Summary Results of a Blinded Study on the Effectiveness and Efficiency of using SPERM HY-LITER™ to Screen Sexual Assault Evidence for Sperm.

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Abstract

The identification of sperm is often a requirement of forensic DNA analysis for the processing and evaluation of sexual assault evidence. **SPERM HY-LITER™** is a new immunofluorescent detection kit specifically designed for forensic DNA laboratories. This kit uses a fluorescent tagged mouse monoclonal antibody with unique specificity for human sperm heads. The sensitivity of the test is such that a single sperm is easily and quickly identified on a microscope slide.

Forensic laboratories are naturally, and appropriately, skeptical of new and emerging technologies. At the request of the Department of Forensic Genetics of the Danish National Institute for Legal Medicine (the centralized forensic DNA laboratory of Denmark), Independent Forensics agreed to perform a blinded study on the specificity, sensitivity and work flow efficiency of **SPERM HY-LITER™** for the microscopic screening of sperm from sexual assault evidence.

The Danish National Institute for Legal Medicine (DNILM) prepared a duplicate series of swabs and stains with forty-four (44) samples prepared from a variety of sources including duplicate cuttings from sexual assault evidence kits retained at the Danish National Institute for Legal Medicine. All samples were blinded and number coded to both laboratories. An identical protocol for the extraction, processing and staining of all samples was chosen prior to testing – the provided **SPERM HY-LITER™** protocol was followed with the addition of a short, water-bath sonication extraction step and Spin-Eze™ aided recovery of the resultant cell pellet. The Department of Forensic Genetics used both **SPERM HY-LITER™** staining and their standard histological staining (i.e., H&E) for the evaluation of all samples.

Upon completion of the testing and the exchange of data, sample codes were revealed. Sample types tested and evaluated included negative controls, low sperm count samples, dense samples, epithelial, vaginal, washed semen stains, mixtures of blood, menstrual blood, soil, saliva, and bacterial with seminal fluid all formed part of the tester set.

Data comparison of the three sets of samples (**SPERM HY-LITER™** staining at IFI, **SPERM HY-LITER™** staining at DNILM, and H&E staining at DNILM) revealed that **SPERM HY-LITER™** was clearly the most sensitive of the staining methods.

The most dramatic result of the study is the time savings seen with **SPERM HY-LITER™**: the two weeks required by DNILM to stain and scan the forty-four samples using their standard H&E histological method was reduced to ***three (3) days*** with **SPERM HY-LITER™.** ***This represents an 80% time saving with increased sensitivity as compared to standard forensic technique.***

Methods

Extraction

All samples were extracted using an identical protocol with standardized volumes, times and conditions. Briefly, swabs and cuttings were soaked in phosphate buffered saline (PBS) for 1-2 hrs at room temperature, and incubated in a water bath sonicator for twenty (20) minutes and the extract recovered, and cells pelleted using Spin-eze and centrifugation. Cell pellets were resuspended in 50-200 µL of PBS.

Staining

All samples were stained using **SPERM HY-LITER™** as described by the manufacturer (IFI) and using the standard DNILM method (H&E).

Documentation

Slides were scored visually and communicated between IFI and DNILM before coded information was revealed.

Time for Analysis

Total time –
IFI: three (3) days
DNILM: two (2) weeks

Time per sample –
IFI: 32 min
DNILM: 109 min

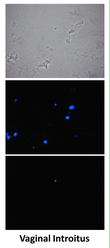
Results

Negative Controls

	IFI	DNILM
Buccal Swabs	No	No
Vaginal Introitus	No	No
Vaginal Fornix	No	No
Rectum	No	No
Epithelial	No	Yes (cont)
Vaginal with blood	No	No

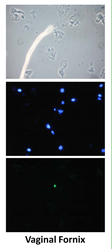
Low Copy Samples (1-10 spermatozoa per prep)

Buccal Swabs	No	No
Vaginal Introitus	Yes	No
Vaginal Fornix	No	No
Rectum	No	No
Epithelial	No	Yes
Vaginal with blood	No	No


Vaginal Introitus

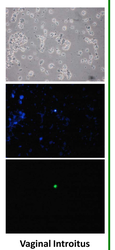
Standard Samples, Dense Epithelia

Buccal Swabs	Yes	Yes
Vaginal Introitus	Yes	No
Vaginal Fornix	Yes	No
Rectum	No	Yes
Epithelial	Yes	Yes
Vaginal with Blood	Yes	No


Vaginal Fornix

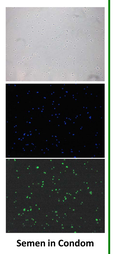
Degraded Samples (incubated at 37°C)

	IFI	DNILM
Buccal Swabs	Yes	No
Vaginal Introitus	Yes	Yes
Vaginal Fornix	No	Yes
Rectum	No	No
Epithelial	Yes	Yes
Vaginal with blood	Yes	Yes


Vaginal Introitus

Experimental Samples

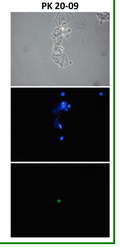
Semen stain on white bed linen	No	Yes
Semen stain on white paper towel	Yes	Yes
Semen stain on white toilet paper	No	Yes
Semen mixed with soil	No	No
Semen swab from concrete	Yes	No
Semen stain on denim fabric	Yes	Yes
Semen stain on swab with blood	Yes	Yes
Semen stain on swab with menstrual blood	Yes	No
Rectum stain with semen and blood	Yes	No
Semen in new condom (1-3 days)	Yes	Yes
Seminal fluid (no spermatozoa) swab	--	--
Semen stain on washed trousers (Normal wash, 40°C)	Yes	Yes


Semen in Condom

Case Work Samples

PK18-09 Fornix swab	Yes	Yes
PK19-09 Rectal swab	Yes	Yes
PK19-09 Fornix swab	Yes	Yes
PK19-09 Introitus swab	Yes	Yes
PK19-09 Buccal swab	Yes	No
PK20-09 Fornix swab	Yes	Yes
PK40-09 Introitus swab	No	No
PK40-09 Introitus posterior swab	No	No
PK40-09 Rectal swab	No	No

PK 20-09



Conclusions

Negative Controls
IFI - All negative controls correct
DNILM – one false positive

Low Copy Samples
IFI - 1 of 6
DNILM – 1 of 6

Standard Samples
IFI – 5 of 6
DNILM – 3 of 6

Degraded Samples
IFI- 4 or 6
DNILM – 4 of 6

Experimental Samples
IFI-14 of 20
DNILM 12 of 20

Sensitivity:
SPERM HY-LITER™: 30 of 44
DNILM: 25 of 44

Specificity:
SPERM HY-LITER™: 0 false positives
DNILM: 1 false positive

Efficiency:
SPERM HY-LITER™: 80% time saving
Three (3) times faster screening of SAE.

Independent Forensics
DNA Testing & Technologies