Journal of Forensic Research

Borges et al., J Forensic Res 2017, 8:3
DOI: 10.4172/2157-7145.1000380

Research Article Open Access

Evaluation of the SPERM TRACKER™ for Semen Stains Localization on Fabrics

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Received date: May 12, 2017; **Accepted date:** May 30, 2017; **Published date:** May 31, 2017

Abstract

Semen stains localization is an important challenge and a prerequisite to genetic identification during forensic investigations. Often performed using either non-specific techniques such as CrimeScope or/and acid phosphatase specific methods such as naphtyl, these detection requirements were in need of a new technique and procedure: rapid, simple, specific and non-toxic. We are here presenting the results of a comparative study between mini-CrimeScope and the newly launch product Sperm Tracker™. Evidence are given with regards to the specificity, sensitivity and ease of use of the new product. Genetic analysis were also performed right after localization and demonstrated the full compatibility of the method with the subsequent DNA analysis.

Keywords: Acid phosphatase; CrimeScope; Fluorescence; Genetic analysis; Product evaluation; Semen stains

Introduction

Within forensic labs worldwide and more particularly in the French "Institut National de Police Scientifique", one of the first step in the identification of semen traces is the orientation method which rapidly enables the localization of semen traces, subsequently sampled for DNA analysis [1]. This test can be either based on the intrinsic fluorescence of semen traces, upon light excitation (mini-CrimeScope) and/or based on the detection of the acid phosphatase activity present in semen. A test can be used based on the ability of this enzyme to catalyse the hydrolysis of organic phosphates like alpha naphtyl phosphoric acid, generating a reaction product that will subsequently react with a chromogen diazonium salt and induce a colour change [2,3]. A positive reaction is recorded upon rapid development (less than 15 seconds) of a purple colour, but the procedure is long (30-40 minutes), needs special handling care of carcinogenic reagents and does not allow a precise location of small seminal liquid spots since the detection is performed on a transferred image from the fabric.

However, when using CrimeScope, the procedure is immediate but really lacks of sensitivity and often generates signal from many other stains than only semen traces, giving only presumptive semen detection which has to be validated using an additional technique [2, 4,5].

Alternative methods were also extensively studied such as emerging spectrometric techniques applied for the forensic analysis of body fluids. These techniques include the use of ultraviolet-visible, infrared (IR), Raman [6], X-ray fluorescence [7], nuclear magnetic resonance spectroscopy and mass spectrometry [8] to investigate blood, semen, saliva, urine, vaginal fluid or sweat stains. Although all these spectrometric techniques seem to have a high potential to differentiate body fluids prior to DNA extraction, IR and Raman spectroscopy have shown the most promising results for discriminating stains from body fluids [9].

The aim of the present study is to evaluate the use of a new commercial product (Sperm Tracker™) specially developed in collaboration with the French "Institut National de Police Scientifique" (INPS/LPS69) for the detection of semen traces, directly on fabrics. The product presents the specificity of naphtyl reaction toward semen acid phosphatase, but also an ease of use almost equivalent to the CrimeScope. Indeed, only water spraying and pressure application are required to obtain clear results since no toxic compound and no complex protocol in fume hood.

Sensitivity, specificity, compatibility with subsequent genomic analysis will be reported and compared to the widely used mini-CrimeScope technique.

Materials and Methods

Materials

Human semen samples were supplied by three voluntaries of known genotype. The three samples of sperm were mixed and stored frozen at -20°C. 10 m^2 rolls of Sperm Tracker™ were obtained from AXO Science SAS (Lyon, France). Human saliva, blood, vaginal secretion, faeces and urine were obtained voluntarily and anonymously. Phosphate Buffer Saline (PBS) tablets were purchased from Applichem GmbH (Darmstadt, Germany).

Standardized fabric was composed of cotton cloth which can be assimilated to a blue jean.

