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

B. R. B. Chapman1*, S. J. Blackwell1,2, L. H. Müller1

1 Medical, Molecular, and Forensic Sciences
   Murdoch University
   Perth, Western Australia
   Australia

2 Forensic Biology Department
   PathWest Laboratory Medicine WA
   Perth, Western Australia
   Australia

* Corresponding author: Mr. Brendan Chapman, Medical, Molecular, and Forensic Sciences, Murdoch University, Perth, Western Australia, Australia; +61 8 9360 2270 (voice); brendan.chapman@murdoch.edu.au.

TABLE OF CONTENTS

INTRODUCTION ................................................................. 106

I. DIFFERENTIAL EXTRACTION: PRIMARY METHOD FOR ISOLATION AND LYSIS OF SPERMATOZOA ......................................................... 107
   A. Modifications to Traditional Differential-Extraction Method .......... 107
   B. Challenges of Differential Extraction and of Method Comparison .... 110

II. OTHER METHODS FOR ISOLATING SPERMATOZOA ....................................... 110
   A. Laser-Based Methods ................................................... 111
   B. Microdevices ............................................................. 111
   C. Affinity-Based Methods .................................................. 111

CONCLUSIONS ................................................................. 112
REFERENCES ................................................................. 112
ABOUT THE AUTHORS ...................................................... 116
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 a 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.
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.

Having worked for over 10 years in Australian government departments in both a forensic DNA laboratory and with law enforcement at crime scenes, Mr. Chapman has attended thousands of major crime scenes and was involved in countless investigations and body recovery activities associated with homicides in Australia prior to his appointment as an academic. His expertise and research interests lie in forensic genetics and DNA, homicide and sex crime investigation, clandestine gravesite recovery, and cold-case reviews. Mr. Chapman has published 12 peer-reviewed articles since 2018 along with numerous conference proceedings and has supervised over 35 master’s degree completions. He is a member of the International Society of Forensic Genetics and Australian and New Zealand Forensic Science Society and represents Murdoch University at various national meetings with regards to the future of forensic science.

Shannen Blackwell received her BSc in molecular biology/biomedical science, BSc in forensics, and MSc of forensic science (professional practice) from Murdoch University. Since graduating from the master’s degree program in 2017, she has been employed as a technical assistant at PathWest Laboratory Medicine (Nedlands, Western Australia, Australia), within the Forensic Biology Department. Her main research interests lie in forensic DNA analysis, sexual assault sample collection and processing, the forensic application of cell isolation techniques, and future forensic techniques. She is a member of the Australian and New Zealand Forensic Science Society.

Linda Helena Müller completed her bachelor’s degree studies in molecular biomedicine at the Rheinische Friedrich-Wilhelms-University (Bonn, Germany), with specialization in the field of genetics and genomics. Having performed her thesis at the Institute for Legal Medicine of Bonn, Linda gained more profound knowledge about forensic genetics during that time period. After graduation, she expanded her practical experience in the area of forensic science at Murdoch University, in terms of a three-month internship. Further, she plans to enroll in a master’s degree program focused on genetics in Scandinavia.