Increased Accuracy and Precision in the Detection of Human Semen on Clothing Fabrics using the STK ™ Sperm Tracker STK Lab

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Introduction

The detection and identification of seminal fluid is a critical step in forensic analyses, where these stains can be present on items collected at a crime scene or from a sexual assault evidence collection kit. The alternate light source, or ALS, is the first analytical tool that is used to screen evidence for the presence of body fluids, including semen. Also, presumptive tests, such as the acid phosphatase spot test, are often employed to provide support for the use of future confirmatory tests or DNA analysis. However, ALS and the AP spot test are not quite specific or sensitive, as other types of body fluids and chemical reagents can fluoresce under ALS and react with the AP test, making the search and identification of semen stains potentially difficult and time-consuming. Additionally, diluted semen or semen mixed with other bodily fluids can go unnoticed. This study investigates the sensitivity and specificity of the STK Sperm Tracker - STK Lab paper for semen stains on clothing made from a diverse array of fabric types of varying colors and thicknesses. This product has chemical reagents impregnated onto one side of the paper that reacts with human seminal acid phosphatase, producing a bright fluorescence when viewed under a 365nm UV light. The STK Lab paper is compared to the ALS method in the screening of evidence for the presence of semen on clothing fabrics.

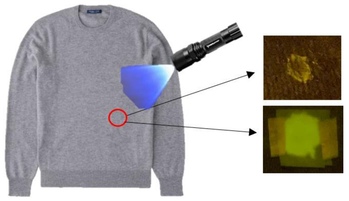


Figure 1. Using ALS to visualize semen stains by itself vs. with STK Lab paper

- Evaluate the sensitivity, and specificity of STK Sperm Tracker – STK Lab in the detection and identification of semen on clothing fabrics
- Compare the fluorescence of 455nm Arrowhead Forensics ALS to STK Lab with 365nm Vilber VL-6.L ALS
- Assess the capability of STK Lab to detect and identify semen on clothing fabrics of various colors, thicknesses, and material, as well as when semen is mixed with another bodily fluid, blood.

Methods

Preparation and Deposition of Biological Samples: Neat semen, neat blood, 1:1 ratio of semen and blood, and 1:10 and 1:50 semen dilutions were prepared in 5 mL test tubes. The 1:10 and 1:50 semen dilutions were made with PCR water. 50 µL of each sample were deposited in triplicate onto 11 different clothing items (Figure 2). Total of 15 stains per clothing item. The stains were deposited blindly so the experimenter was not aware of the stain locations prior to screening.

Observation of Stains with ALS: The clothing items were screened for stains using the Arrowhead Forensics (455nm) handheld ALS. Viewed through the orange filter goggles, pictures were taken with an iPhone 13 Plus. A fluorescence grading system was created based on the fluorescence of each stain on the clothing item with Arrowhead (Figure 3). Each stain was assigned a number, 0 to 3, based on their fluorescence using the fluorescence grading system (Figure 3). The average fluorescence of stains with Arrowhead based on their stain type, fabric color, and fabric material were calculated (Table 1).

Item Number	Clothing description
1	Black and grey plaid fleece pullover sweater
2	White/ivory acrylic knitted scarf
3	Red with white polka dots satin blouse
4	Grey wool sweater
5	Red cotton flannel shirt
6	Dark blue cotton denim jeans
7	Black polyester leggings
8	Black faux fur coat
9	White lace underwear
10	Black nylon + spandex underwear
11	White cotton socks

Figure 2. The 11 clothing items and their description.

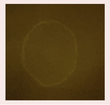
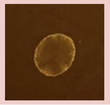
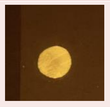
Rating	Example Reference Photo
0 (no fluorescence)	
1 (slight fluorescence)	
2 (moderate fluorescence)	
3 (high fluorescence)	

Figure 3. Fluorescence grading system with Arrowhead 455nm light source.

Observation of Stains with STK Lab: Following the STK Lab protocol provided by AXO Science, the absorbent side of pieces of STK Lab was saturated with distilled water and taped onto the stains. The clothing items were sandwiched in the following order bottom to top: one piece of plexiglass, the ziplock bag with the cloth, the clothing item, second piece of plexiglass, and a 32lb microscope stand (Figure 4). After 7 minutes of pressing, the STK Lab was viewed with the Vilber ALS (365nm), taking pictures of all stains with Vilber and orange filter goggles. Each stain was assigned a number, 0 to 3, based on their fluorescence using the fluorescence grading system. The average fluorescence of stains with Vilber ALS based on their stain type, fabric color, and fabric material were calculated. A paired t-test was conducted with a confidence interval of 95% for the overall Arrowhead and STK Lab + Vilber results by stain type for their significance regarding fluorescence of stains (Table 1).