Other fabrics such as cotton, sweater, blue-jeans, towelling, pillow and towelling with patterns were obtained from various clothes and fabrics randomly selected in order to establish a panel of different colours and thicknesses.

Artificial staining of fabrics with pooled sperm

In order to generate artificially stained clothes, the fabrics to be tested were spotted with 50 µl of semen diluted or not, in double distilled water. The fabrics were then allowed to air dry overnight and were then ready to be used.

For cross-reactivity study, stains were generated by spotting 20 µl of semen, drying and then spotting 30 µl of a body fluid contaminant, except for faeces and vaginal secretion which were performed by solid material deposition.

For negative control, 50 µl of physiological saline solution were spotted on the fabric and allowed to air dry overnight.

Direct visualization of semen on fabric using mini-Crime Scope

Mini-CrimeScope-400 (HORIBA Scientific, France) was used with CSS (wavelengths between 390 and 540 nm) and orange glasses (HORIBA Scientific, France) to illuminate and visualized stained fabrics. Experiments were conducted in a dark room. The fabrics were directly observed under the lamp. Visualization is considered positive if a luminescence appears.

Images were obtained using a Canon G12 Powershot camera, directly through an orange filter-plate (HORIBA Scientific, France).

Fluorescent visualization of semen on fabric using Sperm Tracker™

Sperm Tracker™ was used as received and following the supplier instructions. First the absorbent side of the product was evenly sprayed with double distilled water; a volume of 150-200 ml per m^2 is necessary.

Fabrics were then brought into contact with the moisturized Sperm Tracker™ paper and kept 3 minutes under pressure (650 Kg/m^2). Sperm Tracker™ paper and specimen were not separated before observation of the fluorescent signal coming from the sperm stains.

The fluorescent visualization of the semen stain on fabric material was obtained with a Camag UV lamp (366 nm, CAMAG, USA). The visualization is done by simply looking at the Sperm Tracker™ through its transparent side without removing the sample or the paper. Visualization is considered positive if a clear and bright blue luminescence is visible. A negative result is considered when no distinction between background fluorescence and a blue fluorescence coming from a stain can be seen using naked eye. It is usually obvious for any user to state the presence of a positive result. Images were obtained using a Canon G12 Powershot camera.

Spermatozoa detection and genomic analysis

Each fabric sample, collected following the Sperm Tracker™ detection procedure, was analysed by the French "Institut National de Police Scientifique" (INPS/LPS69) standardized procedure (COFRAC ISO 17025). Briefly, samples were cut out from the stained fabric and soaked for 1 hour in 400 µl of PBS solution. The samples were then dyed using the Christmas Tree (Supplementary information 2) [10]. Spermatozoa were then separated from the other cells using differential lysis and their DNA purified using the Qiamp DNS mini kit (Qiagen, Hilden, Germany). Purified DNA was then quantified using Quantifiler Duo kit (Applied Biosystems, Foster City, USA) and amplified using the Identifier and kit (Applied Biosystems, Foster City, USA).

The PCR products were then analyzed using capillary electrophoresis (3130XL, Applied Biosystems, Foster City, USA) and validated using the Genemapper ID (software Applied Biosystems, Foster City, USA).

Results

In order to evaluate the ability of the Sperm Tracker™ product to be used routinely in a forensic laboratory, the performances of the orientation system were compared to the widely used CrimeScope apparatus. Thus, the different figures of merit such as specificity, sensitivity, potential interferences and repeatability were assayed. For this purpose, a standardized fabric composition, (blue jeans like cotton) was used.

Specificity of the detection

First of all, specificity of the semen detection was evaluate by comparing the signal obtained on stains produced using different body fluids (pure). False positive related compounds found in the literature are vaginal secretions, saliva, faeces, some tea stains, cauliflower, broccoli, potato, some mushrooms, male urine, sweat, some contraceptive creams and wood [11,12]. Were then tested: semen, saliva, blood, vaginal secretion, urine and faeces. Potato and wood were also tested without any effect on the sperm detection (data not show). Two series of staining (Figure 1A and 1B) were performed on the standardized fabric used all over the study.

