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The effect of mark enhancement techniques on the subsequent detection of semen/spermatozoa

Rory Simmons [a], Paul Deacon [b], Darren J. Phillips [a], Kevin Farrugia [a,*]

[a] School of Science, Engineering & Technology, Division of Computing and Forensics, Abertay University, Bell Street, Dundee DD1 1HG, UK
[b] C/o School of Science, Engineering & Technology, Division of Computing and Forensics, Abertay University, Bell Street, Dundee DD1 1HG, UK

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ABSTRACT

Fingermarks, footwear marks, blood and semen are amongst the most commonly encountered types of evidence at crime scenes. Previous work has extensively investigated fingermark and blood enhancement techniques and a sequence developed to maximise evidence recovery; however, there is limited research as to the effect of these techniques on the subsequent detection of body fluids such as semen.

In this study, seven fingermark and blood enhancement techniques (e.g. powder suspension, cyanoacrylate fuming and acid violet 17) were employed followed by the subsequent detection of semen/spermatozoa. Other variables included in the study were the use of two substrates (white ceramic tiles and grey laminate flooring), a depletion series and ageing periods of 1, 7, 14 and 28 days. The effect these techniques had on the subsequent detection of semen was assessed by visual and fluorescence examination followed by presumptive and confirmatory testing for semen and spermatozoa.

The results found that protein stains (acid violet 17 and acid yellow 7) caused a loss in presumptive test reactivity; however, sperm heads were still observed using microscopic examination after extraction and staining. The use of black magnetic powder, Bluestar® Forensic Magnum luminol, Lumicyano™ 4% and cyanoacrylate fuming followed by basic yellow 40 staining did not hinder subsequent presumptive and confirmatory tests for semen and sperm heads. Powder suspension caused a loss in both presumptive test reactivity and sperm heads from the substrate. In general, the enhancement techniques resulted in the improved visualisation of the semen stains under white and violet/blue light. The results from this study aim to provide a strategy to maximise evidence recovery and improve efficiency in an integrated forensic approach.

1. Introduction

Fingermarks, footwear marks and biological fluids are routinely collected from crime scenes to aid criminal investigations in the identification of offenders [1,2]. Current methodology for the recovery of latent (not visible to the naked eye) fingermarks and footwear marks can adversely affect the subsequent detection of biological fluids and vice versa. Despite the importance of these types of evidence, an examination of the literature and discussions with forensic practitioners has indicated that, currently, there are no guidelines or recommendations for the maximum recovery of latent marks and biological fluids. If an item of evidence is suspected of having latent fingermarks on it as well as semen deposits, the application of two different tests will be required; however, which forensic test is applied first and whether the application of one test affects the other has not been fully investigated.

1.1. Speculative searching for semen

Dry stains of semen will fluoresce under excitation light sources of wavelengths 300–480 nm [3]. Fluorescence is useful as an initial and non-destructive tool that may be used for speculative searching of semen; however, certain light sources and wavelengths may be damaging to DNA. The use of Quaser high intensity light sources has been proven non-destructive to DNA at all wavelengths for exposures of up to 30 min [4].

1.2. Presumptive test for semen

The acid phosphatase (AP) reagent was developed in 1957 by Stuart Kind [5] and is widely used as a presumptive test for semen

* Corresponding author. Tel.: +44 01382 308689; fax: +44 01382 308663.
E-mail address: kevin.farrugia@abertay.ac.uk (K. Farrugia).

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by reacting with AP present in seminal fluid to give a purple colour. The AP reagent is only considered as a presumptive test for semen due to the number of potential false positive reactions including those from semen-free vaginal material, faecal material, foods and beverages such as tea [6]. The use of the AP test assists in narrowing down areas for further examination and testing for seminal material which can be used for subsequent DNA analysis. The AP reagent consists of sodium acetate, acetic acid, α-naphthyl phosphate disodium salt and brentamine fast black K salt. In the presence of acid phosphatase, α-naphthyl phosphate is hydrolysed to produce α-naphthol. This then combines with the fast black K to produce a purple azo dye [7]. Since first described by Kind in 1957, the application process of the AP reagent has remained largely unchanged. Until recently, it was believed that 2 min was sufficient time to allow for a purple colouration to develop and indicate a positive AP reaction. Additionally, a negative reaction was generally recorded if no purple colour was developed or if the time required for the colour to develop was greater than 2 min. Recent studies have suggested that this 2 min cut off was insufficient to detect some dilutions of semen as high as 1 in 20 [7]. Based on a number of studies [7–9], it is now suggested that reaction times of at least 10 min, and up to 15 min, be allowed as to not overlook potential dilute seminal evidence.

