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Forensic Techniques for the Isolation of Spermatozoa from Sexual Assault Samples — A Review

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Forensic Techniques for the Isolation of Spermatozoa from Sexual Assault Samples — A Review

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ABSTRACT: The challenge of profiling spermatozoa from samples containing a mixture of male and female cells has been extensively discussed within the forensic community. Various techniques have been developed for the analysis of sexual assault evidence with the aim to generate a single-source male DNA profile. Multiple methods practiced for the isolation of the male component are discussed in this review, with a focus on differential extraction. Benefits of alterations that have been made to the original differential method to increase the efficiency are highlighted. Although improvements were achieved, it is ascertained by this review that these methods are limited in their overall success rate or their applicability. Perhaps future approaches and research should concentrate on more efficient, cost-effective, and time-saving techniques to individually sort or isolate spermatozoa.

KEYWORDS: Differential extraction, DNA analysis, forensic, sexual assault, spermatozoa.

INTRODUCTION

The term "sexual assault" refers to any unwanted, coerced, or forced sexual contact that occurs between two or more people, without the victim's consent [92]. Due to the severe physical and mental trauma that sexual assault victims suffer, society mandates that the perpetrators of such crimes be arrested and prosecuted within the law enforcement and justice systems [14,31,32,78,92]. In order to link an accused offender to a crime by DNA evidence, three primary sample types are collected: Samples from the crime scene, samples from the victim`s body, and reference samples from the suspect(s) and victim for comparison purposes. This review will focus on evidence collected from the victim`s body.

When a victim visits a hospital or sexual assault clinic for medical care and forensic evidence collection, swabs of different body parts are taken during the examination [73,115]. The acquisition of vaginal, anal, and oral swabs is prioritized, as the presence of semen on these swabs provides substantial evidence that sexual contact has occurred [1,7,39,58,75,91]. Nevertheless, swabs of other body areas such as the neck or the breast are also potentially helpful to the investigation owing to deposited buccal cells of the perpetrator after kissing, licking, or biting [6,99]. If a fight or resistance has occurred, the DNA profile of the assailant might also be obtainable by collecting and analyzing accumulated epithelial cells from the victim`s fingernails [111].

For verifying the presence of semen on sexual assault swabs, commercially available immunochromatographic assays are routinely used as presumptive tests. In the case of a positive result, dye-conjugated antibodies lead to a colored test line on the strip after binding a semen-specific protein such as semenogelin (e.g., RSID™-Semen test) or the prostate-specific antigen (e.g., ABAcard® p30 test) [13,84]. In order to confirm the result through the visualization of spermatozoa, a portion of the sample is transferred to a microscope slide, the cells are histologically stained, and the slides are searched for spermatozoa [87]. Once the presence of semen and/or sperm is verified, DNA can be extracted from the swabs and undergo STR profiling to assist with the identification of the perpetrator [20].

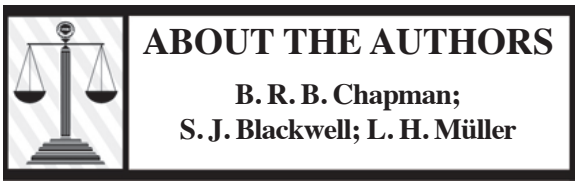
In intimate swab samples, a cellular mixture is inevitably collected, with a high proportion of the female victim's material along with deposited male cells from the offender. Before performing DNA profiling, ideally, the male cellular fraction should be isolated from this mixture. Failure to separate the male fraction may result in one of two outcomes:

- There will be a greater detection of the female DNA whereby no male alleles can be observed [112]; or
- A mixed male/female DNA profile will be generated, making interpretation difficult [108].

These outcomes are a result of the significant excess of female epithelial cells in the sample and a comparatively small number of male cells.

Over the years, diverse techniques have been developed in order to isolate spermatozoa from the cell mixture; an overview including laser-based, affinity-based, and microdevice-based approaches is provided herein. The primary method used by contemporary forensic DNA laboratories to separate the male component of sexual assault samples is differential cell lysis. Both the original technique and novel differential lysis approaches will be reviewed in detail. In particular, the limitations are highlighted in order to establish a case for further research.

zerland; 2003.
116. Xu K, Clark CP, Poe BL, Lounsbury JA, Nilsson J, Laurell T, Landers JP: Isolation of a low number of sperm cells from female DNA in a glass-PDMS-glass microchip via bead-assisted acoustic differential extraction; *Anal Chem* 91:2186; 2019.
117. Xu Y, Xie J, Chen R, Cao Y, Ping Y, Xu Q, Hu W, Wu D, Gu L, Zhou H, et al.: Fluorescence- and magnetic-activated cell sorting strategies to separate spermatozoa involving plural contributors from biological mixtures for human identification; *Sci Rep* 6:36515; 2016.
118. Yakoo MN: The Development and Optimization of a Direct Lysis Differential Extraction Method (master's thesis); Boston University School of Medicine: Boston, MA; 2017; *https://hdl.handle.net/2144/26954* (Accessed April 25, 2020).
119. Yoshida K, Sekiguchi K, Mizuno N, Kasai K, Sakai I, Sato H, Seta S: The modified method of two-step differential extraction of sperm and vaginal epithelial cell DNA from vaginal fluid mixed with semen; *Forensic Sci Int* 72:25; 1995.
120. Zhao X-C, Le Wang, Sun J, Jiang B-W, Zhang E-L, Ye J: Isolating sperm from cell mixtures using magnetic beads coupled with an anti-PH-20 antibody for forensic DNA analysis; *PLoS ONE* 11:e0159401; 2016.
121. Ziegler AD: Modification of a Novel Temperature Controlled Differential Extraction Procedure for Better Application in Forensic Casework (master's thesis); Boston University: Boston, MA; 2019; *https://hdl.handle.net/2144/38734* (Accessed June 23, 2020).



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Brendan Chapman received a BSc (Hons) in molecular biology from Murdoch University (Western Australia, Australia). He is currently a senior lecturer in forensic science at Murdoch University and the academic chair of the University's Postgraduate Forensic Science Coursework.

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