Detection of Semen Stains on Household and Vehicle Fabrics Using STK Sperm Tracker Lab

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Introduction

The detection of semen is critical in the investigation of sexual assaults. Initial screening for semen alternative light source (ALS) and the detection of potentially relevant stains can be influenced by the type of fabric on which semen stains may be deposited, some of which make the detection with an ALS more difficult. The ability to detect semen stains is greatly affected by the circumstances, since stains may appear different on different fabric colors and materials.[1] Additional difficulties are experienced when semen is mixed with another fluid, such as blood, since there may be no fluorescence of the mixed stains under ALS.

Beyond the use of ALS, a current test method for the presence of semen is the Acid Phosphatase (AP) test. There is a cut-off time to observe a positive reaction, which has shown to be insufficient to obtain positive results for some semen dilutions.[2] The reaction time increases with dilutions, and in some cases, semen stains of dilutions greater than and equal to 1:20 have yielded negative results due to the reaction occurring after the cut-off time.

This research project focused on the use of STK Sperm Tracker Lab along with ALS as a presumptive test for human semen. STK Lab is impregnated paper that reacts with acid phosphatase, making it specific to semen. This product does not damage DNA, allowing for subsequent DNA testing.[3]

Objective: **To compare STK Lab + ALS with ALS alone in the detection of semen stains on various household and vehicle fabrics. Specifically, compare the fluorescence of semen stains under ALS with and without STK Lab.**

Methods

Fabrics: Nine fabrics were used: white bedsheet, black satin pillowcases, gray microfiber bathmat, brown medium pile carpet, gray flat rug, white leather, black cloth car headrest, black vehicle carpet, and blue/beige decorative pillows.

Stains: Five stain types were used: neat semen, 1:10 semen dilution, 1:50 semen dilution, 1:1 blood-semen, and neat blood. Three stains of each solution were deposited on each fabric, for a total of 15 stains/fabric. They were deposited without prior knowledge to the primary screener.

Stain Detection: All items were searched with Arrowhead 455nm, Rofin Flare 2 365nm, and Vilber 365nm ALS. Detected stains were photographed under ALS with an orange filter.

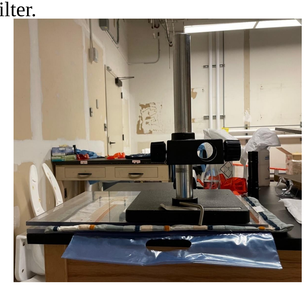


Figure 1. Visual depiction of the pressing method.

STK Lab: For each ALS detected stain, a piece of STK Lab paper was cut to an appropriate size. The absorbing side of the paper was wet with deionized water, placed absorbing side down on the stain, and taped in place. The item was pressed with uniform weight (~32 lb) for 7 minutes (figure 1).

STK Lab + ALS: After pressing, without removing the STK Lab paper, the stains were viewed with 365nm ALS. The stains were photographed under ALS with an orange filter. Any stains that fluoresced were circled directly on the paper, with precise boundaries.

