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Evaluation of Sperm Tracker® Spray for Semen Stain Localization

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Abstract

Alternative-light sources (ALS) are widely used in both forensic laboratories and crime scenes for semen detection. This non-specific strategy is effective despite a few limitations, leading to both false negative and false positive results. To detect semen stains, specific acid-phosphatase (AP) tests can also be implemented in a controlled laboratory environment. However, they are not appropriate for direct crime scene investigations. A newly commercialized product could overcome all those limitations: Sperm Tracker (STK) Spray®, an AP-spray test for non-textile items.

In this study, we assessed its specificity, sensitivity, and compared its effectiveness with those of two well-known ALS on a wide range of materials, focusing on 32 different surfaces that can be commonly encountered on crime scenes. We found STK spray® easy to use, with a rapid fluorescent signal appearing in the presence of semen. It is specific, detects pure semen spots down to 1 µL and semen dilutions down to 1/20. Results showed that the fluorescent signal may be blocked by feces or blood but not by any of the other body fluids we tested. Comparison with ALS showed encouraging results, particularly on diluted semen stains for which ALS were less sensitive. This new reagent does not interfere with the Christmas tree spermatozoa staining method or with the process leading to DNA profiling.

In conclusion, the present study showed convincing results regarding the new STK spray® reagent and its further direct use on real crime scenes.

Keywords: Acid-phosphatase • Alternative-light source • Genetic profile • Product evaluation • Semen stain detection • Sperm Tracker® • Spermatozoa detection

Introduction

Identification of body fluids is a central step of forensic investigations. Especially in rape cases, detection of non-visible semen stains is crucial. Presumptive tests are used to locate potential semen stains, prior to subsequent microscopic spermatozoa visualization, to confirm the presence of semen. Several strategies exist, mainly alternative light sources (ALS) and acid phosphatase (AP) tests.

Dry semen has fluorescence properties, with a wide excitation spectrum of 300-500 nm and an emission spectrum of 400-700 nm. Many ALS procedures combine several wavelengths, depending on the background fluorescence [1,2]. However, a 450 nm (blue) excitation combined to an orange observation filter is the most used process. ALS are non specific, as photoluminescence spectra of different body fluids overlap [3,4] and many commercial products display fluorescence properties [5,6]. False positives are described and well known [7] and can be identified with confirmatorytests such as Prostate-Specific Antigen tests or microscopic screening, but it does result in a waste of time for the forensic examiner. False negatives are also described and appear to differ according to background color and material, as well as the type of lamp used [8,9].

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Those limitations may encourage the forensic examiner to rely on AP tests, at least as a second line test [10]. These presumptive tests also exhibit false positives [11] such as cauliflower, sprouts, and vaginal secretions, while false negatives are described mainly on washed items [12]. We previously described Sperm Tracker lab® (STK lab®, Axo Science, France) as a nontoxic AP press test appropriate for fabrics analyses [13]. It allows the treatment of clothing items and bed sheets without moving the reagent sheet away from the tested item. It is suitable for laboratory analyses and is commonly used in Lyon public forensic laboratory in France.

Recently, Axo Science released an aerosol version of the reagent: STK spray®. According to the manufacturer, it is as specific and sensitive as STK lab® and easily usable in crime scenes, only on non-pressable items. STK spray® has been compared to STK Lab® and naphtyl reaction [14]. It has not yet been compared to forensic light sources in realistic forensic situations.

The purpose of the present study is to assess STK spray® sensitivity, specificity and compatibility with subsequent DNA analyses. A comparison between STK spray® and forensic light sources commonly used on crime scenes is also performed, focusing on both LED (Crime-lite® 2 from Foster + Freeman) and Xenon (HandScope® from Horiba) technologies.

Materials and Methods

Materials

Human semen, saliva, vaginal secretions, feces and urine were supplied by staff donors of known genotypes with voluntary consent. Collected samples and data were anonymized. Sperm samples were mixed and stored frozen at -20 °C. Human blood was provided by the French blood donors organization and stored frozen at -20 °C.

