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A pilot study: The effects of repeat washing and fabric type on the detection of seminal fluid and spermatozoa

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ABSTRACT

In sexual assault cases and more specifically those involving childhood sexual abuse (CSA), victims may have had their potentially semen-stained clothing washed multiple times before a criminal investigation commences. Although it has been previously demonstrated that spermatozoa persist on cotton clothing following a single wash cycle, items of clothing washed multiple times are not routinely examined in these cases because of the assumption that the laundering process would have removed all seminal fluid and spermatozoa. The aim of this study was to examine the persistence of seminal fluid and spermatozoa on a range of fabric types including cotton, nylon, terry towel (100% cotton), polyester fleece, satin and lace which were laundered up to six times. Three techniques were used for the detection of seminal fluid and spermatozoa: an alternative light source, acid phosphatase test and microscopy. The study demonstrated that spermatozoa persisted on cotton and terry towel following six wash cycles. This data emphasises the need to recover and examine items of clothing and bedding of victims for semen, even if the item has been washed multiple times.

1. Introduction

In 2004, childhood sexual abuse (CSA) was declared a "silent health emergency" by the World Health Organisation (WHO) [1]. The long term negative effects of CSA have been found to be extensive, including increased risk for violence, depression and suicide [2]. A 2010 Australian study, surveying 1745 adolescents (812 males and 933 females) found 17% of females and 7% of males were identified as having some type of unwanted sexual contact (touching, fondling, kissing, display of sex organs, etc.) prior to age sixteen [1].

Studies have found that children involved in sexual abuse rarely acknowledge their own victimisation for several reasons; this could include fear, shame and normalisation of sexual behaviours. In such cases, extended periods of time would separate the alleged offence and police investigations, further complicating forensic analyses [3].

Despite the knowledge of potential health risks and the forensic significance of semen, a multi-perpetrator rape study identified that only 20% of offenders wore a condom during a sexual attack [4]. Similarly, a study of child sex trafficking (CST) cases found that offenders did not commonly use condoms but would instead ejaculate directly onto the body, clothing or bedding of the victim [3]. It was identified that in some CST cases, victims hid their clothes for extended periods of time before washing, or washed items of clothing multiple times to remove any visible stains in order to avoid having to discuss the assault with a parent or carer [3].

A small number of studies have demonstrated that seminal fluid is undetectable using the acid phosphatase (AP) test or alternative light sources (ALS) when items of clothing have been laundered in a washing machine, using various detergents and washing protocols [5–8]. Other studies have demonstrated that spermatozoa continue to persist following washing [9–11]. Interestingly however, none of these studies have investigated the persistence of seminal fluid and spermatozoa beyond one wash cycle or on a fabric type other than cotton, simulating circumstances more commonly encountered in CSA and CST cases. Therefore, the aim of this study was to examine the persistence of seminal fluid and spermatozoa on a range of fabrics types including cotton, nylon, terry towel, polar fleece, satin and lace that were laundered up to six times by utilising an alternative light source, AP test and microscopy.

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2. Materials and methods

2.1. Preparation of stains

Seminal fluid was collected from one healthy 29-year-old male, over a two-week period, stored at 4 °C and used as a homogenous stock. The stock was evaluated for sperm count in accordance with WHO guidelines for the routine counting of spermatozoa [12] in order to establish that it was a representative sample of the normal male population. Fabric samples were chosen to best provide a range of samples encountered in casework. For experiments whereby ALS was utilised, black fabric was selected in order to limit the effect of fabric autofluorescence and false positive results. A mixed 1 mL sample of seminal fluid was deposited onto a range of coloured fabric test materials including cotton, lace, polar fleece, satin, nylon and terry towel; this was done in duplicate for all fabric types and labelled A and B. Control samples (unwashed seminal fluid stains) were also made in duplicate and stored in paper bags for the duration of the trial.

In preliminary trials, it was noted that the different fabrics influenced the manner of seminal fluid spread due to various absorptivity and wicking rates. To accommodate this, for stains made on multi-coloured fabrics, the stained area was encircled using a black marker and divided into six equal parts for microscopy sampling to ensure that sampling was uniform. Stains made on black fabrics were encircled using a yellow wax pencil. The entire spread of the seminal fluid was marked as best as possible given the difficulty in evaluating it visually against the black background. For this reason, any attempt at dividing the stain into equal portions for microscopy would have been error-prone and therefore the black fabric swatches were used for AP and ALS testing only and not microscopy. The seminal fluid stain was air-dried for 12 h at room temperature before being stored in paper bags until examination.

