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LETTER TO THE EDITOR

Forensic DNA research: keeping it real

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Introduction

With any research project, a successful outcome is dependant firstly on a proper understanding of the problem you are trying to solve. It pays to think long and hard about the way an experiment should be designed, and in a forensic context, the design should emulate as far as possible the real world scenarios likely to be encountered.

In our experience, the choice of sample used in a research project is critical to the successful implementation of a DNA-based technique into forensic science. Whilst the crude development of an end-to-end process can be performed using a general sample type, it is essential that the optimisation of the process is performed on samples that mimic the real sample type as closely as possible.

A number of sample types might be discussed, including unadulterated blood vs EDTA anticoagulated blood, DNase-degraded DNA samples vs degraded body fluid samples, and post-coital samples vs cellular samples seeded with semen. As an example, we consider the consequences of the sampling decisions we make when developing protocols for the examination of post-coital (PC) samples submitted for DNA analysis.

Post-coital samples

There are numerous reports in the literature of novel or improved methodologies for the differential extraction of sperm and epithelial cell DNA and subsequent analysis, e.g. [1–4], many of them using vaginal or even mouth buccal swabs spiked with known amounts of semen. Whilst it might be more convenient to procure samples of such swabs and load these with semen, rather than collect true PC swabs, we have demonstrated that such mock samples do not behave in the same way as a true PC sample. We have found that the quality of the recovered sperm fraction varies greatly between post-coital samples, many of which result in mixtures made up of both the male and female donors of post-coital swabs (see Fig. 1). In this example, samples were taken 48 h PC and processed using the method described by Gill [5] and using the Differex™ procedure (Promega Inc., Madison, USA). Comparative work was performed on vaginal swabs which had been seeded with 50 μL of 1:50 dilution of semen in water. The samples from the PC set showed a much higher contribution of DNA from the female donor, compared with the seeded set, independent of the method of preferential lysis. Indeed, when cells are isolated by laser capture microdissection, a very selective method of cell separation, it is not uncommon to observe mixtures in the resultant DNA profiles [6, 7]. In previous work using fluorescence-assisted cell sorting [8], we have observed reproducible incidences of ten sorted sperm providing a full single source profile of the female donor with no trace of the male DNA, just 8 h post-coitus. By comparison, mocked-up samples (adding semen to a vaginal or buccal swab) routinely produced full single source male profiles with 34 cycle SGMPlus™.

The vaginal environment is extremely hostile to sperm, and immediately following ejaculation, the sperm are protected by proteins and other molecules, perhaps polyamines and glycosaminoglycans, present in seminal fluid. As these molecules are degraded, the sperm is exposed to the environment which includes female extracellular DNA.

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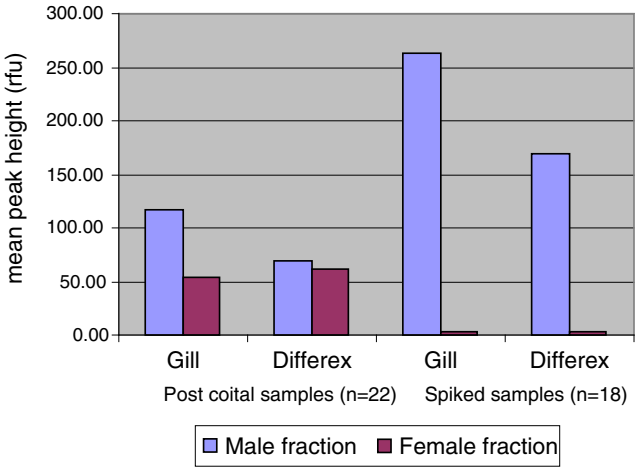

Fig. 1 Preferential lysis was performed on 22 post-coital (t=48 h) samples from different donors and on 18 vaginal swabs (six from each of three donors) seeded with 50 μL 1:50 semen diluted in water. Preferential lysis was performed in accordance with Gill et al. [5] or using the Differex™ kit in accordance with the manufacturer's directions

It is likely that extracellular DNA can bind to the sperm head via a class of 30- to 35-kDa DNA-binding proteins [9] and MHC class II proteins [10]. Studies have also demonstrated that extracellular DNA can be internalised into the sperm nucleus [11], a process which begins within minutes of DNA binding [12]. Exposure to foreign DNA also activates nucleases within the cell which catalyse a localised degradation of sperm DNA, eventually leading to cell death [13]. A further challenge to the sperm in the vaginal tract is via phagocytosis by neutrophils [14]. The phagocytotic process involved in the complete digestion of a relatively resistant spermatozoon may result in the cytolysis of the fragile neutrophil with the release of partially digested sperm into the extracellular fluid. Multiple episodes of phagocytosis of the same sperm are possible.

In our experience, post-coital samples are more likely to result in a mixed DNA profile constituting alleles from both sexual contributors, than swabs of buccal or vaginal cells spiked with semen, owing to the complex biochemical processes which take place in the vagina post-coitus.

Whilst mocked samples can be useful to evaluate the crude performance of a process requiring preferential extraction of spermatozoa, it is likely that any method devised using only seeded samples will perform less well when real post-coital samples are encountered. We would expect to see a higher incidence of mixed DNA samples from real samples which might impact on the success rates of the DNA process in terms of DNA profile quality and timeliness (requirement for mixture analysis and/or rework) and indeed might result in the requirement to further optimise and revalidate the laboratory process.

Sample types approximating the samples for forensic analysis might be an appropriate starting place for the development of a technique, but it is imperative that the assay should then be fully evaluated, optimised and validated using samples that emulate the forensic submission if the system is to be properly optimised and characterised for a casework sample.

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