Phosphate Buffer Saline (PBS) tablets (ThermoFisher, Oxoid) were mixed in sterile water (Versol) according to the manufacturer's instructions.

All 32 materials representing floors and walls commonly found both indoors and outdoors, a sink, as well as various leather-type items were donated from staff members. Reference material (dark car rug) was chosen for its properties regarding semen detection, specifically that no signal is emitted under UV fluorescence alone.

Artificial staining of materials

For both STK spray® sensitivity study and ALS/STK spray® comparison, 50µL of pure or diluted semen were spotted and air dried at least 24h.

For specificity study, semen, blood, urine or saliva spots were created using 50µl of liquid and air dried overnight. Feces and vaginal secretions were spread by solid material deposition. To assess a possible masking effect of these fluids, 50 µl of air dried pure semen were covered with 75µl of blood, urine or saliva. Feces and vaginal secretions were directly applied to semen stains using a swab.

To mimic the effect of cleaning, 50µL of fresh, pure semen were spotted on chosen materials and immediately wiped by hand or with a slightly watered paper towel. Materials were then air dried as previously described.

All liquid spots were done using a micropipette (Gilson). For negative control, 50µl of sterile waterwere spotted and air dried overnight.

Fluorescent visualization of semen using sperm tracker spray®

Sperm Tracker spray® (Axo Science, France) was used in a dark room according to the supplier instructions. Briefly, a single dose packet was diluted in 100mL of sterile water and transferred in a spray bottle. Prior to use, to assess the presence of unspecific background fluorescence that may disturb the experiment, the tested surfaces were observed with a 365 nm UV light (VILBER, France). STK spray® was then sprayed under UV light for a first reading at t=0 min after vaporization. A second spray was performed at t=5min to evaluate the reagent. This two-steps method is referred as "STK spray® treatment" in the ALS/STK comparison experiment.

Results were considered positive when a clear and bright blue fluorescence was observed within seconds after using the reagent. Results were considered negative when no distinction between stains and background fluorescence were seen. Images were obtained using a Canon G12 Powershot camera.

Fluorescent visualization of semen using ALS on 32 surfaces

HandScope® Xenon (Horiba, USA) was used according to the manufacturer's instruction, using CSS filter (filtering light from 390 to 540 nm, with a peak around 455 nm) combined to orange visualization goggles to detect body fluids.

Foster + Freeman (England) recommends wavelengths from UV to blue for body fluids detection. We chose to focus on the Blue Crime-lite® 2, which has an emission peak at 445 nm. Observation was done using orange goggles. Images were obtained using a Canon G12 Powershot camera.

A fluorescent signal was considered as positive results. Non-fluorescent stain rings were considered as negative results.

Spermatozoa detection and genomic analysis

Samples from the sensitivity study were collected and analyzed by the public forensic laboratory of Lyon, France, according to its standardized procedure (COFRAC ISO 17025). The procedure is briefly summarized below: samples were cut out and soaked in 430 µl of PBS solution for 1 h at 37 °C under continuous agitation at 900rpm. For microscopic visualization, 30 µL were spread on a glass slide and dyed using the Christmas Tree staining method (nuclear fast red/picroindigocarmine). The remaining 400µL was processed by differential lysis and DNA purification (Qiamp DNA mini kit, Qiagen, Hilden, Germany). DNA was quantified using Quantifiler Duo kit (Applied Biosystems, Foster City, USA) and amplified using the Globalfiler kit (Applied Biosystems, Foster City, USA). PCR products were analyzed using capillary electrophoresis (3500XL, Applied Biosystems, Foster City, USA) and analyzed using the Genemapper IDX software (Applied Biosystems, Foster City, USA).