Methods: *Continued*

Figure 4. Overview photos demonstrating the pressing method. Stains on Item #8 were pressed, front and back.

Results

ALS Overall: Neat semen fluoresced the greatest with a slight to moderate fluorescence (1.45 ± 1.18). Almost slight fluorescence for 1:10 and 1:50. No fluorescence with 1:1 semen + blood (Table 1).

STK Lab Overall: Neat semen and 1:1 semen + blood fluoresced the greatest with a moderate to high fluorescence. Maintained a slight to moderate fluorescence with both 1:10 and 1:50 semen stains. The most significant finding was the fluorescence of the 1:1 semen + blood stains which exhibited no fluorescence with Arrowhead (455nm) and moderate to high fluorescence with STK Lab + Vilber (Table 1).

T-test: To determine if there is a significant difference between the average fluorescence results of the Arrowhead (455nm) and STK Lab + Vilber, a t-test was performed. The null hypothesis is Arrowhead and STK Lab + Vilber produce the same results, resulting in no difference in fluorescence values of semen stains. The p-values of the neat semen, 1:10, 1:50, and 1:1 semen + blood are all significant ($\alpha = 0.05$) (Table 1). These results provide support for the hypothesis that STK Lab + Vilber allows for better detection and visualization of semen stains.

Stain	# of stains	Mean Fluorescence Arrowhead 455 nm	Mean Fluorescence STK Lab + Vilber 365 nm	Δ Fluorescence	*p*-value ($\alpha = 0.05$)
Neat Semen	**33**	**1.45 ± 1.18**	**2.82 ± 0.58**	**1.37**	**< 0.05**
1:10 Semen	**33**	**0.45 ± 0.75**	**1.48 ± 1.35**	**1.03**	**< 0.05**
1:50 Semen	**33**	**0.18 ± 0.46**	**1.12 ± 1.17**	**0.94**	**< 0.05**
1:1 Semen + Blood	**33**	**0.00 ± 0.00**	**2.82 ± 0.58**	**2.82**	**< 0.05**
Neat Blood	**33**	**0.00 ± 0.00**	**0.06 ± 0.24**	**0.06**	**N/A**

Table 1. Average fluorescence of stains by stain type with Arrowhead 455nm ALS and STK Lab + Vilber 365nm and t-test results.

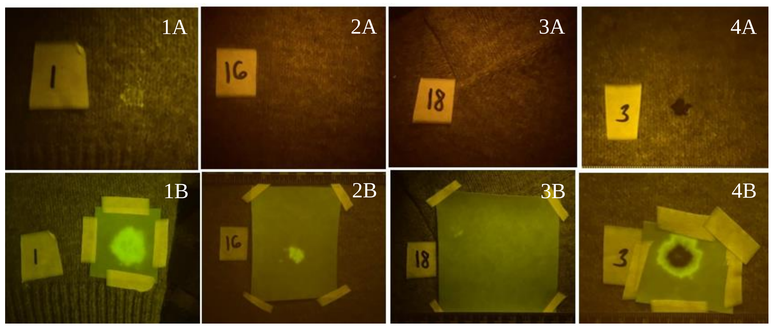


Figure 5. Example stain photos of Item 4 (grey wool sweater). For each pair of photos, (A) represents stains with Arrowhead 455nm and (B) with STK Lab + Vilber 365nm. 1 is neat semen, 2 is 1:10, 3 is 1:50, and 4 is 1:1 semen + blood.

Results: *Continued*

ALS by Fabric Color: Fabric colors are categorized as follows: light colors (white and grey), red, and dark (black and dark blue). Higher average fluorescence was detected with light colors compared to red and dark colors (Figures 6 and 7). Neat, 1:10, and 1:50 fluoresced with light colors while only neat semen fluoresced with red and dark (data not shown).

