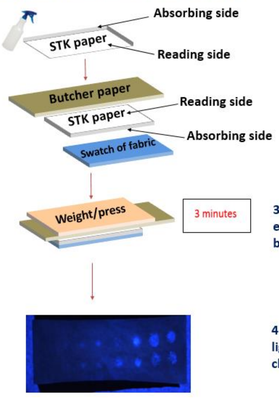
Internal Validation of the STK Sperm Tracker Procedure

Jennifer Paynton, B.S., Lauren Lukens, M.S., and William Hudlow, M.S.
State of California, Department of Justice, Jan Bashinski DNA Laboratory

Abstract

Locating and identifying semen stains on evidence items can be the most labor intensive step in processing items for DNA analysis. This is especially true for large items that either fluoresce or quench, and/or contain numerous stains that fluoresce, under an alternate light source. Traditional overlay methods used in seminal fluid searches depend on colorimetric methods to indicate the presence of acid phosphatase, but are not specific to seminal acid phosphatase, and may be masked by the presence of other materials deposited on the surface of the substrate. The fluorescence based *STK Sperm Tracker* method is reported to have a higher specificity to seminal acid phosphatase, and may have a number of advantages over the traditional overlay methods.

STK Sperm Tracker Basics: 4 Simple Steps



1. Moisten absorbing side of STK paper with DiH_2O

2. Place absorbing side on the item with the reading side up and a barrier, like butcher paper over the STK paper

3. Apply pressure by placing a weight or press, e.g. an ~ 18" x 18" kitchen tile on top of the butcher paper, and set a timer for 3 minutes

4. Read the results of the STK paper using a light source with a 365 nm wavelength and clear filter to visualize the semen stains

Materials and Methods

Examination of Biological Stains:
Consisted of a visual examination, an Alternate Light Source (ALS) examination, and an STK Sperm Tracker examination using STK paper.
The STK Sperm Tracker paper is manufactured by AXOScience

Alternate Light Source (LEEDS LSV2 system):
For ALS examination – 455 nm - blue light – orange filter was used.
For STK examination – 365 nm - UV light – clear filter was used.

GBC Overlay Method
The α-naphthyl phosphate/GBC overlay method consists of moistening a piece of filter paper with DiH_2O and applying the moistened side of the filter paper in contact with the item, applying weight/pressure for 3 minutes, and then spraying the filter paper with the α-naphthyl phosphate/ GBC chemical reagent, which is a mixture of α-naphthyl acid phosphate, Fast Garnet GBC salt, and Citric Acid Buffer. After applying the chemical solution to the filter paper, the paper is monitored and any color changes are recorded for up to 5 minutes.

Samples:
Semen, blood, saliva, urine, sweat, vaginal, rectal, and case type samples were provided by laboratory staff with informed consent.

Mixture: Known semen from a single source was diluted in blood, saliva, urine and water at mixture ratios of 1:2, 1:10, 1:20, 1:30, 1:40, 1:50, and 1:60. 25 µL aliquots of each dilution were transferred in duplicate to two black and two gray fabric swatches, which were allowed to air-dry overnight. Each stained fabric swatch was examined with the 455 nm Leeds alternate light source prior to testing one of each of the fabric swatches with either the α-naphthyl phosphate/GBC overlay method or the STK Sperm Tracker/Leeds 365 nm ALS method.

Sensitivity: Semen dilutions (1:10, 1:20, 1:30, 1:40, 1:50, 1:60, 1:70, 1:80, 1:90, and 1:100) from three known sources were transferred in duplicate in 25 µL aliquots to two sets of three fabric swatches, which were allowed to air-dry overnight. The fabric swatch sets were examined with the 455 nm Leeds alternate light source (ALS) and subsequently tested with either the α-naphthyl phosphate/GBC overlay method or the STK Sperm Tracker/Leeds 365 nm ALS method.

Stability: Known semen from a single source was diluted in blood, saliva, urine and water at mixture ratios of 1:10, 1:20, 1:30, 1:40, 1;50 and 1:60. 25 µL aliquots of each dilution were transferred in duplicate to each of five gray fabric swatches, which were allowed to air-dry overnight. One stained fabric swatch was processed as a zero time point with additional testing at monthly intervals to test the stability of the STK Sperm Tracker paper beyond the one-year and the acid phosphatase activity in various diluents.

Specificity: Blood, saliva, urine, sweat, semen free vaginal secretions, and fecal material from multiple individuals were either transferred in 25 µL aliquots to a white fabric substrate or collected with cotton swabs and allowed to air-dry overnight. The stains were examined with the 455 nm Leeds alternate light source (ALS) and then processed with the STK Sperm Tracker/Leeds 365 nm ALS method.