As a matter of fact, stains were all visible upon white light illumination without any possible discrimination between the different body fluids. Then, mini-CrimeScope was shown to be able to detect pure semen but with a limited luminescence signal. This low luminescence can be explained by the properties of the standardized fabric which is one of the most challenging for semen detection. Performances of the mini-Crime Scope technique were then lower than expected but still representative of the real case performances. Moreover, when paying attention to the specificity, the mini-CrimeScope was shown, on this particular fabric, to generate a fainted false positive signal for semen detection from the urine and saliva stains.

Getting to the Sperm Tracker™ results, a strong luminescent signal was observed, emitting only from the semen staining. Also, none of the other fluids were shown to generate false positive and dark staining upon illumination were observed, as when using the mini-CrimeScope technique, on blood and faeces samples.

Finally, from this first row of experiments, another feature of the Sperm Tracker™ can be presented: the very low diffusion of the stains during the revelation procedure. Indeed, while in the case of the naphtyl coloration procedure for semen detection, large diffusion smears are usually observed, in the present procedure, since the amount of humidity needed to generate a signal is low, a precise localisation of the semen traces is possible. Also, the direct observation of the stains through the Sperm Tracker™ paper enables precise localisation of even small semen spots.

Potential interference of the detection

In this second part, the masking effect of the concomitant presence of semen and other body fluids within a stain was evaluated. As

previously, saliva, blood, vaginal secretion, urine and faeces were tested but this time in the presence of semen. The semen was always spotted below the contaminant and this will have a strong effect, particularly when the contaminant is a thick dark matter such as faeces.

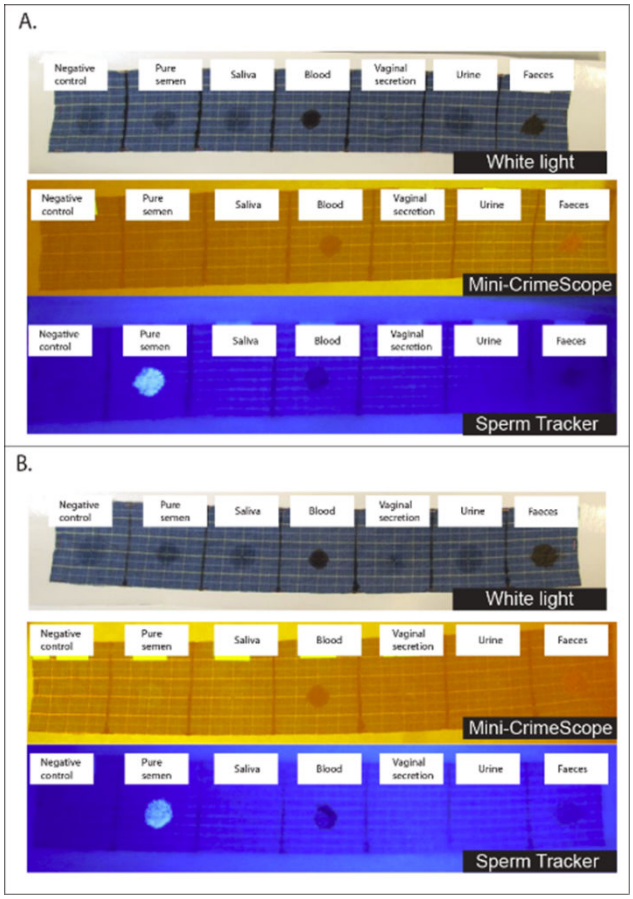

Figure 1: Specificity study of the mini-CrimeScope and Sperm Tracker™ techniques. A: and B: are two series of experiments. All body fluids were stained pure.

