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Improved detection of semen by use of direct acid phosphatase testing ☆

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ABSTRACT

Acid phosphatase (AP) reagent (Fast Black) is used as a presumptive test for the presence of seminal fluid on exhibits submitted in allegations of sexual assault. Research was carried out to determine whether the direct application of AP reagent to exhibits is a viable alternative to the traditional indirect (blot) testing method used routinely in the laboratory. The relative sensitivity of the indirect and direct testing methods was investigated as was the effect of AP reagent on histological staining of spermatozoa, the incidence of false positives from vaginal material and saliva, and the effect of AP reagent on subsequent DNA testing. Also included are the results of specificity studies from validations of the direct AP testing method. The results of this research show that, provided the incidence of false positives is borne in mind, direct AP testing can be especially useful when screening exhibits which are difficult to indirectly (blot) AP test or when it is problematic to relocate an AP positive stain. Direct application of AP reagent can also be beneficial for locating dilute semen stains. Three case examples are given which illustrate the use of direct AP testing in laboratory casework.

1. Introduction

The location and identification of semen stains is a fundamental requirement for the forensic examination of items in allegations of sexual assault. Acid phosphatase (AP) is a water-soluble enzyme generally found in high concentrations in seminal fluid [1]. The identification of AP provides a quick and straightforward means to locate possible semen stains. The indirect AP Brentamine-based test is routinely used to screen items for the presence of semen. The indirect (blot) screening involves pressing dampened filter/blotting paper onto the surface of the item to transfer a proportion of any seminal fluid present to the paper. The paper is then tested with the chemical reagent, which changes colour from orange to purple in the presence of acid phosphatase [2–5]. This colour change is due to hydrolysis of the α-napthyl phosphate to produce α-napthol, which couples with a Brentamine Fast Black salt, resulting in a purple azo dye. This colour reaction develops with time and the time taken for the colour to develop as well as the colour of any reaction are key, along with the alleged case circumstances, in determining whether or not the colour change could be due to the presence of semen. The AP test is a presumptive test for semen as other substances are known to give positive purple or other coloured reactions. As this test is not specific for semen [4], the presence of semen is then confirmed by microscopic identification of spermatozoa or by use of a chemical test, such as the Florence Iodine test [6], if interpreted in conjunction with the AP test result.

The Association of Forensic Service Providers Body Fluid Forum (AFSP BFF) commissioned research and compiled data from member organisations to determine the effect of directly applying AP reagent onto the surface of an item rather than using the indirect method. Research commissioned by the AFSP BFF was carried out at LGC Forensics (LGC) and Cellmark Forensic Services (CFS). This work included comparing the sensitivity of indirect and direct AP testing, the effect of the presence of AP reagent on histological staining of spermatozoa and on the incidence of obtaining false positive AP reactions from vaginal material. It also included an initial investigation into the effect of AP reagent on subsequent DNA testing.

Further work, compiled by the AFSP BFF and carried out at CFS, determined the specificity of indirect and direct AP testing using different body fluids, various household products and lubricants. CFS and the Forensic Science Service (FSS) also provided data arising from further studies, including looking into the effect of AP reagent on subsequent DNA testing.

☆ Research commissioned and data compiled by the Association of Forensic Service Providers Body Fluid Forum (AFSP BFF). This paper details the results of research and validations carried out over a number of years and in different organisations.

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Table 1
Extraction methods.