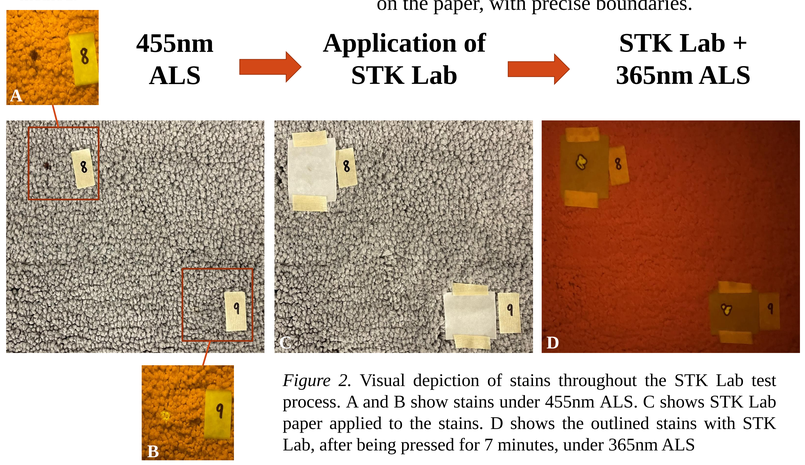


Figure 2. Visual depiction of stains throughout the STK Lab test process. A and B show stains under 455nm ALS. C shows STK Lab paper applied to the stains. D shows the outlined stains with STK Lab, after being pressed for 7 minutes, under 365nm ALS

Methods: *Continued*

Data Analysis: A fluorescence intensity rating system of 0-3 was created, see table 1. All stains were graded according to this fluorescence rating system; for each stain, a rating was given for each ALS before and after STK Lab.

The average fluorescence rating was calculated for each stain type. The change in average fluorescence rating was calculated between the Arrowhead 455nm ALS and the STK Lab + Vilber 365nm ALS. The average fluorescence ratings with and without STK Lab were compared using paired t-tests, alpha 0.05.

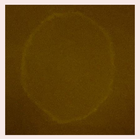
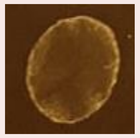
Rating	0 (No fluorescence)	1 (Slight fluorescence)	2 (Moderate fluorescence)	3 (High fluorescence)
Example				

Table 1. Fluorescence rating examples, Arrowhead 455nm LED light source

Results

Overall Results: For all stains containing semen, the average fluorescence was greater with STK Lab than without (table 2). Neat blood did not fluoresce with or without STK Lab. The greatest increase in fluorescence with the use of STK Lab was observed for the 1:1 blood-semen mixture stains, which did not fluoresce with ALS alone but had clear fluorescence with STK Lab + ALS.

		Average Fluorescence			
Stain	# of Stains	Arrowhead 455nm ALS	STK Lab + Vilber 365nm	ΔFluorescence	P value (alpha 0.05)
Neat semen	27	2.04 ± 1.02	2.85 ± 0.60	0.81	< .001
1:10 semen	27	1.00 ± 0.83	2.33 ± 0.83	1.33	< .001
1:50 semen	26*	0.62 ± 0.70	1.50 ± 1.24	0.89	< .001
1:1 blood-semen	27	0.00 ± 0.00	2.96 ± 0.19	2.96	< .001
Neat blood	27	0.00 ± 0.00	0.00 ± 0.00	0.00	

Table 2. The average fluorescence of stains for each stain type with and without STK Lab. The p values were calculated using paired t-tests. *One 1:50 semen dilution stain was not found with ALS or STK Lab.

