Verification Study of STK® Sperm Tracker Spray

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ABSTRACT

The current protocol for the Los Angeles Police Department (LAPD) Field Investigation Unit when investigating a potential rape scene includes the use of an Alternate Light Source (ALS) with the Acid Phosphatase (AP) test to locate semen traces. The AP test for semen detection is very useful as a screening test; however, non-prostate sources of acid phosphatase exist that cause false positive results. In addition, the dye used in conjunction with AP is a carcinogenic hazard and requires proper disposal. Similar to the AP test, STK® Sperm Tracker Spray (STK® Spray) is a screening tool for the precise localization of semen traces at a potential rape scene by visually distinguishing semen from other bodily fluids. This verification study provides research conducted on STK® Spray (AXO Science) in collaboration with the LAPD Forensic Science Division to evaluate the product's usefulness for the Field Investigation Unit. The sensitivity and specificity of the STK® Spray were investigated. Furthermore, the luminescence intensity and effectiveness of the STK® reaction on various substrates were studied to evaluate the spray's performance across a range of potential rape scene locations.

The results showed that dilutions up to and including 1/1000 demonstrated a bright and lasting blue luminescence when exposed to UV light excitation, indicating a positive result. Five false positives were observed, including one bodily fluid (breast milk) and four cleaning agents (LA's Totally® Awesome All Purpose, LA's Totally® Awesome Orange, PINE-SOL®, and Scrubbing Bubbles®). Testing showed that semen traces deposited on non-porous substrates (metal, plastic, wood, and glass) provided superior luminescence results. The research presented in the present study demonstrated that STK® spray is efficient, sensitive, specific, and maintains a bright and persistent luminescence, allowing samples to be located efficiently and accelerating the current procedure used to locate semen traces. Previous studies state that STK® Spray does not interfere with DNA recovery rates. Additional testing is in process for confirmation, and the results will be presented to seminar attendees.

RESEARCH QUESTIONS

- Under what circumstances does STK® Spray produce the best results and when does it fail?
- Will it allow Criminalists to easily screen and document an area that cannot be taken back to the laboratory?

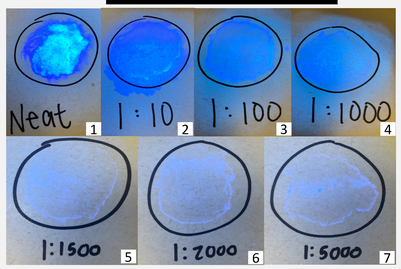
MATERIALS

- STK® Sperm Tracker Spray from AXO Science
  - One box with 10 pouches of STK® Spray
  - One box with 10 positive control sheets
- Flairosol® Spray bottle
- Crime-lite® 82S 350-380nm UV light
- Transparent UV goggles
- Poster paper
- Filter paper
- Swabs
- Nanopure® water
- Pipettes
- Semen from anonymous donors
- 32 different substrates
- Acid Phosphatase (AP) tests
- For specificity testing:
  - Bodily fluids: blood, saliva, urine, breast milk, and vaginal secretions
  - Food: Apple, carrot, and banana
  - 12 household cleaning products

METHODS

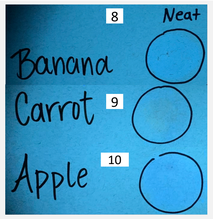
- **Sensitivity:**
  - Dilutions: NC, Neat, 1:10, 1:100, 1:1000, 1:1500, 1:2000, 1:5000
    - 1mL solute to 9mL DI water
    - Vortex 30 seconds
    - 500uL deposit
- **Substrates:**
  - 500uL deposit of (1:10)semen directly onto each substrate
- **Specificity:**
  - 50uL deposit of each bodily fluid
  - 50uL deposit of 1:5 diluted semen
  - Food applied directly to poster paper
  - 1mL deposit of cleaning product
- **Specificity Mixtures:**
  - NC, Neat, 1:5 semen dilution
  - Vortexed 25uL (1:5)semen + 25uL bodily fluid
  - 50uL (1:5)semen on 50uL bodily fluid
  - 25uL (1:5)semen deposit on each food and 75uL of each cleaning product
- **STK Spray preparation:**
  - Combine one pouch with 100mL DI water in a spray bottle
  - Shake lightly to mix and wait 30 s
  - Spraying samples at least 8 in. away
  - View results with 365nm UV light and transparent UV goggles in darkness and with overhead lighting
- **DNA Recovery:**
  - Hole punch size of each sample: NC, references, Neat semen with STK® Spray, dilutions, semen + vaginal secretions mixture
    - EZ2® with DNA Investigator® Kit and Tip Dance protocol
    - QuantStudio® 5 with Quantifiler® HP DNA Quantification kit
    - MiSeqFGx® with ForenSeq® DNA Signature Prep Kit

