

Internal Validation of the STK® Sperm Tracker STK Skin and its application in the examination of victims of sexual assaults

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Background

The proper identification and collection of semen stains on victims of e.g. sexual assault is of huge benefit for reconstructing a crime. Moreover, the identification of a perpetrator's DNA can add to the victim's mental recovery by strengthening their credibility and overall position during the preliminary proceedings as well as the trial. However, identification of sperm traces with the naked eye might be time consuming, if not impossible, even for trained forensic physicians. Eventually, many samples of a victim's body are taken at random.

AXO Science introduced the STK® Sperm Tracker STK Skin, which is a non-toxic presumptive test for the detection of human acid phosphatase on human skin and hair. Semen stains can be visualized using a 365 nm UV light. Supposedly, STK Skin does not alter DNA and further molecular analyses of collected semen stains. The STK® Sperm Tracker STK Skin was examined in regards to laboratory requirements and possible influences on DNA analysis. Moreover, its performance was tested in mock cases that (forensic) physicians may be confronted with.

Material & Methods

STK® Sperm Tracker STK Skin	1000 mg powder solubilized in 100 mL (full volume) or 500 mg in 50 mL (half volume), respectively and sprayed on body (parts) / illumination with Crime-lite® X (Foster+Freeman Ltd.), 365nm
Sensitivity:	5 µl of semen applied on forearm and dried for 1h up to 24h / neat semen, 1:2, 1:10, 1:20, 1:50 dilutions (all used in duplicates except undiluted semen samples)
Shelf life:	Prepared solution was stored up to several weeks in the dark at 5°C
Specificity / cross reaction:	Blood, saliva, urine (and 1:1 mixtures with semen), body lotions, sunscreen, deodorants, lubricating gel, beverages (e.g. beer, liquor, coke, energy drink)

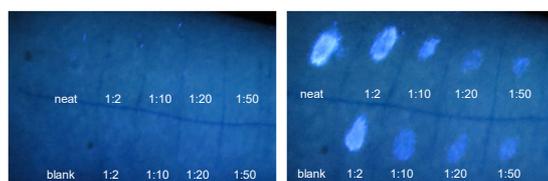
For the following experiments, we applied 5 µl semen on a subject's forearm(s) (neat semen, 1:2, 1:10, 1:50 dilutions) and compared sprayed and swabbed samples against not sprayed only swabbed samples:

Presumptive test / histological staining:	Galantos RSID™ Semen / Baecci staining
DNA extraction / Quantification / PCR:	Qiagen EZ1 Advanced / EZ1 & EZ2® DNA Investigator® Kit / Investigator® Quantiplex® Pro Kit / Promega PowerPlex® ESX 17 Fast System

4 Mock cases examined by different physicians without knowledge

Results

Sensitivity: Semen stains after utility of STK Skin were clearly visible in all experiments / no difference over time or between full and half volume / all results were both reproducible and repeatable on light and dark skin



Dilution series without application of STK Skin at 365nm (full volume)

Dilution series with application of STK Skin at 365nm (full volume)

Shelf life: No loss in sensitivity and/ or fluorescence intensity within the first week after solubilization / positive fluorescence up to three months (only tested in full volume)

Specificity / cross reactions: No false positives / stains containing semen with good fluorescence / no fluorescence in 1:1 semen-blood mixtures

no fluorescence after
Washing or sweating!

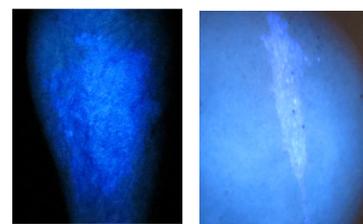
Presumptive Test / histological staining: No false negatives / no differences microscopically

DNA analysis: comparable amounts of DNA but slightly higher when using STK Skin / slightly increased degradation indices of STK Skin-treated samples compared to non-treated samples at low DNA amounts / full single-source STR profiles even in 1:10 dilutions with STK Skin and partial male profiles even at 1:50 diluted samples

Mock cases: All traces containing semen were easily discovered by the physicians / a darkened environment during examination is recommended. A suitable light source for detection, such as the used Crime-lite® X, proved to be of advantage compared to a conventional light source, due to its broader and more intense cone of light

Dilution Sperm-H ₂ O	DNA quantity (mean) [pg/µl]		DNA degradation index (mean)		DNA profile quality	
	Yes	No	Yes	No	Yes	No
STK Skin	Yes	No	Yes	No	Yes	No
Undiluted	100	160	2	2	FP	FP
Ø 1:2	100*	85	3	3	FP	FP
Ø 1:10	10	7	4	2	FP / PP (3 dropouts)	FP
Ø 1:20	4	1.5	5	3	PP (3, 6 dropouts)	NP
Ø 1:50	2	0.5	6	4	PP / NP (6 dropouts)	NP

Kit-specific degradation threshold with a default setting index of 10; *pipetting error FP = full DNA profile, PP = partial profile, NP = no profile (i.e. more than 13 allelic dropouts)



Palm sized application of 1 mL undiluted sperm on Shinbone

1 mL undiluted sperm on chest

Conclusion

STK® Sperm Tracker STK Skin is easy to apply and use. It reliably allows visualization even of miniature amounts of sperm. (Forensic) Physicians were able to successfully apply the product with little explanation and detected all stains during the mock case examinations. STK Skin gave a good fluorescence signal even three months after solubilization. No cross reactions was observed. Presumptive tests for semen, as well as histological staining worked as intended. However, the manufacturers claim of trace-detection after washing (i.e. showering) could not be verified. Furthermore, DNA analysis revealed a slightly increased degradation of DNA compared to samples not treated with STK Skin, prompting long-term experiments regarding DNA-integrity when storing collected swab samples. So far, STK Skin does not seem to have an impact of profile quality. Influences on RNA integrity and subsequent analysis will be tested in the near future.