Extraction protocol	Pre centrifugation	Spinaroo	Spin basket	Centrifugation speed	Post centrifugation
A Stains on fabric	Fabric placed in 1.5 ml screw topped tube with 250–300 μl of deionised water. Manually agitate for 2 min and vortex for 1–2 min.	✓	–	11,000g 5 min	Supernatant removed without disturbing the pellet and the pellet re-suspended in 25 μl of supernatant. 2.5 μl of re-suspended pellet used to prepare a slide.
B Swabs	As protocol A with 200 μl deionised water	✓	–	11,000g 5 min	Supernatant removed without disturbing the pellet
C Swabs or stains on fabric	Fabric placed in 1.5 ml screw topped tube with 200 μl of deionised water. Manually agitate for 2–5 min and vortex for 2 min.	–	✓	11,000g 5 min	Supernatant removed without disturbing the pellet
D Swabs or stains on fabric — creating epithelial supernatant used for presumptive tests [10]	Place the swab into a 2 ml screw topped tube with 750 μl of Mo-Lite buffer. Vortex for 20 s and pulse centrifuge. Incubate the tube(s) at room temperature for 30 min. Then vortex for 20 s and pulse centrifuge to remove droplets from the tube lid. Transfer the swab in a spin-basket and place basket in the tube. Centrifuge.	–	✓	13,200g 5 min	Epithelial supernatant removed without disturbing the epithelial pellet
E Swabs or stains on fabric — creating seminal pellet [10]	After creating the epithelial pellet and supernatant using Protocol D, place the swab head in 2 ml screw top tube with 780 μl Mo-Classic buffer. Vortex 20 s and pulse centrifuge. Incubate at room temperature for 2 h. After 1 h of incubation vortex for 20 s and pulse centrifuge. Following 2 h incubation sonicate the tube(s) for 5 min. Then vortex the ISP tube(s) for 20 s and pulse centrifuge. Transfer the swab in a spin-basket and place basket in the tube. Centrifuge.	–	✓	13,200g 5 min	Remove the spin-basket and place swab head into another 2 ml screw topped tube. Seminal supernatant removed without disturbing the seminal pellet. Seminal pellets combined in a 1.5 ml screw topped tube with centrifugation as before.
F Stains on fabric	Soak, mash and agitate fabric in 1 ml PBS for 30 min using a mechanical shaker. Centrifuge.	–	–	13,000g 5 min	Supernatant removed without disturbing the pellet

As this work was carried out in different organisations and over a number of years, the methods for extracting fabric and swabs ([7] and Table 1), applying AP reagent and performing DNA tests varied between studies. The different methods used for applying AP reagent were borne in mind when assessing the results of this research. It should also be noted that these studies were limited in size. In all instances a positive AP reaction was noted when any colour change was observed, although the length of time for observation of the AP result did vary [8] and is detailed for each study. A false positive is denoted by a purple colour reaction to any substance other than semen.

Case examples were provided by CFS, Forensic Science Northern Ireland (FSNI) and LGC.

2. Method

2.1. Sensitivity

A series of semen dilutions (1 in 50, 1 in 100, 1 in 250, 1 in 500, 1 in 750, 1 in 1000, 1 in 1500, 1 in 2000 and 1 in 3000) were prepared from a fresh unfrozen semen sample using deionised water. This semen sample along with all other body fluids provided for all of the studies in this paper were provided by fully consenting donors.

100 μl of each semen dilution was seeded onto the synthetic main body and cotton gusset of each of three pairs of newly purchased knickers and left to air dry in a fume cupboard. The knickers were then packaged in brown paper bags and left at room temperature for a week before testing.

For each dilution of semen one pair of knickers was indirect AP tested and the other two pairs were direct AP tested. Two methods of application were used for the direct AP testing: a spray bottle and an aerosol (see Fig. 1). In the results tables the latter are designated 'direct spray' and 'direct aerosol'. For the indirect AP test, only the screening paper was dampened with water rather than both the screening paper and the knickers [9].

A positive AP reaction was noted when any colour change was observed. The screening papers for the indirect testing and the garments themselves with the direct testing were observed for at least 2 min and up to a maximum of 13 min.

2.2. The effect of AP reagent on haematoxylin and eosin staining of spermatozoa

AP positive areas identified by indirect and direct testing in the sensitivity study 2.1 were excised completely along with a small amount of the adjacent fabric. All excised fabric was then extracted

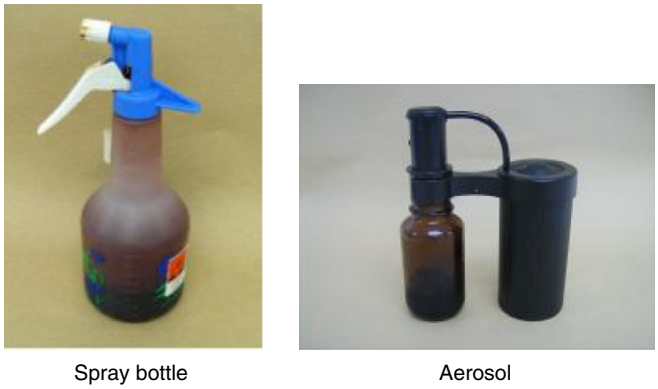

Fig. 1. Equipment used to apply AP reagent.