2.2. Observation of fluorescence using a light source

The light source employed was the Polilight-Flare® II Plus (Rofin, Australia). The Polilight-Flare® II Plus intensity and beam profile of the light can be changed to suit the application, making it ideal for the examination of crime scenes and exhibits [13]. Only stains deposited on the black control and test fabric swatches were visualised at a wavelength of 415 nm and 450 nm and observed with yellow and orange filters, respectively; this was to avoid any false positives due to background fluorescence of the lighter coloured fabrics [6]. Fluorescence was recorded on an arbitrary scale as either strong positive (++), weak positive (+) or negative (−).

2.3. Acid phosphatase test

A one litre solution of acid phosphatase was made by dissolving 10 mL of glacial acetic acid, 20 g sodium acetate, 2 g sodium 1-napthyl phosphate and 4 g fast black practical grade in distilled water. The solution was refrigerated overnight, filtered and then adjusted with concentrated sodium hydroxide to pH 5. Each black coloured control and test fabric sample was swabbed with a cotton swab previously moistened using distilled water and the acid phosphatase solution dropped directly onto the swab. Multi-coloured fabrics were not used to avoid any chance of further dilution of the sample before microscopy. Results were recorded using a non-linear scale ranging from '+++' to '−' (Table 1).

Table 1
Scoring method for acid phosphatase test.

Cut-off	Result	Score
30 s	Strong positive	+++
2 min	Positive	++
10 min	Weak positive	+
10+min	Negative/positive result after 10-min cut-off	–

2.4. Detection of spermatozoa through microscopy

Each control (unwashed) and test multi-coloured sample was swabbed with a moistened cotton swab and microscopy slides prepared with Christmas tree stain in order to visually identify any cellular materials, including spermatozoa, associated with the stain. The Christmas tree stain is a reliable confirmatory visual test for the the presence of semen and is based on the differential staining ability of the sperm head and tail using nuclear fast red and picroindigo-carmine, yielding a crimson colour to the head and green-blue-grey colour to the tail. Nuclear fast red was commercially procured (Sigma Aldrich) and picroindigocarmine was prepared by dissolving 40 g picric acid and 10 g indigo carmine in distilled water. In addition to slides prepared from the swabs, spermatozoa were isolated from the multi-coloured samples by excising a portion of the stain and vortexing it in 150 μL of distilled water for 120 s; portion sizes differed depending on the spread of the seminal fluid on the fabric during initial deposition. The fabric was then removed and the sample was centrifuged at 14,000 rpm for three minutes. 2 μL of the cell pellet was then transferred to 1–1.2 mm thick clear glass/frosted end microscopic slides which were then prepared with a Christmas tree stain [14]. Spermatozoa were identified based on morphological and staining characteristics (green tail and a red head with light pink cap). Sperm density was recorded using a non-linear scale ranging from 'few' to '+4' (Table 2). Each rating referred to the number of spermatozoa identified per microscopic field of view (FOV) at 400× magnification on an Olympus BX51 compound microscope fitted with an Olympus DP70 camera. Fields of view that contained spermatozoa with a tail were recorded as 'T'.

2.5. Effects of repeat washing

The semen stained fabrics (all except control samples) were washed independently in a non-biological (OMO-Sensitive) detergent using a domestic LG Inverter Direct Drive 9.5 kg top loader washing machine. The washing machine programme included a 15-min wash cycle, 15-min rinse cycle and 12-min spin cycle in cold water (20 °C). Additional untreated items such as pants, tops, socks and tea-towels were added in duplicate to simulate a normal washing load. Once washed, samples were air-dried on a clothes airer at room temperature overnight. The laundered fabrics were then placed individually into brown paper bags and stored at room temperature until further examinations. Samples were washed, stored and examined up to six times, if seminal fluid or spermatozoa continued to be detected.

3. Results and discussion

3.1. Observation of fluorescence using a light source

In 1991, Stoilovic demonstrated that the excitation spectrum of semen was broad and that the fluorescence could be generated with wavelengths ranging from 350–500 nm. It was demonstrated that the detection of seminal stains was based on their

Table 2
Scoring method for microscopy.

Spermatozoa number	Score
Less than 5 spermatozoa per slide	Few
1 spermatozoa in some fields	+1
1–5 spermatozoa in most fields	+2
5–10 spermatozoa in most fields	+3
10+ spermatozoa per field	+4