Results

STK spray® specificity assessment

In order to evaluate STK spray® performances and reliability, artificial staining of a reference material (dark car rug) was performed. The product's semen specificity was determined focusing on human fluids that are most commonly encountered in rape cases: blood, urine, saliva, feces and vaginal secretions (Figure 1). Only feces showed a slight natural fluorescence under UV light alone. With STK spray®, semen's fluorescence signal appeared almost instantaneously and was intensified by a second application of the product at t=5 min. Other fluids showed no fluorescence after neither of the two sprayings

The potential masking effect of fluid mixtures was also investigated, concealing semen stains with other body fluids (Figure 2). Semen's fluorescence signal was quickly detected when covered with saliva, urine or vaginal secretions. For the latter, signal spread and appeared diffused, most likely due to the sample preparation method. Both feces and blood blocked semen specific signal at t=0 min. A second application at t=5 min was effective at revealing semen covered with blood, but non-effective at overcoming the natural fluorescence of feces.

STK spray® sensitivity assessment

To determine the reagent's detection threshold, two separate sets of experiments were performed on a dark car rug. First, decreasing volumes of pure semen were revealed using STK spray® (Figure 3). All spots comprised in a range of 50 to 1 µL were successfully detected.

Secondly, pure and diluted semen stains were revealed using STK spray® (Figure 4). Semen was successfully detected down to 1/20 dilution. No signal was found at 1/50 dilution and beyond.

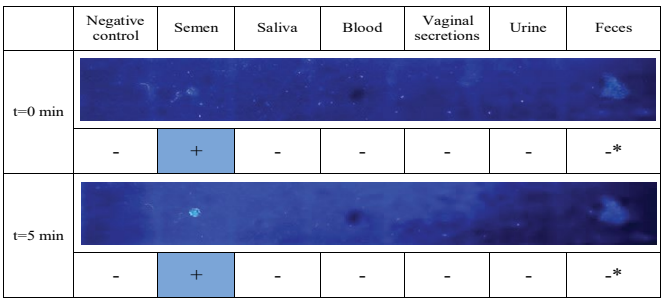


Figure 1. Body fluids spotted on a dark rug and visualized under a 365 nm wavelength after using STK spray® once at t=0 min; a second time at t=5 min, in duplicates. Only one of the duplicates is shown here as photographs and associated interpretations. *: unspecific background fluorescence.

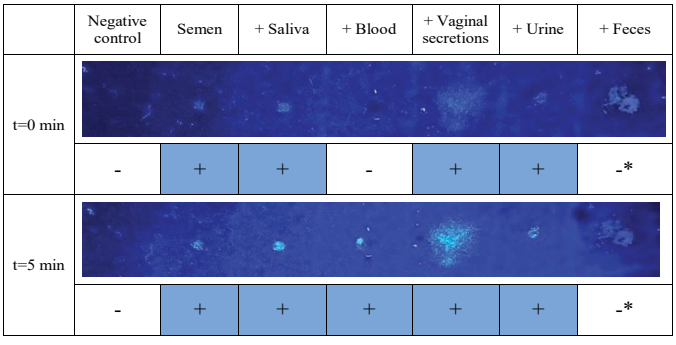


Figure 2. Spots of dried semen on a dark rug covered or not with body fluids and visualized under a 365 nm wavelength after using STK spray® once at t=0 min; a second time at t=5 min, in duplicates. Only one of the duplicates is shown here as photographs and associated interpretations. *: background unspecific fluorescence.

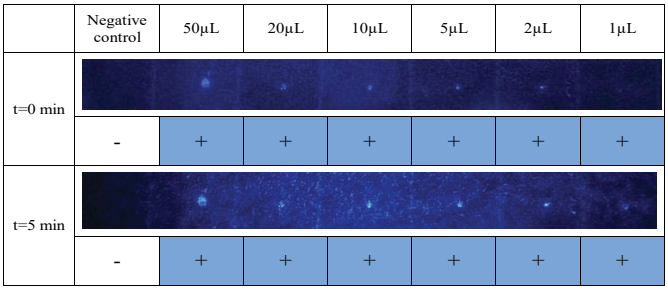


Figure 3. Spots of pure semen on a dark rug at decreasing volumes under a 365 nm wavelength after using STK spray® once at t=0 min; a second time at t=5 min, in duplicates. Only one of the duplicates is shown here as photographs and associated interpretations.