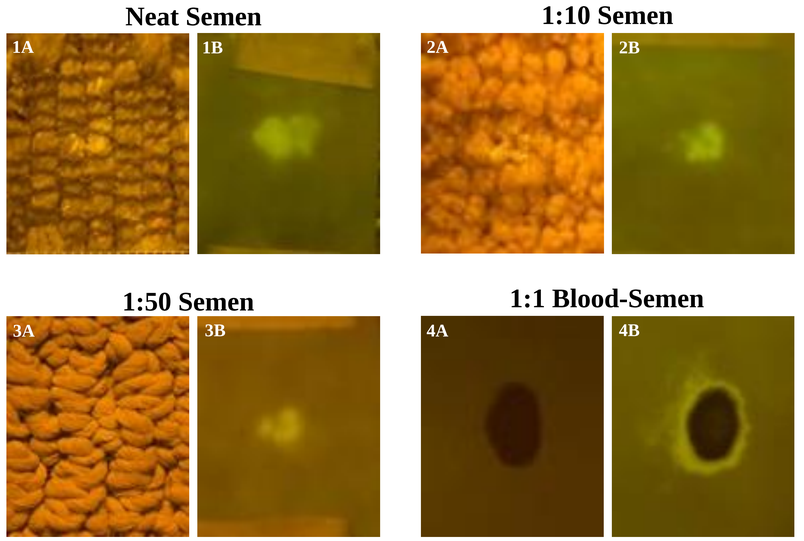


Figure 3. For each pair of images, (A) represents stains with 455nm ALS only and (B) represents stains with STK Lab + 365nm ALS. 1 is a neat semen stain on a flat gray rug; 2 is a 1:10 semen dilution stain on a gray bathmat; 3 is a 1:50 semen dilution stain on a brown medium pile carpet; 4 is a 1:1 blood-semen stain on a white bedsheet.

Results: *Continued*

For all stain types containing semen, there were stains that were undetected with 455nm ALS only but detected with STK Lab + 365nm ALS. STK Lab resulted in the fluorescence of an additional 2 neat semen stains, an additional 7 1:10 semen dilution stains, an additional 6 1:50 semen dilution stains, and an additional 27 1:1 blood-semen stains.

With STK Lab, there was a greater increase in the fluorescence of stains on darker fabrics than on lighter fabrics (figure 4).

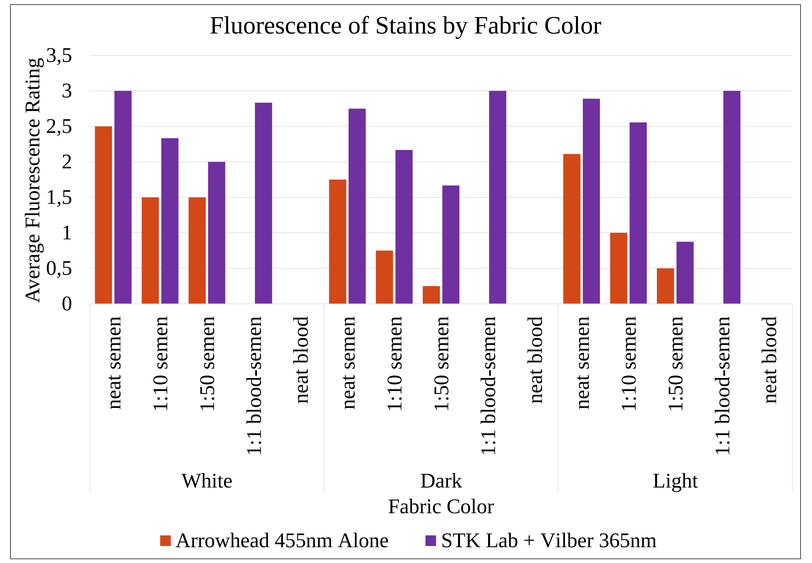


Figure 4. Visualization of the average fluorescence of stains before and after STK Lab, by fabric color. White fabrics included the bedsheet and leather; black fabrics included the pillowcases, vehicle carpet, and car headrest; light fabrics included the rug, bathmat, and decorative pillows; and dark fabrics included the medium pile carpet.

Considerations: The pressing method used was developed based on a series of test samples. The method has not been validated, so any errors or inconsistencies associated with this method are unknown.

Conclusion

Current methods of testing for the presence of semen, such as the AP test, are effective with neat semen stains but may be unreliable at detecting dilute semen within the allotted time.[2] This creates uncertainties in if all semen stains were detected, regardless of dilution. In addition, the use of the paper may be an easier process, where test solutions don't need to be prepared daily.

In this study, STK Lab has been shown to be effective at detecting various semen stains, including dilutions of up to 1:50 and mixtures with human blood. The use of STK Lab with 365nm ALS resulted in significantly more fluorescence of semen stains than the use of ALS alone. Greater fluorescence of semen stains would allow for better detection of the stains.

References

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[3] AXO Science. STK Sperm Tracker: STK Lab: Directions for use. STK_Lab_notice_EN_V1.2. 2022 Jan 7.

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