IMPROVED RECOVERY OF SPERM DONOR PROFILES FROM VAGINAL SWABS USING LASER MICRODISSECTION (LMD)

Melanie Richard, Tamara Forde, Al Marignani, John Wilson and Jonathan Millman.

Centre of Forensic Sciences (CFS), Biology Section, Toronto, Ontario, Canada

The CFS uses a direct-to-DNA (DTD) approach for internal and external genitalia swabs, collected in duplicate as part of a sexual assault examination. One swab undergoes differential extraction and STR DNA profiling without prior examination for the presence of semen. This approach has proven to be efficient, cost-effective, and sensitive. However, differential extraction does not always sufficiently separate female donor DNA from spermatozoal DNA to enable the development of a male profile in the sperm fraction. This can be an issue even when many spermatozoa are present; particularly with vaginal swabs due to the abundance of epithelial cells. In order to improve the recovery of sperm DNA profiles from these samples, the CFS evaluated LMD, culminating in the development of a workflow for elution, microscopic identification, dissection and DNA analysis of spermatozoa from vaginal swabs. Samples are eluted using a three step protocol incorporating a passive 2hr soak at 4°C in PBS/Sarkosyl buffer followed by two 1hr incubations at 37®C on a heated shaker in a TNE-SDS-ProK buffer. This method has been found to effectively recover spermatozoa, while minimizing the co-recovery of epithelial cells, which can impede microscopic identification and dissection. The sperm cell pellet is applied to a Polyethylene Napthalate membrane on a glass slide and stained using a modified Haematoxylin-Eosin method. Other staining methods, including the CFS standard Nuclear Fast Red and Picroindigocarmine protocol, generated DNA profiles that appeared to be somewhat degraded or inhibited. Microscopic identification and dissection of spermatozoa is subsequently performed on a Leica LMD7000 system. DNA is isolated using the sperm fraction buffer from a CFS standard differential extraction and amplified in the Identifiler Plus® STR typing system using standard PCR cycling parameters. Following this protocol 15 STR locus profiles can be expected from DNA analysis of 300 dissected spermatozoa. Where fewer sperm are available for dissection, highly discriminating DNA profiles can still be obtained. In a comparative study using post-coital vaginal swabs collected in duplicate from volunteers, the value of LMD was clearly demonstrated: In 3 of the 10 vaginal swabs processed by DTD, a sperm donor profile was not obtained. By contrast, processing the swabs by LMD resulted in complete single source sperm donor profiles in all instances, suitable for upload to the National DNA Databank and, therefore, capable of providing otherwise unobtainable investigative information.