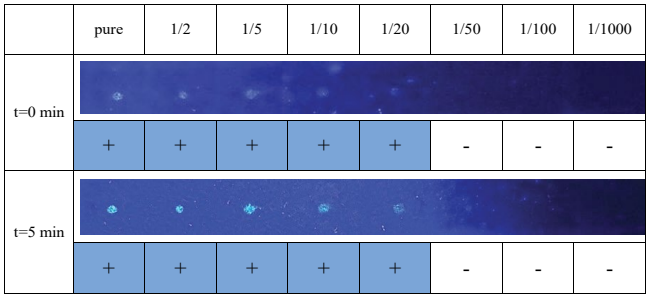


Figure 4. Spots of pure and diluted semen on a dark rug visualized under a 365 nm wavelength after using STK spray® once at t=0 min; a second time at t=5 min, in duplicates. Only one of the duplicates is shown here as photographs and associated interpretations.

For both experiments, all spots were visible on the first STK spray® application, and the fluorescent signal was improved by a second revelation at t=5 min.

STK spray® compatibility with spermatozoa detection and genetic profiling

In the laboratory, microscopic visualization of spermatozoa is crucial for semen characterization. The reagent's ability to not damage spermatozoa nor interact with cell coloring is then mandatory. One of each diluted semen spot from the sensitivity assessment experiment on the dark car rug was collected and analyzed. Results are shown in Table 1. Both spermatozoa visualization and genetic profiling were successfully carried out for spots down to the 1/1000 dilution, which is way below the detection threshold.

Comparison with ALS

If STK spray® is to be used on crime scenes it appears relevant to compare its effectiveness with forensic light sources. We focused on both Xenon HandScope® using the CSS filter and LED Blue Crime-lite® 2.

The three methods were compared on 32 materials stained with pure and diluted semen. Detailed results are shown in Table 2. We found some differences between materials, some allowing semen detection for all methods and dilutions, others only for pure semen. Two representative materials' photographs are shown in Figure 5.

ALS was mostly efficient on smooth surfaces (sink, tiles…) and exhibited some false negatives (grass, faux leather handbag…). All pure semen spots were successfully detected using STK spray®,whereas 78 to 85% of stains were detected using ALS (Figure 6). It should be noted that 1 of the 32 tested surfaces showed brighter results with ALS: grey vinyl flooring.

While the effectiveness of all three methods decreased with semen dilution, STK spray® allowed 87% of positive results on 1/20 diluted semen while ALS efficiency drops down below 30% (Figure 6).

A final experiment was performed in order to mimic semen cleaned by a perpetrator. We focused on 4 materials which gave satisfactory results for all

Table 1. Spermatozoa detection and genomics analysis of stains collected from the sensitivity assessment experiment. Each collected sample is soaked in PBS, a portion of which is spread on a microscopic slide dyed using the Christmas tree method. The remaining solution undergoes a differential extraction. Fraction of present alleles is calculated with reference to expected profiles.

Sample	Spermatozoa detection	Male DNA Concentration(ng/µL)	IPC acceptable range: 27.7-33.1	Fraction of present alleles
Extraction negative control	/	0.0000	29.11	/
Extraction positive control	/	8.7198	30.51	/
Pure semen	positive	47.9457	**35.18**	100%
1/2 diluted semen	positive	13.1711	30.95	100%
1/5 diluted semen	positive	4.6219	30.61	100%
1/10 diluted semen	positive	3.4031	31.46	100%
1/20 diluted semen	positive	1.9308	32.07	100%
1/50 diluted semen	positive	0.5012	31.37	100%
1/100 diluted semen	positive	0.1052	29.18	100%
1/1000 diluted semen	positive	0.0174	29.49	95.40 %

detection methods (Figure 7 and Figure 8). This cleaning experiment showed that wet wiping, without any detergent, made semen stains difficult to reveal. None were detected with the Handscope®, only one with the Blue-Crime-lite® 2. Three were positive using STK spray®. Stains on grey vinyl flooring were not detected by any of the three methods. Dry wiping by hand is less effective at masking semen stains. Two materials were found positives with the Handscope®, three with STK spray®, while all were detected with the Blue-Crime-lite® 2, though with a low signal for two of them.