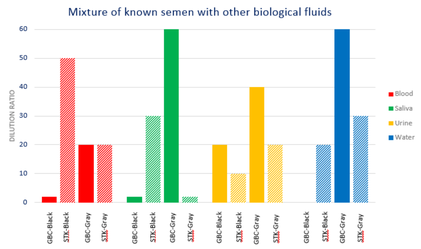
Reproducibility & Repeatability: Semen dilutions (1:2, 1:10, 1:20, 1:30, 1:40, 1:50, and 1:60) from one known source were transferred in duplicate in 25 µL aliquots to three black fabric swatches, which were allowed to air-dry overnight. Each of two analysts tested one stained fabric swatch with the STK Sperm Tracker/Leeds 365 nm ALS method to demonstrate the reproducibility of the STK Sperm Tracker method and one analyst tested two of the stained fabric swatches to demonstrate the repeatability of the STK Sperm Tracker method.

Case Type Samples: Six case type samples (e.g. underwear that had been worn under various conditions and/or spotted with 1:2 and 1:10 semen dilutions) that may or may not contain semen were examined as follows. Visual, 455 nm Leeds alternate light source (ALS) and STK Sperm Tracker/Leeds 365 nm ALS method examinations of each of six items. These blind samples consisted of three pairs of panties and three pairs of cotton briefs with one of each being worn post-coitus (M1 & F1), one of each being worn without coitus (M2 & M3) and one of each being worn/washed with 1:2 and 1:10 semen dilutions being transferred to 50 uL and 100 uL aliquots.

Results

Mixture Study

The STK Sperm Tracker method (STK) was observed to perform as well, or better than the α-naphthyl phosphate/GBC method (GBC) with the blood. This is potentially due to the colorimetric GBC test being masked by the red blood cells.



The lower sensitivity of the STK Sperm Tracker method on the gray fabric is likely due to the limited absorptive ability of the surface of the synthetic material and the wicking of the semen into the under layer. When working with substrates that fluoresce, reading the STK paper off the substance on a dark background is recommended as was done with the gray fabric in this study.

Specificity Study

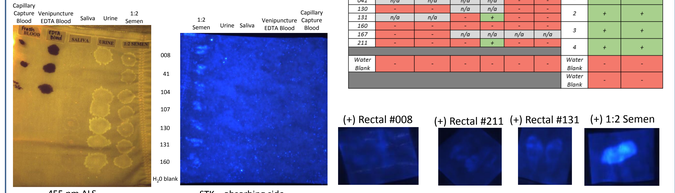
The specificity of the STK Sperm Tracker method was found to be very good with only the rectal swabs (3 of 4) yielding weak positive results. As expected, the STK method was more specific than the ALS examination for detecting biological stains with high concentrations of acid phosphatase.

White Fabric:

Donor QCA#	Blood (Capillary Puncture) ALS	Blood (Capillary Puncture) STK	Blood (Venipuncture/EDTA) ALS	Blood (Venipuncture/EDTA) STK	Saliva ALS	Saliva STK	Urine ALS	Urine STK	1:2 Semen (Positive Control) ALS	1:2 Semen (Positive Control) STK
008	-	-	-	-	+	-	+	-	+	+
041	-	-	-	-	+	-	n/a	n/a	+	+
104	-	-	-	-	+	-	+	-	+	+
107	n/a	n/a	n/a	n/a	-	-	+	-	+	+
130	n/a	n/a	n/a	n/a	+	-	+	-	+	+
131	n/a	n/a	n/a	n/a	+	-	+	-	+	+
160	n/a	n/a	n/a	n/a	+	-	+	-	+	+
Water Blank	-	-	-	-	-	-	-	-	-	-

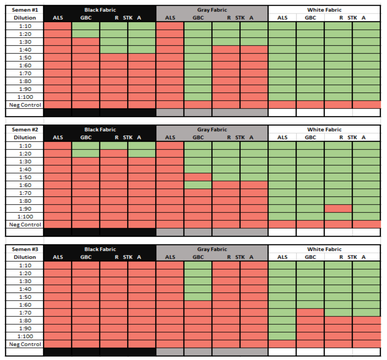
Swabs:

Donor QCA#	Semen free vaginal swabs ALS	Semen free vaginal swabs STK	Rectal swabs ALS	Rectal swabs STK	Sweat swabs ALS	Sweat swabs STK	Swab #	1:2 Semen (Positive Control) ALS	1:2 Semen (Positive Control) STK
008	n/a	n/a		+	n/a	n/a	1	+	+
041	n/a	n/a	n/a	n/a	-	-			
130	-		n/a	n/a	-	-	2	+	+
131	n/a	n/a	-	+	-	-			
160	-	-	-	-	-	-	3	+	+
211	-	-	-	+	-	-			
							4	+	+
Water Blank	-	-	-	-	-	-	Water Blank	-	-
							Water Blank	-	-



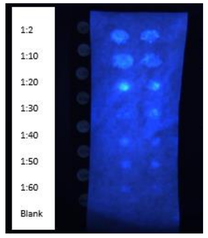
Sensitivity Study

The STK Sperm Tracker method was comparable to the GBC method in detecting acid phosphatase from known semen dilutions. Both the GBC and STK methods performed better than ALS in detecting stains on dark fluorescent fabrics.

