



Short report

Comparative study of spermatozoa detection using the genital swab versus bedside smear slide technique in sexual assault patients



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ABSTRACT

Background: Specimen collection from sexual assault victims is an essential part of practice in forensic medicine. Semen analysis is a forensic test used to confirm sexual contact. Two methods of specimen collection were compared in this study: the genital swab and bedside slide smear.

Materials and methods: A retrospective chart review was performed in the Division of Clinical Forensic Medicine, Siriraj Hospital, Thailand. Data were collected on microscopic spermatozoa detection in the external and internal genitalia, the time interval from assault to visit, and the history of condom use and genital cleansing. McNemar's test was used for comparison between the genital swab and bedside slide smear techniques.

Results: In total, 724 case files from 2009 to 2013 were reviewed. The genital swab technique yielded a higher detection rate than did the bedside slide smear technique in both the external genitalia (37.0% vs. 31.8%, respectively) and internal genitalia (40.8% vs. 34.1%, respectively). Collection of a genital swab only missed positive cases in 4%–6% of negative swabs. When considering both the external and internal genitalia, the genital swab still yielded a higher rate of positive results than did the slide smear (45.9% vs. 39.0%, respectively). Examination of the victim within the first 3 days produced the highest spermatozoa detection rate (46.3%).

Conclusions: The genital swab is the preferred specimen collection method for spermatozoa detection in adult sexual assault victims. However, collection of both a swab and slide specimen may be considered in some cases. Collection of specimens later than 3 days after the assault greatly decreases the rate of spermatozoa detection.

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1. Introduction

Sexual assault against women and minors is a worldwide problem. In addition to medical treatment, specimen collection from an alleged sexual assault victim is an essential part of practice in clinical forensic medicine. The legal definition of rape or sexual assault varies among jurisdictions. The definition usually has two essential components necessary to make a conviction. One is sexual intercourse through a body orifice, and the other is that the sexual intercourse was nonconsensual or against the victim's will. The attending physician may be asked to give an opinion or testify during a criminal trial regarding whether the alleged act has occurred. Forensic physicians have a pivotal role in specimen

collection in institutions in which such physicians are available. Gynecologists, emergency physicians, or pediatricians may also be involved in the specimen collection process during emergency care. Retrieval of forensic specimens seems to have an influence on the charges filed.^{1,2}

Semen analysis is the most common forensic laboratory test performed to prove the occurrence of alleged sexual intercourse. Semen can be divided into two components: the protein-rich seminal fluid and the spermatozoa. Seminal fluid testing can be performed by protein detection methods using acid phosphatase, choline, spermine, leucine aminopeptidase, and other agents.³ One milliliter of seminal fluid contains 20 to 50 million spermatozoa (normal volume = 2–5 mL).⁴ Unlike reproductive evaluation, forensic spermatozoa testing warrants only determination of the presence or absence of spermatozoa. This can be accomplished by microscopic examination of the specimen. Because spermatozoa

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are only present in semen, detection of spermatozoa is a confirmatory test and indicates the presence of ejaculation at the collection site.

Microscopic examination of spermatozoa requires a smear slide and various staining methods. Very few detailed guidelines on specimen collection have been written. An Australian guideline recommends sterile gauze maybe swabbed from vulvar area and sent to local police for forensic test,⁵ whereas a guideline from Canada recommends that the physician collects both a vaginal swab and slide.⁶ Thailand currently has no national recommendation on how to collect forensic specimens from sexual assault victims. The authors' hypothesis is that genital swabs may yield more positive results and that there is no need to collect bedside smear slides because the laboratory extracts the swab and prepares a smear after the extraction. This practice would decrease the collection procedure and examination cost.

Therefore, the objectives of this study were to (1) compare the spermatozoa detection rates between two specimen collection methods, namely the genital swab and bedside slide smear techniques, and (2) estimate the spermatozoa detection rate in the bedside slide smear technique in which a genital swab yields a negative result (missing rate if a genital swab is collected only).

2. Material and methods

2.1. Study design

This study was a cross-sectional retrospective chart review. The data collection process was approved by the Institutional Review Board of the Faculty of Medicine Siriraj Hospital.