Discussion

STK spray®, recently launched to detect semen on objects and hard surfaces, has been evaluated. We found this new product easy to prepare and easy to use. The fluorescent signal appears rapidly, within a few seconds, in the presence of semen. The examiner does not need a specific filter to read the result nor to photograph the evidence. Florescence persistence varied from a few minutes to a few days, depending on the tested materials, leaving enough time for the examiner to photograph and sample the stain.

Its specificity to semen was confirmed compared to most body fluids. Its sensitivity is similar to the one of STK Lab® [13].

When compared to ALS, it outperformed both the Handscope® and the blue Crime-lite® 2, at least on tested materials. Moreover STK spray® offered a clearer result as the spot "lighted up" under UV light, confirming its nature, wich did not happen with ALS as they are not specific to semen.

In addition, STK spray® did not prevent spermatozoa from being microscopically detected and did not disturb further DNA analysis. It should be noted finally that the pure semen sample led to a complete DNA profile despite a high internal PCR control (Table 1), which could be explained by the sample's high DNA concentration (primer competition may be unfavorable to the internal control).

Thus, STK spray® reagent appears to be a valuable asset for sexual assault cases. However, STK spray® still demonstrated some limitations. First, a meticulous observation of the scene/material under UV light is essential prior to STK spray® use in order to assess the presence of unspecific fluorescence.

Table 2. Results of pure and diluted semen spots on 32 materials observed with either a HandScope® (CSS filter), a blue Crime-lite 2® both combined with an orange observation filter or STK spray® treatment. ++: clear and bright fluorescent signal. +: low fluorescent signal. -: no fluorescent signal. Negative controls are not shown for readability reasons (all were negatives).

		CSS Handscope®	Blue Crime-lite®	STK spray®
Sink Tent	Pure semen	++	++	++
	1/10 diluted semen	++	++	++
	1/20 diluted semen	++	++	++
Grey glazed-tile Grey tile	Pure semen	++	++	++
	1/10 diluted semen	+	++	++
	1/20 diluted semen	+	+	++
Tent floor Door handle	Pure semen	++	++	++
	1/10 diluted semen	+	+	++
	1/20 diluted semen	+	+	++
Grey laminated flooring Black shoe sole	Pure semen	++	++	++
	1/10 diluted semen	+	+	++
	1/20 diluted semen	-	+	++
Brown shoe sole White shoe sole Black sport shoe	Pure semen	++	++	++
	1/10 diluted semen	+	+	++
	1/20 diluted semen	-	-	++
"Dark wood" vinyl flooring	Pure semen	++	++	++
	1/10 diluted semen	-	+	++
	1/20 diluted semen	-	+	++
Grey vinyl flooring	Pure semen	++	++	+
	1/10 diluted semen	+	+	+
	1/20 diluted semen	-	-	-
Concrete block	Pure semen	++	++	++
Tree branch Tree leaves	1/10 diluted semen	-	-	++
White leather shoe	1/20 diluted semen	-	-	++
Wood paneling	Pure semen	++	++	++
	1/10 diluted semen	-	-	++
	1/20 diluted semen	-	-	+
Dark car rug Blue carpeting	Pure semen	+	++	++
	1/10 diluted semen	-	-	++
	1/20 diluted semen	-	-	++
"Light wood" vinyl flooring Beige suede leather shoe	Pure semen	+	+	++
	1/10 diluted semen	-	-	++
	1/20 diluted semen	-	-	++
Pebbles set in a concrete stone	Pure semen	+	+	++
	1/10 diluted semen	-	-	++
	1/20 diluted semen	-	-	+
Concrete paving stone	Pure semen	+	++	++
	1/10 diluted semen	-	-	++
	1/20 diluted semen	-	-	-
Asphalt sidewalk	Pure semen	-	++	++
	1/10 diluted semen	-	-	++
	1/20 diluted semen	-	-	+
Red carpeting	Pure semen	-	+	++
	1/10diluted semen	-	-	++
	1/20 diluted semen	-	-	++
Sea grass flooring Grass Black suede leather shoe Faux leather handbag	Pure semen	-	-	++
	1/10 diluted semen	-	-	++
	1/20 diluted semen	-	-	++
Wallpaper	Pure semen	++	++	++
	1/10 diluted semen	-	-	-