As can be seen in Figure 2, none of the contaminant body fluids totally hinders the detection of the semen traces using the Sperm Tracker™ technique. Nevertheless, lowering of the signal was observed and the higher effect was obtained, as expected, for faeces contamination, directly followed by blood contamination.

Comparing these results with the mini-CrimeScope series, obvious differences appeared. Indeed, apart from the lower signal generated from pure semen, a strong lowering of the signal was observed for both faeces and blood, leading to false negative results. For Sperm Tracker™, a slight lowering of the signal is observed with faeces; however it does not hinder the detection of a clear positive signal. The decrease of the specific signal compared to pure semen can be attributed to the masking effect of faeces, i.e. it is likely that the amount of biological materials interferes with either the fluorescent dyes and/or the Alkaline Phosphatase activity itself.

Sensitivity of the detection

Once specificity and potential interference studied, the next step of the validation study was the evaluation of the sensitivity of the Sperm Tracker™ technique. Different dilutions of pure semen were then spotted on the standardized fabric, from pure to 1/10000, dried and detected. As can be seen in Figure 3, semen dilutions down to the 1/20 can be detected using the present method.

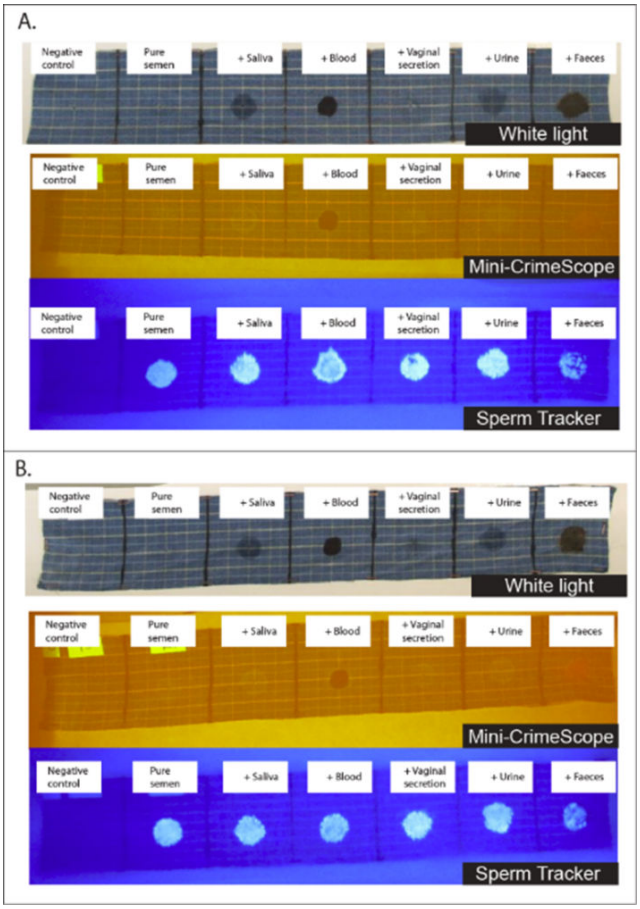

Figure 2: Interference study of the mini-CrimeScope and Sperm Tracker™ techniques. A and B are two series of experiments. All body fluids were stained pure on top of the semen stain.

Moreover, and this is true also for the two previous studies, no signal was observed from the negative control stain, evidence of the very low background of the method. For sake of comparison, mini-CrimeScope results are also shown in Figure 3, evidencing the low sensitivity with only the detection down to the 1/2 sperm dilution.

Impact of the fabric composition

When it comes to the fluorescent detection of semen directly on fabric, an important point is the ability of the method to work on various fabric composition, both at the material level and the texture level. Indeed, some fabrics will generate intrinsic fluorescence or absorb fluorescent signal (which is a problem when performing detection directly on fabric upon light excitation) when other will have diffusion tendency when wet or stained by fluids. In order to

demonstrate, as far as possible, the ubiquity of the Sperm Tracker™ technique for the localisation of semen stain on fabric, a series of 6 fabrics (cotton, sweater, blue-jeans, towelling, pillow and towelling with patterns) was tested. In this experiment, different amounts or different dilutions of spotted semen were used in order to evidence the diffusion effect of the fabric but also to demonstrate the efficiency of the technique on various fabric thicknesses.

