An Efficient and Effective Protocol for Identifying Sperm From Anal Swabs Using **SPERM HY-LITER™** ***EXPRESS***

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Microscopical sperm searching has long been a painful, slow and less than robust task for forensic analysts. Searching for sperm from anal/rectal swabs poses an even greater challenge due to the interference of fecal matter.
Recently, a far more specific, sensitive and scientifically defensible method of microscopical sperm searching using **SPERM HY-LITER™** and the related **SPERM HY-LITER™** ***EXPRESS*** kits have provided the first scientifically defensible identification of sperm from sexual assault evidence (SAE). These immunofluorescent-based staining kits have made the detection of sperm from SAE considerably faster, easier and more robust; however, even this technique can be stymied by fecal contamination which among other factors, increases background fluorescence from anal/rectal swab extracts.

Here we report an update on the staining/processing of anal/rectal swabs that considerably improves the ability of **SPERM HY-LITER™** and **SPERM HY-LITER™** ***EXPRESS*** to provide clear signals from samples containing fecal matter.

The updated protocol was developed from anal/rectal samples obtained from an academic collaboration studying receptive anal intercourse. The protocol is simple and greatly increased the final immunofluorescent signal making sperm easily identifiable regardless of the amount of fecal matter present on the initial anal/rectal swab.

Forensic Workflow

1) Evaluate presence of fecal matter on evidence by visual determination:
(i) significant, (ii) some, (iii) little or no

Significant fecal matter | Some fecal matter | Little to no visible fecal matter

2) Prepare cuttings from swabs:
Cut 1/3 of swab and place in 1.7 mL tube for **SPERM HY-LITER™ EXPRESS** staining
Cut 1/3 of swab and place in 1.7 mL tube for **RSID™-Semen** testing
Reserve remaining 1/3 swab for additional testing

3) Prepare extract for staining
incubate swab cutting in 500 µL PBS, 1 hour, recover cell pellet with spin basket

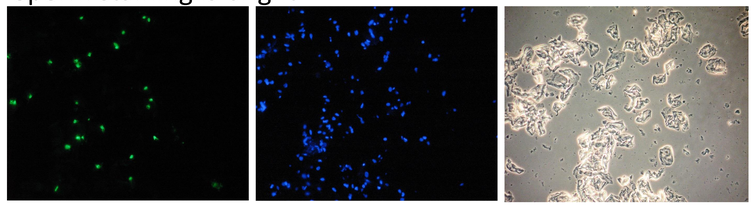
4) Add PBS to recovered cell pellet as indicated for level of fecal material

add 100 µL PBS ↓ | add 40 µL PBS ↓ | add 25 µL PBS ↓

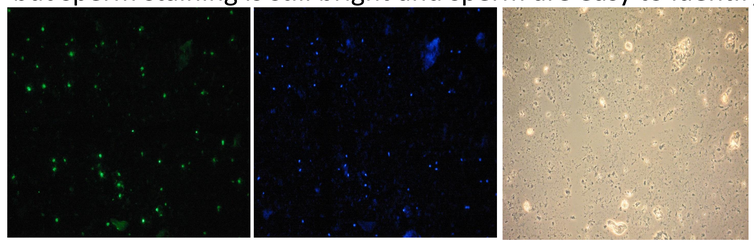
5) Resuspend pellet and add 25 µL of resuspension to sample well of **SH** slide

6) **Proceed with SPERM HY-LITER™ staining as per protocol**

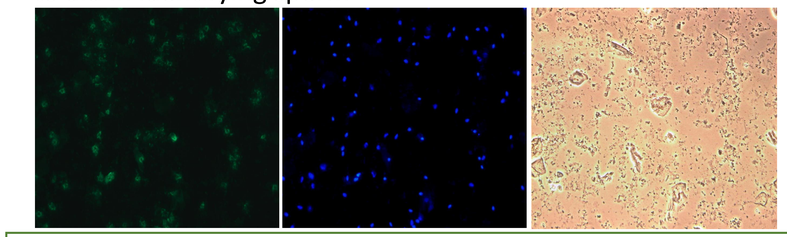
Little to no fecal matter – Fluorescent background is low and Sperm staining is bright



Some fecal matter – Fluorescent background may be increased but sperm staining is still bright and sperm are easy to identify



Significant fecal matter – Fluorescent background is increased and sperm staining may be decreased; DAPI is important tool to aid in identifying sperm



Conclusions:
Using this protocol to stain anal swabs from actual forensic samples, identification of sperm was efficient and effective regardless of the amount of fecal material present.
DAPI is an important tool in the identification of sperm in samples with increasing amounts of fecal material.

SPERM HY-LITER™ *EXPRESS* Protocol for Fecal Samples

1) Evaluate presence of fecal matter on evidence by visual determination

Significant fecal matter | Some fecal matter | Little to no visible fecal matter

2) Prepare cuttings from swabs:

Cut 1/3 of swab and place in 1.7 mL tube for **SPERM HY-LITER™ *EXPRESS*** staining
Cut 1/3 of swab and place in 1.7 mL tube for **RSID™-Semen** testing
Reserve remaining 1/3 swab for additional testing

3) Prepare extract for staining

incubate swab cutting in 500 µL PBS, 1 hour, recover cell pellet with spin basket

4) Add PBS to recovered cell pellet as indicated for level of fecal material

(i) significant
add 100 µL PBS

(ii) some
add 40 µL PBS

(iii) little or no
add 25 µL PBS

5) Resuspend pellet and add 25 µL of resuspension to sample well of **SH** slide

6) Proceed with SPERM HY-LITER™*EXPRESS* staining**

****Important: DTT levels are increased to 12.5 µl 1M DTT per drop Sample Prep solution.***