	1/20 diluted semen	-	-	-
Brown leather sandals	Pure semen	-	-	++
	1/10 diluted semen	-	-	-
	1/20 diluted semen	-	-	-

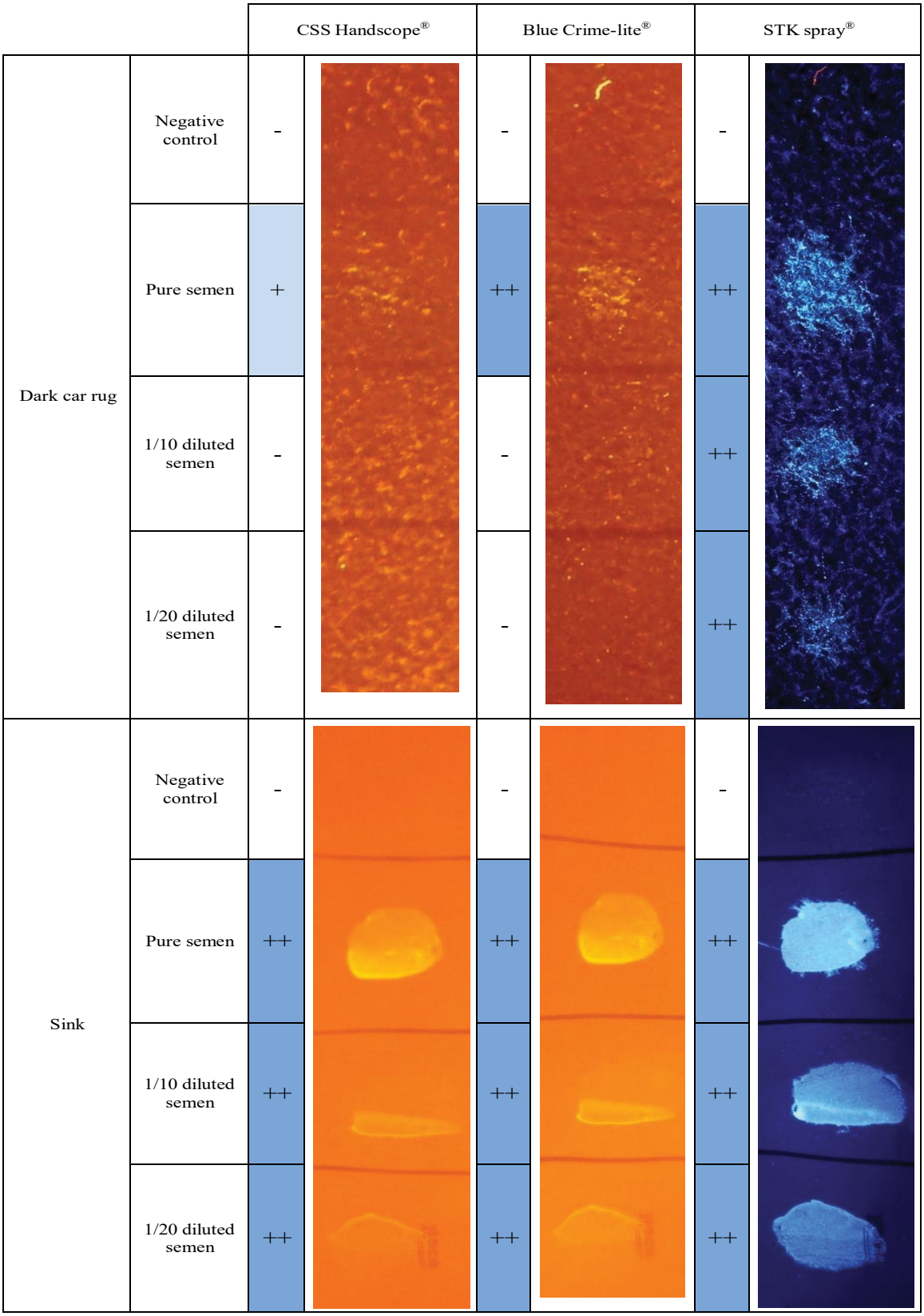


Figure 5. Photographs and associated interpretations of pure and diluted semen spots on 2 chosen materials observedwith a HandScope® (CSS filter), a blue Crime-lite 2® both combined with an orange observation filter or with STK spray® treatment. ++: