then the presence of blood cannot be said with confidence, therefore it cannot be said that the DNA profile was obtained from the blood. Thus, there is a limitation in that to reliably associate the DNA profile with the bloodstain, the blood itself needs to be visible – a factor that limits the sensitivity of a robust DNA result.

There are efforts to develop more sensitive assays for blood and other body fluids, with the most sensitive so far being the use of genetic strategies whereby messenger RNA, microRNA and DNA methylation patterns are being characterised 7-9. Such approaches are highly specific and highly sensitive and will represent the gold standard of body fluid identification. One major aspect of this research was to develop co-isolation strategies whereby RNA and DNA could be extracted from the same sample without compromising each other7, 10. This was extended further with significant efforts being made to co-analyse DNA and RNA simultaneously and getting a single result with both the DNA profile and the RNA profile (whether it is mRNA or miRNA)7, 11. Being able to do this would increase confidence in body fluid attribution. These efforts have also been extended to body fluid mixtures, whereby the body fluids could be deconvoluted and whether a body fluid could be associated with a major or minor DNA profile12. Whilst this could be shown where there was a large difference between the major and the minor contributor, there is a great deal of uncertainty when the contributions are more equal.

There is a lot of effort to improve the identification of various body fluids using genetic strategies, however, these would be limited for screening and locating body fluid stains on items or at crime scenes due to the relative cost and need for specialist equipment. Thus, there is a real need to establish and improve the locating of body fluid stains at crime scenes. These need not be confirmatory but should be cheap and non-destructive and most importantly, it should not interfere with the DNA profiling process. Whilst there is a substantial body of work being conducted looking into crime scene searching for body fluids13, there is no real effort to evaluate its impact upon the subsequent DNA profile.

Finally, with improved and novel BFID test becoming available, there is a need to better understand the transferability and persistence of such body fluids, especially vaginal fluids and such work needs to be transformative. For example, there is plenty of research available exploring the effects of laundering upon the persistence of such body fluids14-18 but there needs to be more fundamental understanding about the transfer of the body fluid in the first place. For example, can body fluids such as semen and vaginal fluid travel through fabric and be deposited on the surface underneath?

**References**

1. Rothwell TJ, Harvey KJ. The limitations of the Lugol's iodine staining technique for the identification of vaginal epithelial cells*. Journal of the Forensic Science Society*. 1978;18(3-4):181-184.

2. Fujimoto S, Manabe S, Morimoto C, et al. Distinct spectrum of microRNA expression in forensically relevant body fluids and probabilistic discriminant approach*. Scientific reports*. 2019;9(1):1-10.

3. Van den Berge M, Carracedo A, Gomes I, et al. A collaborative European exercise on mRNA-based body fluid/skin typing and interpretation of DNA and RNA results*. Forensic Science International: Genetics*. 2014;10:40-48.

4. Allery J, Telmon N, Mieusset R, Blanc A, Rougé D. Cytological detection of spermatozoa: comparison of three staining methods*. Journal of Forensic Science*. 2001;46(2):349-351.

5. Glaister J. The Kastle-Meyer Test for the Detection of Blood: Considered from the Medico-Legal Aspect*. Br Med J*. 1926;1(3406):650.

6. Gutman AB, Gutman EB. “Acid” phosphatase and functional activity of the prostate (man) and preputial glands (rat)*. Proceedings of the Society for Experimental Biology and Medicine*. 1938;39(3):529-532.

7. Haas C, Hanson E, Morling N, Ballantyne J. Collaborative EDNAP exercises on messenger RNA/DNA co-analysis for body fluid identification (blood, saliva, semen) and STR profiling*. Forensic Science International: Genetics Supplement Series*. 2011;3(1):e5-e6.

8. Vidaki A, Giangasparo F, Syndercombe Court D. Discovery of potential DNA methylation markers for forensic tissue identification using bisulphite pyrosequencing*. Electrophoresis*. 2016;37(21):2767-2779.

9. van der Meer, Dieudonné J, Williams GA. Performing body fluid identification with microRNAs using capillary electrophoresis*. Forensic Science International: Genetics Supplement Series*. 2015;5:e592-e594.

10. Omelia EJ, Uchimoto ML, Williams G. Quantitative PCR analysis of blood-and saliva-specific microRNA markers following solid-phase DNA extraction*. Anal Biochem*. 2013;435(2):120-122.

11. Van der Meer D, Uchimoto ML, Williams G. Simultaneous analysis of micro‐RNA and DNA for determining the body fluid origin of DNA profiles*. J Forensic Sci*. 2013;58(4):967-971.

12. Uchimoto ML, Beasley E, Coult N, Omelia EJ, World D, Williams G. Considering the effect of stem-loop reverse transcription and real-time PCR analysis of blood and saliva specific microRNA markers upon mixed body fluid stains*. Forensic Science International: Genetics*. 2013;7(4):418-421.

13. Virkler K, Lednev IK. Analysis of body fluids for forensic purposes: from laboratory testing to non-destructive rapid confirmatory identification at a crime scene*. Forensic Sci Int*. 2009;188(1-3):1-17.

14. Kulstein G, Wiegand P. Comprehensive examination of conventional and innovative body fluid identification approaches and DNA profiling of laundered blood-and saliva-stained pieces of cloths*. Int J Legal Med*. 2018;132(1):67-81.

15. Noël S, Lagacé K, Rogic A, et al. DNA transfer during laundering may yield complete genetic profiles*. Forensic Science International: Genetics*. 2016;23:240-247.

16. Kulstein G, Schacker U, Wiegand P. Old meets new: comparative examination of conventional and innovative RNA-based methods for body fluid identification of laundered seminal fluid stains after modular extraction of DNA and RNA*. Forensic Science International: Genetics*. 2018;36:130-140.

17. Schlagetter TG, Glynn CL. The effect of fabric type and laundering conditions on the detection of semen stains. 2017.

18. Mayes C, Houston R, Seashols-Williams S, LaRue B, Hughes-Stamm S. The stability and persistence of blood and semen mRNA and miRNA targets for body fluid identification in environmentally challenged and laundered samples*. Leg Med*. 2019;38:45-50.