SENSITIVITY RESULTS



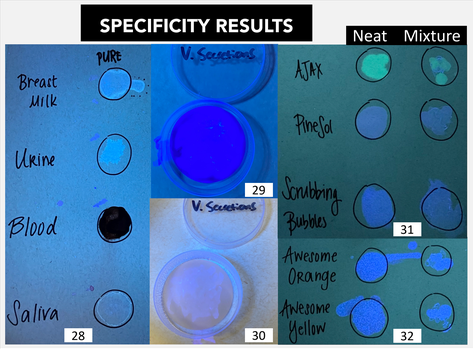
Figures 1-7. Serial dilutions of semen sprayed with STK® Spray. Neat, 1:10, 1:100, 1:1000, 1:1500, 1:2000, 1:5000. A positive blue glow is seen throughout but progressively diminishes.

SPECIFICITY RESULTS



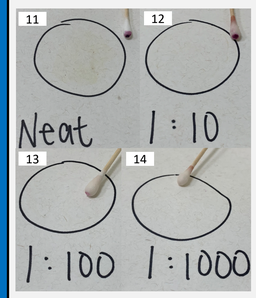
Figures 8-10. Foods (Banana, Carrot, Apple) sprayed with STK Spray revealed negative results.

SPECIFICITY RESULTS



Figures 28-30. Neat bodily fluids breast milk, urine, blood, saliva, and vaginal secretions. Breast milk and vaginal secretions presented false positives.

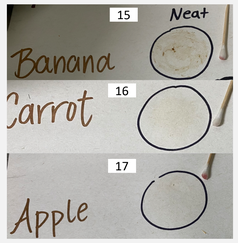
Figures 31-32. Neat cleaning supplies (PINE-SOL®, Scrubbing Bubbles®, LA's Totally® Awesome Orange, LA's Totally® Awesome Yellow) provided false positives. Neat Ajax® was bright green but positive blue glow could still be seen in the mixture



AP COMPARISON

Figures 11-14. Acid Phosphatase tests on serial dilutions of semen; Neat, 1:10, 1:100, 1:1000. Strong positive result observed for Neat and 1:10, positive result for 1:100 and negative result for 1:1000.

Figures 15-17. Acid Phosphatase tests performed on food; Banana, Carrot, Apple. False positive results shown for Banana and Apple.



SUBSTRATE RESULTS

Table 1

Substrate/Surface	Fluorescence In Dark	Fluorescence in Light
Porcelain from Toilet	Positive	Positive
Concrete	Negative	Negative
Granite	Positive	Positive
Cabinet Wood	Positive	Positive
Brick	Light Positive	Light Positive
Rubber Car Floor Mat Flat	Positive	Positive
Rubber Car Floor Mat Textured	Positive	Positive
Glass Pane	Positive	Positive
Plastic Divider from Police Vehicle	Positive	Light Positive
Drywall sheet	Negative	Negative
Fiberglass	Light Positive	Light Positive
Ceramic Tile Piece	Positive	Positive
Carpet	Positive	Light Positive
Rubber Bottom of Traffic Cone	Light Positive	Light Positive
Metal Sheet	Positive	Positive
Leather	Positive	Positive
Cardboard	Negative	Negative
Wood Block	Light Positive	Light Positive
Car Windshield Cover	Positive	Positive
Seat Cover- Outside	Positive	Positive
Seat Cover- Inside	Negative	Negative
Liverpool® Blue Jeans: 39% Cotton, %31 Rayon, 28% Polyester, 2% Spandex	Negative	Negative
Aerie® XS Black Leggings	Negative	Negative
White and Brown Shirt: 95% Rayon, 5% Spandex	Negative	Negative
NY&C® S Black Sweater: 100% Acrylic	Negative	Negative
Active Basic 2XL Black Dress: 92% Cotton, 8% Spandex	Light Positive	Negative
Black and White Berness Shoe	Positive	Positive
Black Shoes for Crews® Shoe	Light Positive	Light Positive
Grass	Negative	Negative
Leaves	Positive	Positive
Wet Dirt	Negative	Negative
Dry Dirt	Negative	Negative