clear and bright fluorescent signal. +: low fluorescent signal. -: no fluorescent signal.

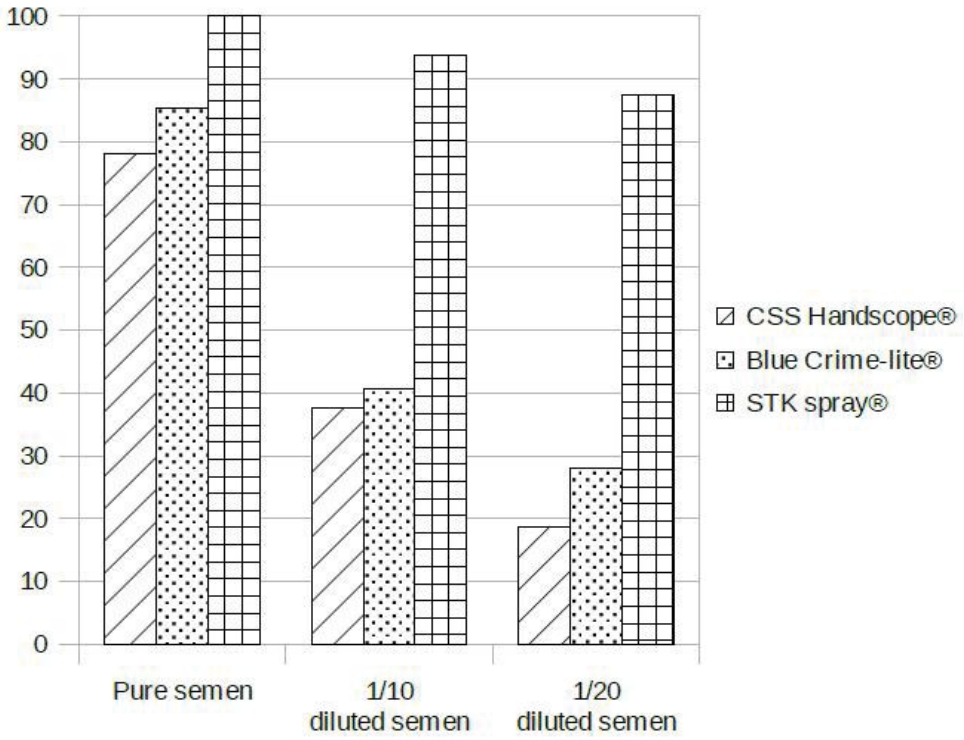


Figure 6. Percentage of pure and diluted semen spots on 32 materials successfully detected with a HandScope® (CSS filter), a blue Crime-lite 2® both combined with an orange observation filter or with STK spray® treatment. Both + and ++ results are considered positive results.