2.2. Population

The medical records of sexual assault victims in the Division of Clinical Forensic Medicine were reviewed. Inclusion criteria were an age of >18 years and the collection of both a vaginal swab and bedside slide smear. Thus, spermatozoa detection result was retrieved from slide smeared in the field (bedside slide smear) and the slide smeared in the lab (vaginal swab). Patients who visited the hospital later than 14 days after the assault were excluded.

The data collected from the medical records included the results of both the vaginal swab and bedside slide smear. The sites of specimen collection were divided into two groups: the external and internal genitalia. Thus, each case comprised four spermatozoa detection results: external genital swab, external genital slide smear, internal genital swab, and internal genital slide smear. Other data collected included the acid phosphatase test results, time interval from assault date to examination date, history of condom use, and history of genital cleansing.

2.3. Sample size calculation

The sample size was calculated by nQuery Advisor[®] (Statistical Solutions Ltd., Boston, MA, USA). The medical files of 40 sexual assault victims revealed a spermatozoa detection rate of 35%. The authors expected a 5% increase in the detection rate to be of clinical significance (difference in proportion = 0.05). McNemar's test of equality of paired proportions with a two-tailed significance level of 0.05 and 80% power revealed a calculated sample size of 192.

The expected proportion of negative swabs but positive slides that would be small enough to cancel the need for bedside slide smear collection was 1%. If the confidence level was set at 95%, the authors would need 381 negative bedside slide smears to prove the hypothesis. The chance of finding a negative result was estimated to be 50%. Thus, the authors required 760 paired samples.

2.4. Statistical analysis

Statistical analyses were performed using PASW[®] Statistics 18.0 (SPSS Inc., Chicago, IL, USA) and STATA[®] version 13.1 (StataCorp LP, College Station, TX, USA). Categorical data were collected as percentages and later analyzed by McNemar's test for paired data and Pearson's chi-squared test for independent variables.

2.5. Swab extraction technique

Phosphate buffered saline was used as the preferred extraction method for genital swabs. A part of the specimen was nicked with a scalpel and placed in a 1.5-ml microtube. Phosphate buffered saline was later used to fill the microtube and cover the specimen. The specimen was left incubated in a water bath at 37 °C overnight. After incubation, the 1.5-ml tube was pinched with an 18-gauge needle and placed in a 2.0-ml microtube. The tubes were centrifuged at 8000 rounds/min for 1 min and then 14,000 rounds/min for 1 min. An auto-pipette was used for aspiration and dispersion of the sample onto a slide. The slide underwent hematoxylin and eosin staining for spermatozoa detection by a forensic scientist in the Forensic Evidence Laboratory. For the bedside slide smear, the specimen went directly to the staining process.

3. Results

3.1. General data

In total, 724 sexual assault victims during a 5-year period (2009–2013) were included in this study. The rates of seminal fluid detection by each method varied from 30% to 40%. Condom use was reported in 10.2% of cases, no condom use was reported in 59.8% of cases, and an unknown history was reported in 30.0% of cases. The patients reported self-cleansing after the incident in 27.1% of cases, no cleansing of the genital area in 63.1% of cases, and an unknown history of cleansing in 9.8% of cases.

3.2. Comparison of genital swab and bedside slide smear techniques

When comparing the spermatozoa detection rate of the bedside slide smear and genital swab techniques using a 2 × 2 square crosstab and McNemar's test, the genital swab technique detected significantly more spermatozoa at both specimen sites (p < 0.01). Tables 1 and 2 show the results of specimens from the external genitalia and internal genitalia, respectively.

3.3. Comparison of specimens and genital swabs only

The authors compared the sperm detection rate between the genital swab and specimen techniques to determine the usefulness of each technique in clinical practice. Because tests for forensic purposes only require the presence of spermatozoa, the result during a criminal trial is considered positive whether it is detected

Table 1
Comparison of spermatozoa detection rates between external genital swab and external genital slide smear (p < 0.01).

	External genital slide smear		Total swab count
	Positive	Negative	
External genital swab Positive	199	69	268 (37.0%)
Negative	31	425	456 (63.0%)
Total slide count	230 (31.8%)	494 (68.2%)	724 (100.0%)

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