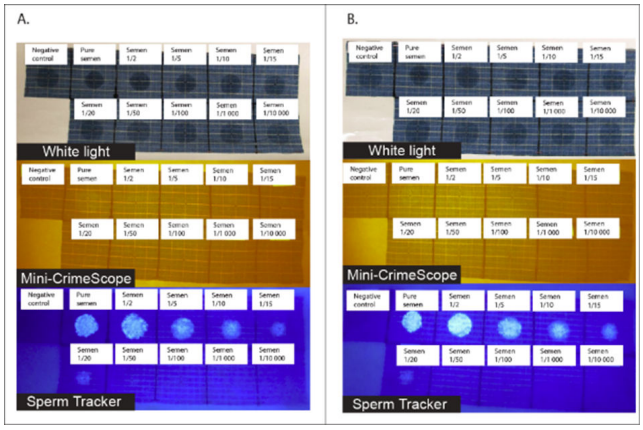

Figure 3: Sensitivity study of the mini-CrimeScope and Semen Tracker™ techniques. A and B are two series of experiments.

As a matter of fact (Figure 4), semen stains were detected on all tested fabrics, but with various signal intensity or diffusion. Pillow was the most diffusive fabric with large area covered with strong semen stain signal. Then, came the cotton fabric from which a quite strong intrinsic fluorescence was observed together with a diffusion of the specific signal.

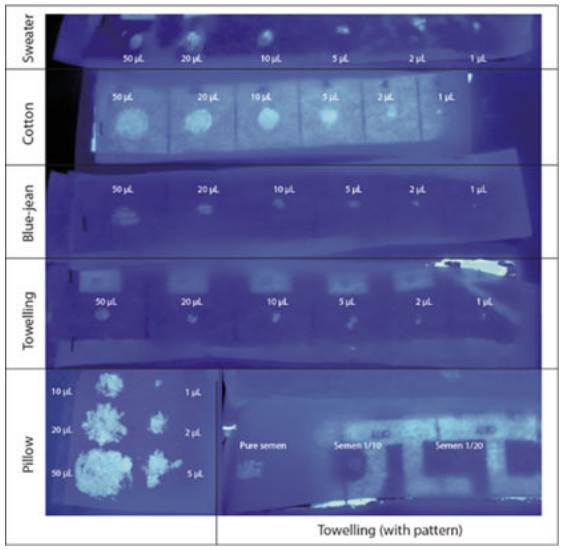

Figure 4: Effect of the fabric composition on the Sperm Tracker™ signal. Different volumes or different dilutions of pure semen were deposited on each fabric.

Finally, the last four fabrics (sweater, blue-jeans, towelling and towelling with patterns) were giving well localized signals, even if the patterns of the towelling with patterns fabric were giving local intrinsic fluorescence background. It is also worth to mention that for the particular cases with auto fluorescent patterns on fabric, the operator still have the opportunity to disassemble the paper from the fabric and to observe only the sperm stain luminescent signal without background from the fabric, looking directly at the absorbent side of the paper.

A last experiment about the impact of fabric on the semen stains detection was performed on a large piece of fabric composed of a complete green velvet trousers (Figure 5). Three 300 µL stains were deposited on three locations of the trousers, one beneath the zipper, one on the right leg and one on the crotch but in the inner side of the trousers (Figure 5). In the three cases, the stains were easily localised using a 1 m^2 piece of Sperm Tracker™. It is worth to mention that these stains were located on areas challenging to detect because uneven. Nevertheless, the technique was shown to be fully operational, thanks to the flexibility of the paper, even if the trousers was composed of different thickness, hardness and composition. Also, as shown in Figures 5 and 6, the trousers was imaged without being cut or reverse since the inner side stain was clearly visible through the fabric.