		CSS Handscope®	Blue Crime-lite®	STK spray®
Grey tile	Pure semen wiped by hand	++	++	++
	Pure semen wiped with a watered paper towel	-	+	++
Grey vinyl flooring	Pure semen wiped by hand	-	+	-
	Pure semen wiped with a watered paper towel	-	-	-
Grey laminated flooring	Pure semen wiped by hand	-	+	++
	Pure semen wiped with a watered paper towel	-	-	++
Sink	Pure semen wiped by hand	++	++	++
	Pure semen wiped with a watered paper towel	-	-	++

Figure 7. Pure semen spots on 4 materials wiped by hand or with a watered paper and observed with a HandScope® (CSS filter), a blue Crime-lite 2® both combined with an orange observation filter or with STK spray® treatment. Results are expressed using ++: clear and bright fluorescent signal, +: low fluorescent signal, -: no fluorescent signal.

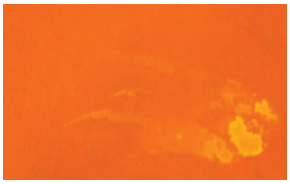
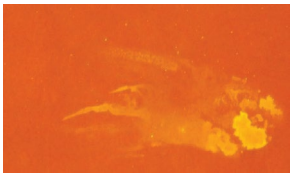
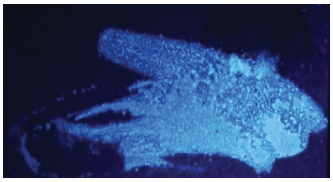
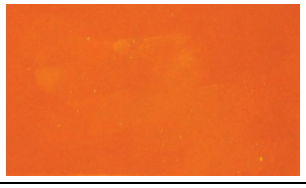
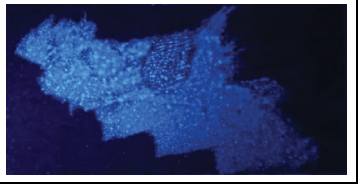
	CSS Handscope®	Blue Crime-lite®	STK spray®
Pure semen wiped by hand			
Pure semen wiped with a watered paper towel			

Figure 8. Photographs illustrating the results of the experiment described on Figure 7, for grey tile.

Then, we also recommend spraying the reagent under UV light, as any increase of the background fluorescence would indicate a positive result.

Second, although a single application of STK spray® appeared sufficient for most tests, a second spraying of the reagent 5 minutes later helped to achieve a better signal, especially at the found limit of detection of the reagent.

Third, when screening for semen, the examiner must be aware of STK spray® detection limits: high semen dilutions down to 1/1000 were not detected, although spermatozoa were found and a quasi- complete DNA profile was obtained. Our results also demonstrated that blood and feces disturbed semen detection. Then, if the examiner suspects the presence of a concealing amount of blood or feces, we would recommend to systematically analyze such stains in a laboratory for spermatozoa screening, even if they appear negative after using STK spray®. However, feces are more often found on pieces of clothes, tissues or toilet paper that should preferably be examined with STK Lab®, which did not show this restriction [13].

Fourth, it should be finally noted that on all the 32 different materials tested here, only grey vinyl flooring showed mixed results: pure semen was successfully detected with STK spray® but with a low signal, and wiped stains were both negative. Then, some specific surfaces could cause limitations, though it seems a less critical issue than with ALS.

Conclusion

To conclude, although we highlighted some limitations to STK spray®, we believe the benefits of using a specific detection method surpass its constraints. This new technique is a useful tool for semen detection. It can be used both on its own and in addition to ALS, on crime scenes as well as in forensic laboratories. STK spray® showed promising results, and is likely to be widely used in a near future.

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Disclosure Statement

No potential conflict of interest is reported by the authors. Lamps used in this study are properties of the french national department of forensic police (SNPS). Axo science is a supplier of the Laboratory of Scientific Police of Lyon (LPS). All products used in this study were commercially purchased.

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