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ScienceDirect
Science and Justice 47 (2007) 160–167
science&justice
www.elsevier.com/locate/scijus

A comparison of methods used in the UK and Ireland for the extraction and detection of semen on swabs and cloth samples

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Accepted 26 September 2007

Abstract

The recent formation of a United Kingdom and Irish working group, the Body Fluids Forum (BFF), highlighted the need to investigate different working practices prior to any inter-laboratory comparison work and identification of best practice. Various dilutions of semen were seeded onto swabs and cloth samples for each BFF member laboratory to test using their standard techniques. The results showed that the detection of acid phosphatase on swabs is best achieved using direct testing rather than on an extract from the swab. Extraction methods for spermatozoa require a balance to be achieved between using a sufficient volume of water to ensure optimal release and minimal volume to ensure a concentrated extract. PSA tests were investigated and found to be more sensitive than Choline. DNA profiles were obtained from samples in which no spermatozoa had been detected during microscopic examination.

Keywords: Forensic science; Semen; Extraction; Swab; Cloth; Body Fluid Forum

1. Introduction

During the investigation of sexual offences, intimate body swabs, clothing and bedding items are routinely submitted for examination by forensic scientists. The detection of semen on such items and the subsequent use of DNA profiling tests are often of vital importance, together with an evaluation as to the significance of the findings. For example, a suspect may accept that his semen is present on a complainant but dispute that it was deposited during sexual intercourse.

The recent formation of a United Kingdom and Irish working group, *the Body Fluids Forum* (BFF), highlighted that different working practices exist in different organisations for semen extraction and identification and that these needed to be investigated before any inter-laboratory comparisons could be pursued. It was anticipated that guidelines regarding best practice would be proposed as a result of this work. The existence of different methods has arisen due to local methods being set up and amended to meet local needs. For example, a laboratory whose customers rarely require DNA profiling tests will need a quick and simple method that enables the detection and identification of semen rather than a

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doi:10.1016/j.scijus.2007.09.010

Table 1
Swab extraction methods

Lab	1	2	3	4	5	6	7	8	9	9
Extraction method	Direct	Extract	Direct	Direct	Extract	Extract	Extract	Extract	Direct	Extract
Amount swab/volume water	Tip in 50 µl	Whole in 1000 µl	Half with 25 µl	20% with 40 µl on slide	Whole in 200 µl	Whole in 400 µl	Whole in 300 µl	Whole in 50 µl then add 100 µl		Whole in 400 µl
Method	Macerate on slide	Soak 60 s, agitate, vortex to produce extract. If weak, extract may be pelleted or swab spinaroo'd.	Leave 60 s, agitate, pipette off 5 µl liquid from swab.	Palpate on slide, squeeze out and wipe swab on paper for AP test.	Agitate vigorously for 2–5 min, vortex, spinaroo, resuspend pellet in 25 µl.	Swab placed in Dolphin filter tube with 200 µl, pummel, repeat with further 200 µl, spin, resuspend pellet in min volume.	Agitate vigorously for 2–5 min, vortex, spinaroo, resuspend pellet in 25 µl	Filter tube, agitate, leave 20 min, spin, remove filter, vortex, 5 µl used for slide	Roll damp swab onto slide	Differential extraction in 450 µl with 50 µl ProK, vortex, incubate, spinaroo, spin, resuspend pellet in 350 µl with ProK, spin, resuspend in 50 µl
Spin speed/time		13,000 g for 5 min if required			11,000 rpm for 5 min	14,000 rpm for 1 min	11,000 rpm for 5 min	8000 rpm for 3 min		13,000 rpm for 4 min twice
Spinaroo		As required 9000 g for 5 min			All	Filter tube	All	Filter tube		With differential extraction
Volume of extract/ resuspended pellet used for slide µl (%)		5 (0.5%)	5 (20%)		2 (10%)	(10%)	2 (10%)	5 (3%)		5 (10%)

more involved extraction to produce a sample for submission for PCR.

The primary methods used for the identification of semen in the participating laboratories are the Acid Phosphatase (AP) Brentamine test [1] and the microscopic detection of spermatozoa using Haematoxylin and Eosin stains [2]. Additional tests such as Choline [3,4], Laurell Rocket electrophoresis [5] and Prostate Specific Antigen (PSA) [6,7 and personal communication Norton] are occasionally used in some laboratories when oligospermic or azoospermic semen are suspected. All participants utilised SGMplus®, the Second Generation Multiplex system, for DNA profiling [8–10].

2. Materials and methods

Nine laboratories were each supplied with a set of eleven swabs and six pieces of cloth stained with various concentrations of semen from neat to 1 in 10 000 (dilutions were made in

Table 2
Cloth extraction methods

Lab	1	2	3	4	5	6	7	8	9
Amount fabric used	3 mm^2	5 mm^2	5 mm^2	5 mm^2	5 mm^2	All of fabric extracted	10 mm^2	10 mm^2	3 mm^2
Volume water (µl)	50	300	25	5	200	200	250	100	10
Soak (room temperature)	10 min	Minimum 60 s	Up to 30 min	30 s	No	No	No	20 min	No
Method	Macerate on slide, spin cloth to remove excess and add to slide	Agitate, vortex	Agitate, pipette off 5 µl for slide	Palpate on slide	Macerate/ vortex/spin	Macerate/spin and put supernatant back through sample and repeat	Macerate/ vortex/spin	Macerate/ spin using filter tube	Macerate in tube
Spin speed/ time	14,000 rpm for 30 s	N/A	N/A	N/A	11,000 rpm for 5 min	14,000 rpm for 60 s	11,000 rpm for 5 min	8000 rpm for 3 min	N/A
Pellet resuspended in water (µl)	N/A	N/A	N/A	N/A	25–50	Approximately 10–20	50	Resuspend in original volume	N/A
Volume extract used for slide	All pellet	5 µl	5 µl	Excess fluid	2 µl	2 µl of resuspended pellet	2.5 µl	5 µl	5 µl