AP is water soluble and often found in high concentrations in seminal fluid; however, this concentration will vary day to day in individuals and between different individuals (intra and inter person variation). There are two methods of AP testing samples from a substrate: direct and indirect. In the direct method, a water-moistened filter paper is pressed onto the area of interest or a moistened swab is rolled over an area of interest and AP reagent is then added to the filter paper or swab. There is also the possibility of applying the AP reagent directly onto the substrate; however, there is an increased possibility of false positives associated with this [10]. For indirect testing, the area of interest may be swabbed using a moistened swab and an extract made from this, containing both seminal fluid and spermatozoa. A drop of the extract is then applied to filter paper before applying AP reagent. AP reagent can be applied as a drop, spray or by aerosol with recent studies suggesting that application by aerosol is the most effective method of application for detection [10].

1.3. Confirmatory test for semen

A common method of confirmatory testing for the presence of spermatozoa in semen is microscopic examination. Following a positive AP reaction, the area of interest can either be cut out (if fabric or similar material) or swabbed (if item cannot be easily cut). The fabric or swab head can then be placed into a microcentrifuge tube and distilled water added. Combined action of the water plus agitation will remove many of the sperm heads (if present) from the fabric or swab head. The resulting supernatant can then be centrifuged to form a sperm pellet, which can then be mounted on a slide for examination [10]. A drawback of this method is that sperm tails are often lost due to the mechanical forces involved [8] and as a consequence it is common to find sperm heads without tails.

Sperm heads, and tails if present, need staining before microscopy to provide contrast and allow for identification. In the UK, this procedure is generally performed using haematoxylin and eosin (H&E) staining. These stains will colour the heads of the sperm purple, and the remaining cellular material a pink colour. Once stained, the slide can then be examined by microscopy at 400× magnification and the presence and concentration of any sperm heads (with or without tails) can be recorded. Alternative staining methods include the 'Christmas tree stain', which uses nuclear fast red and picroindigocarmine to stain sperm a distinctive green and red colour [11,12].

There may be instances where there is a positive AP reaction, but no sperm heads are present during microscopic examination. This may indicate a potential false positive AP reaction, or it may be a result of an oligospermic or azoospermic semen sample (i.e. little or no spermatozoa in the semen). In these situations, alternative confirmatory tests are available in the UK including the choline test [13] and prostate specific antigen (PSA) kits, which are more sensitive than the choline test [8].

1.4. Integrated forensic approach

Previous research has set out recommendations and considerations that should be given in terms of sequencing fingerprint enhancement techniques in order to maximise efficiency, the most prevalent of these being the Manual of Fingerprint Development Techniques (MoFDT) [4]. It is clear that an integrated forensic approach to different types of evidence is necessary to maximise evidence recovery, also discussed in the recently released Fingermark Visualisation Manual by the UK Home Office Centre of Applied Science and Technology (CAST) which supersedes the MoFDT [14]. Examples of work discussed in the new manual include items that are suspected to contain fingermarks that are latent and in blood. Such an example is the use of vacuum metal deposition or powders for the detection of latent fingermarks which does not hinder subsequent treatment of marks in blood with protein stains. The use of cyanoacrylate fuming for the detection of latent fingermarks generally hinders subsequent protein staining (water/ethanol/acetic acid formulation) for the enhancement of fingermarks in blood. The use of methanol-based protein stains; however, assists to penetrate the cyanoacrylate polymer to effectively enhance the blood.

There is limited, although on-going, research with regard to the effect of fingermark enhancement techniques on the subsequent detection of other types of evidence and vice versa. This may result in evidence being missed or destroyed as well as reduced efficiency. A recent study [15] investigated the effect of ninhydrin on the subsequent serological testing of envelopes for the detection of saliva. It was reported that the ninhydrin process did not impact the results of serological testing of the envelopes. Other work assessed the effect of fingermark detection techniques on the subsequent recovery and analysis of explosive residues [16], the effect of chemical, biological, radiological, and nuclear (CBRN) decontamination on the detection of fingermarks on glass [17], the effect of bacteria on fingermark detection [18], the effect of chemical warfare agents on footwear enhancement techniques [19] and the effect of formaldehyde gas on fingermark evidence [20].

This study aims to contribute to this growing area of research of an integrated approach. Seven fingermark and blood enhancement techniques were employed in this study including: acid yellow 7 (AY7), acid violet 17 (AV17), Bluestar® Forensic Magnum luminol, cyanoacrylate fuming with subsequent basic yellow 40 (BY40) treatment, Lumicyano™ 4%, black magnetic powder and black iron-oxide powder suspension. In order to determine the effect of these enhancement techniques on subsequent detection of semen, three methods of detecting semen/spermatozoa were applied: visual examination, acid phosphatase (AP) reagent and microscopic examination following haematoxylin and eosin (H&E) staining. The sequence of mark enhancement followed by the detection of semen/spermatozoa was applied because fingermarks and footwear marks are fragile and any attempt of an AP presumptive test or swabbing for body fluids may result in a loss of detail recovered. Consequently, this study looked at the sequence of mark enhancement followed by the presumptive AP test and confirmatory test for semen.