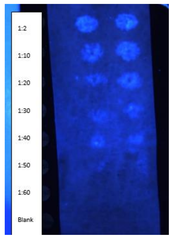


Reproducibility and Repeatability Studies

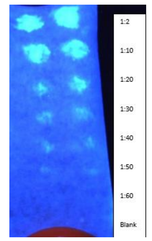
The reproducibility and repeatability of the STK method was observed to be good as detection of the 1:50 known semen dilutions was achieved by each analyst with all three of the stained fabric swatches.



Swatch #1 - Analyst 1 - Day 1
STK absorbing side, detected 1:60



Swatch #2 - Analyst 2 - Day 1
STK absorbing side, detected 1:50



Swatch #3 - Analyst 2 - Day 2
STK absorbing side, detected 1:50

Stability Study

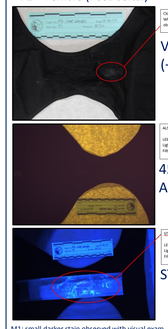
Semen Source QCA# 134 wk	Zero time* Swatch #1		Zero time Swatch #2		One month Swatch #3		Two months** Swatch #4		Additional swatch created & tested		Three months*** Swatch #5		Additional swatch created & tested	
Diluent	Row 1	Row2	Row 1	Row2	Row 1	Row2	Row 1	Row2	Row 1	Row2	Row 1	Row2	Row 1	Row2
Blood	NF	NF	1:20	1:20	1:30	1:20	1:10	1:10	N/A	N/A	1:10	1:10	N/A	N/A
Saliva	NF	NF	1:20	1:50	1:10	1:20	1:40	1:20	N/A	N/A	1:10	1:20	N/A	N/A
Urine	NF	NF	1:30	1:30	1:10	1:10	1:20	1:10	N/A	N/A	NF	1:10	N/A	N/A
Water	NF	NF	1:50	1:60	1:50	1:60	1:20	1:20	1:60	1:60	1:20	1:20	1:60	1:30
Blanks	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF

NF = No fluorescence observed
* The first swatch yielded no results, possibly due to STK package being heat sealed to the STK paper inside, the STK paper being too wet when applied to the fabric, or the extra that was weight applied. Re-tested using swatch #2 with new STK paper package (same lot number), careful not too apply to much water, and not add any extra weight.

A significant decrease in sensitivity was observed at the two and three month data points for the semen/water mixture. After re-testing freshly prepared semen stains with the same lot of STK paper, this was determined to be due to the decrease in the acid phosphatase activity in the samples when the substrate was stored at room temperature.

Case Type Samples

F1 – Female (Post Coital)

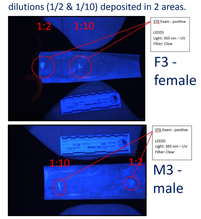


M1: small darker stain observed with visual exam, ALS(-),STK(-) Spermatozoa observed on slide prepared from this stain

F2 – Female (casual wear/no coitus)

M2: visual (-), ALS(-),STK(+) spot in rear crotch

F3/M3: Previously worn and washed with semen dilutions (1/2 & 1/10) deposited in 2 areas.



Five of the six semen stains on the underwear were detected with the exception being M1. The negative STK results for M1 are likely attributed to suboptimal storage conditions; since the underwear were stored at room temperature for ~2 months prior to testing. The M2 fluorescence is likely attributed to fecal material consistent with our results from testing rectal swabs. The STK Sperm Tracker method detected 83% of the stains compared to visual examination 33% and ALS examination 0%.

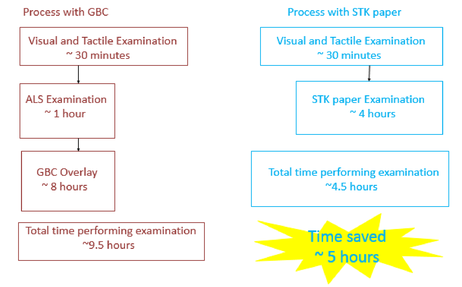
Conclusion

Collectively, the results from the STK Sperm Tracker validation indicate the STK method is a valid alternative to traditional overlay methods. The sensitivity of STK Sperm Tracker method was comparable to the GBC overlay method and both the STK method and GBC overlay method performed better than ALS in detecting stains on dark or fluorescent fabrics. Additionally, the specificity of the STK method and performance in the presence of blood-semen mixtures was observed to be greater than that of the GBC overlay method.

Since the STK method is a simple process that does not involve the mixing or application of chemical reagents it would be a valuable tool at crime scenes. This would be especially true in the examination of large items (e.g. mattresses or carpets) as an aid in narrowing down the evidence that needs to be collected and transported back to the laboratory for further examination.

Traditionally, biological screening for semen progresses from the least complicated and time consuming methods to the more complex and time consuming methods with the acid phosphatase overlay method being the last, most complicated and time consuming option. However, the simplicity of the STK Sperm Tracker method may negate the need for the nonspecific alternate light examination and therefore increase the efficiency of biological screening for semen.

For example: Estimated timeline for examination of a queen sized bedsheet (~90 x 102"):



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Jennifer Paynton
Jan Bashinski DNA Laboratory
1001 W. Cutting Blvd.
Richmond, CA 94804
jennifer.paynton@doj.ca.gov