Table 1. STK® Spray tested on 32 substrates. The fluorescence intensity observed in both darkness and with overhead light

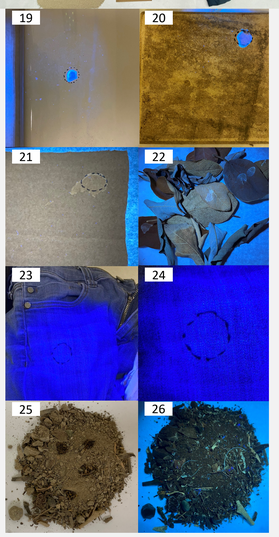


Figure 18. Substrates laid out before use
Figure 19. Deposit on porcelain from a toilet fluorescing positive blue
Figure 20. Deposit on ceramic tile piece fluorescing positive blue
Figure 21. Deposit on cardboard; negative
Figure 22. Deposit on leaves; positive
Figures 23-24. Deposit on Liverpool® Blue Jeans; negative
Figures 25-26. Deposits on dirt; negative
Figure 27. STK® Spray, Flairosol® spray bottle, and Crime-lite® used

DNA RESULTS

Table 2

Sample Name	Quantitation Data (ng/uL)
Reference 1 Poster Board	0.015928647
Reference 2 Oral Swab	8.4030056
Reference 3 V Secretions Filter Paper	3.760875225
Substrate 1 Filter Paper	Undetermined
Substrate 2 Poster Board	Undetermined
Neat w/ STK	1.437517166
1/10 Dilution	0.128123015
1/100 Dilution	0.070711568
1/1000 Dilution	0.017324291
1/1500 Dilution	0.002385529
1/2000 Dilution	0.014393002
1/5000 Dilution	0.003520686
Mixture Immediate	0.4944534
Mixture +1hr	0.124123648

Table 2. Quantitation results for 14 samples which includes controls, references, semen and vaginal secretions mixtures, and dilutions. Undetermined results represent control samples for substrates used in DNA recovery process. Samples will be typed through sequencing to determine whether the application of STK® spray adversely impact the DNA typing results.

DISCUSSION

Sensitivity
- STK® Spray demonstrated to be highly sensitive, obtaining positive results up to and including a semen dilution of 1:5000
- AP tests proved less sensitive providing positive results up to and including a semen dilution of 1:100
- STK® Spray luminescence was visible under both light settings

Specificity
- Bodily fluids breast milk and vaginal secretions provided false positives for STK® Spray in addition to four cleaning agents.
- Foods did not provide false positive results as they did for AP tests

Substrates
- Clothing substrates did not perform well with STK® Spray, as advertised
- Non-porous substrates outperformed porous substrates

DNA Recovery:
- Quantitation recovered DNA from all necessary samples showing STK® Spray thus far does not interfere with DNA typing

Limitations and Future Studies
- Neat bodily fluids, neat cleaning agents, and the substrates were not AP tested for comparison
- Test STK® Spray on aged samples
- Determine if 365nm UV light has any significant degrading affect on semen
- Run duplicates or triplicates of false positives

CONCLUSION

This study performed verification tests on STK® Sperm Tracker Spray to determine the product's usefulness for the LAPD Field Investigation Unit. The sensitivity and specificity of the STK® Spray were investigated. Furthermore, the luminescence intensity and effectiveness of the STK® reaction on various substrates were studied to evaluate the spray's performance across a range of potential rape scene locations. The sensitivity and specificity proved to perform better than the current test being used, Acid Phosphatase. In addition, STK® Spray maintained luminescence for over four months aiding scene photography. Non-porous substrates outperformed porous substrates when used with STK® Spray and clothing substrates provided primarily negative results. In conclusion, due to its high sensitivity and ease of use, STK® Sperm Tracker Spray was verified to be a beneficial product when investigating rape scenes.

ACKNOWLEDGEMENTS

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