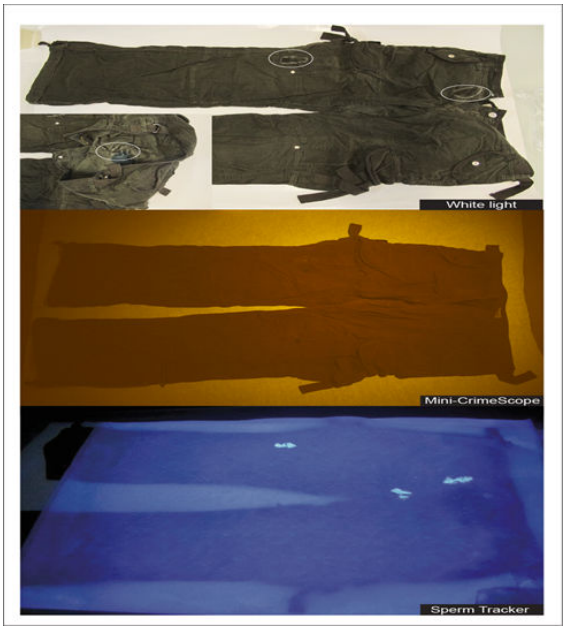

Figure 5: Detection of three semen stains deposited on a large piece of fabric: a complete trousers. Stains were 300 µl pure semen.

In a similar way, three last fabrics (a sock, a nightie and a pullover) were spotted with pure semen and observed using both mini-CrimeScope and Sperm Tracker™ (Figure 6). As can be seen, on these three fabrics, both techniques were able to reveal the presence of the semen traces (except for the stain on the darker part of the pullover which was not detected with Crimescope), with still a higher signal intensity in the case of the Sperm Tracker™ procedure.

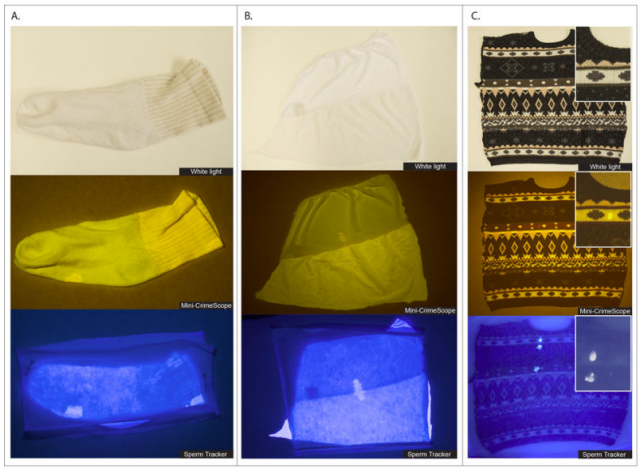

Figure 6: Detection of three semen stains deposited on a large piece of fabric: A: Complete sock; B: Complete nightie; C: Complete pullover and closer view at the stains position. Stains were for A and B 300 µL pure semen and 200 µL for C.

Spermatozoa detection and Genomic analysis

Semen detection is the first step to the genomic analysis and identification of suspects. The compatibility of the detection method with the subsequent sperm cells collection, DNA extraction and PCR amplification is then mandatory. Indeed, numerous reagents can hinder staining, extraction and/or PCR reaction and have to be avoided.

It is worth to mention here that since the Sperm Tracker™ enables the lowering of the pressing time (3-time lower), less impact on the original stain might be observed when compare to the classical naphtyl orientation reaction. Thus, for sperm stains, no interference was observed concerning Christmas tree staining.