STK Lab by Fabric Color: There was an improvement in fluorescence of semen stains with all colors (Figures 6 and 7). Dark colors displayed the most significant improvement with the fluorescence of all types of semen stains: neat, 1:10, 1:50, and 1:1 semen + blood (Figure 7). Light colors did not show as much of an improvement due to the already established high fluorescence values of stains with ALS, but they still displayed moderate to high fluorescence with all semen stains. The red colored clothing exhibited the least improvement in fluorescence. The neat and 1:1 semen + blood presented a moderate to high fluorescence, 1:50 a very small average fluorescence (0.50 ± 1.22), and no fluorescence with 1:10 (data not shown).

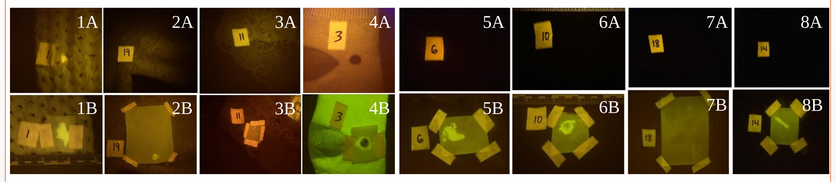


Figure 6. Example photos of light colors. For each pair of photos, (A) represents stains with Arrowhead 455nm and (B) with STK Lab + Vilber 365nm.(1) Item 2 - neat semen, (2) Item 4 - 1:10, (3) Item 9 - 1:50, and (4) Item 11 - 1:1 neat semen + neat blood.

Figure 7. Example photos of dark colors. For each pair of photos, (A) represents stains with Arrowhead 455nm and (B) with STK Lab + Vilber 365nm. (5) Item 7 - neat semen, (6) Item 8 - 1:10, (7) Item 10 - 1:50, and (8) Item 1 - 1:1 neat semen + neat blood.

ALS by Fabric Material: Acrylic and Cotton displayed the highest fluorescence values with neat, 1:10, and 1:50. Polyester and Nylon + Spandex were the most difficult in visualizing semen stains with only neat semen stains shown at slight fluorescence (data not shown). Only neat and 1:10 semen stains fluoresced on Wool.

STK Lab by Fabric Material: There was an improvement in fluorescence of semen stains on all fabrics. Polyester and Nylon + Spandex exhibited the most improvement with semen and semen + blood stains with slight to high fluorescence. All fabric types had a drastic improvement in the fluorescence of 1:1 semen + blood stains with a fluorescence of 2.75-3 on the fluorescence scale (data not shown).

Conclusion

Current presumptive methods for the detection and visualization of semen on pieces of evidence, including clothing, such as ALS and the AP spot test, are not very specific or sensitive. Other body fluids, household chemicals, or topical products can fluoresce with ALS [1]. Also with the AP test, other bodily fluids can give a false positive or positive results do not appear within the approximate 1 minute time frame some forensic laboratories use when looking for a positive result [2]. Diluted semen stains or semen mixed with other bodily fluids like blood can go unnoticed with these presumptive tests and it can be time-consuming to search for these stains. Confirmatory tests that currently exist such as the RSID™ – Semen kit and ABAcard® p30 require various chemical reagents and lab equipment, which can be time-consuming as well [3].

The fluorescence of all semen stains was improved with the use of the STK Lab viewed with the Vilber 365nm ALS. Neat, 1:10, and 1:50 semen stains and 1:1 semen + blood stains exhibited a slight to high fluorescence with STK Lab with Vilber (1.12 ± 1.17 to 2.82 ± 0.58 out of 3) while neat, 1:10, and 1:50 semen stains exhibited slight fluorescence (0.18 ± 0.46 to 1.45 ± 1.18 out of 3), and no fluorescence with 1:1 semen + blood stains with Arrowhead 455nm ALS. The results show that STK is sensitive for semen up to a 1:50 dilution and specific to semen even when mixed with blood. This new method allows for an easy, efficient manner, to detect and identify semen stains on clothing fabrics of various colors, thicknesses, and compositions. These results demonstrate the sensitivity and specificity of STK Sperm Tracker – STK Lab.

Further testing can be performed using the STK Lab paper on clothing fabrics of a wider variety of colors and fabric materials. Additionally, more dilute samples of semen and other body fluids can be utilized to further examine its sensitivity and specificity for semen.

References

[1] Pollitt, E. N., Anderson, J. C., Scafide, K. N., Holbrook, D., D'Silva, G., & Sheridan, D. J. (2016). Alternate Light Source Findings of Common Topical Products. Journal of forensic nursing, 12(3), 97–103. https://doi.org/10.1097/JFN.0000000000000116
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