Genomic analysis, from sperm cells collection to PCR identification, were then performed on materials collected from fabrics first subjected to the Sperm Tracker™ procedure. Different body fluids (semen, blood and saliva) from known origin, classically used of identification were then first spotted on the two different fabrics (blue-jean and cotton), subjected to the semen detection and then collected, extracted and identified. The genomic obtained analysis is presented in Table 1.

Semen samples used for the sensitivity test were obtained by mixing samples supplied by three volunteers thus the profile obtained corresponds to a 3 persons mixed DNA profile.

Stain composition	Spermatozoa detection	DNA quantification (ng/µl)	IPC (acceptable range: 27.7-33.1)	Fraction of alleles present
Extraction negative control	-	0	29.69	0%
Extraction positive control *	-	0.1052	29.58	100%
Blood on cotton	-	0.1379	29.43	100%
Blood on blue-jean	-	1.7793	29.67	100%
Semen on cotton	positive	8.2835	29.51	100%
Semen on blue-jean	positive	12.9014	30.08	100%
Saliva on cotton	-	0.0887	29.61	100%
Saliva on blue-jean	-	0.0789	29.88	100%
Pur Semen	positive	15.0552	30.05	98.20%
Dilution semen 1/2	positive	4.5776	30	100%
Dilution semen 1/5	positive	1.3366	29.99	100%
Dilution semen 1/10	positive	1.037	29.91	100%
Dilution semen 1/20	positive	0.884	29.76	98.20%
Dilution semen 1/50	positive	0.2782	29.49	100%
Dilution semen 1/100	positive	0.0749	29.81	100%
Dilution semen 1/1000	positive	0.0232	29.73	89.60%
*Positive control is composed of 10000 spermatozoa cells.				

Table 1: Spermatozoa and genomic analysis following the Sperm Tracker™ procedure of different body fluids spotted on several fabrics or sperm dilutions used for the sensitivity test.

Ratio between the profiles of the contributors depends from spermatozoa concentration of each sample. One of the three is much less represented than the two others. Lack of alleles can be explain by cut off phenomena (very "weak" alleles not labelled by Genemapper

because of the presence of a very high peak beside) or by few DNA (dilution semen 1/1000). Dilution semen 1/1000 presents relative intensity weaker than the others samples (up to 600 rfu for higher peaks) but number of alleles missing is only 6/58. Finally, the technique, now in routine at the French "Institut National de Police Scientifique", has been used between June 2016 and September 2016 on 50 sexual assault real cases, leading to 66 genetic identifications on various fabrics (5 underwear, 4 bedding elements, 2 boxers, 2 thin tights, 2 gauze samples, 1 sock…).

Discussion

A new technique for the detection of semen stain directly on fabric has been presented and validated. The comparison of the performances of the present method with the classically used mini-CrimeScope technique demonstrates the superiority of the new technique, both at the specificity and sensibility level. Indeed, no cross-reactivity of adverse effect was observed in the presence of various body fluids such as, saliva, blood, vaginal secretion, urine and faeces. Moreover, the technique was also shown to enable the precise detection of semen stains on fabrics of different origins and composition but also on large pieces of fabric presenting different thicknesses such as trousers. Finally, genetic analysis was demonstrated to be fully compatible with the semen detection technique, for semen sampling but also for blood and saliva sampling.

For a more practical point of view, using Sperm Tracker™ procedure (purchase price 87.5€/m) to analyse a standard sheet takes 1 h to 1h30, 10 minutes for an underwear, 35 minutes for trousers and 20-25 minutes for a tee-shirt. No doubt then that this technique will be widely used in forensic laboratories in the near future.

Acknowledgement

The authors want to acknowledge Laurent Pene, Catherine Privat, Christine Sagnal, Nathalie Drouet, Stéphanie Lopez, Sarah Genin, Laurence Dujourdy, Anne Thébault, François-Xavier Laurent and all the DIP team (Departement for Person Identification) from the Laboratory of Scientific Police of Lyon, INPS/LPS69, for their advices and encouragements.

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