Developmental Validation of SPERM HY-LITER™: A Specific, Sensitive, and Confirmatory Screening Method for Human Sperm Detection from Sexual Assault Evidence

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Abstract

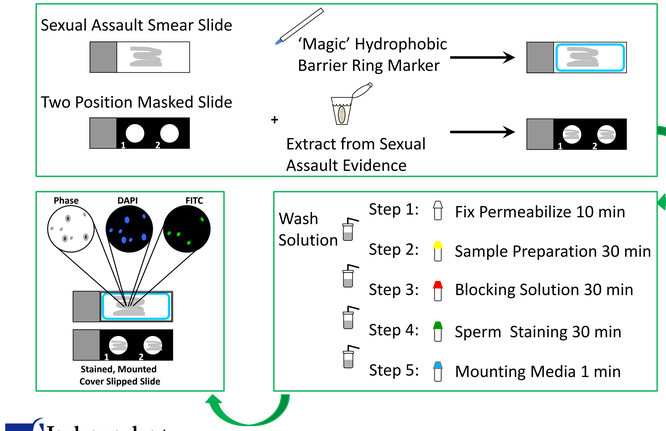
The identification of sperm from sexual assault evidence (SAE), as presently performed, is a labor intensive, time consuming, insensitive, and non-specific technique. In order to satisfy legal and criminalistic requirements for proceeding with DNA-based evidence testing, crime laboratories devote a great deal of effort, time, and resources to identifying sperm from SAE. Commonly employed sperm identification methods are based on non-specific microscopic staining techniques (i.e., KPIC or 'Christmas Tree Stain') that are not amenable to automation or computer-aided searches. Since a large percentage of crime laboratory case work is related to SAE, the effort and expense devoted to sperm screening is substantial. These issues are exacerbated with SAE that has been stored for long periods (e.g., backlogged rape kits) or for samples that have minimal amounts of biological material.

Here we present experiments demonstrating the sensitivity, specificity, and usefulness of Sperm HY-LITER™: a reagent that fluorescently labels human sperm heads. Additionally, we show that Sperm HY-LITER™ provides scientifically justifiable and legally defensible identification of human sperm, and greatly increases the efficiency of microscopic sperm searches.

SPERM HY-LITER™ Specifications

Test Format: Microscopic Screening Method for Sperm
Antigen Detected: Human Sperm Heads Antibodies: Mouse monoclonal
Labeling: Sperm: Alexa 488 / Nuclei: DAPI Test Read-out: Visual
Sensitivity: Single Human Sperm Head
Preparation Time: 100 minutes – 10 minutes 'hands-on'

SPERM HY-LITER™ Staining Protocol



SPERM HY-LITER™ Staining

Cell Type Specificity

Sample: Buccal, Urine & Blood extracts *with* Sperm

Conclusions: Unlike KPIC or H&E staining, SPERM HY-LITER™ is cell type specific as only sperm cells are labeled. Furthermore, the presence of other human body fluids does not interfere with the ability of SPERM HY-LITER™ to specifically detect human sperm.

Sensitivity & Specificity

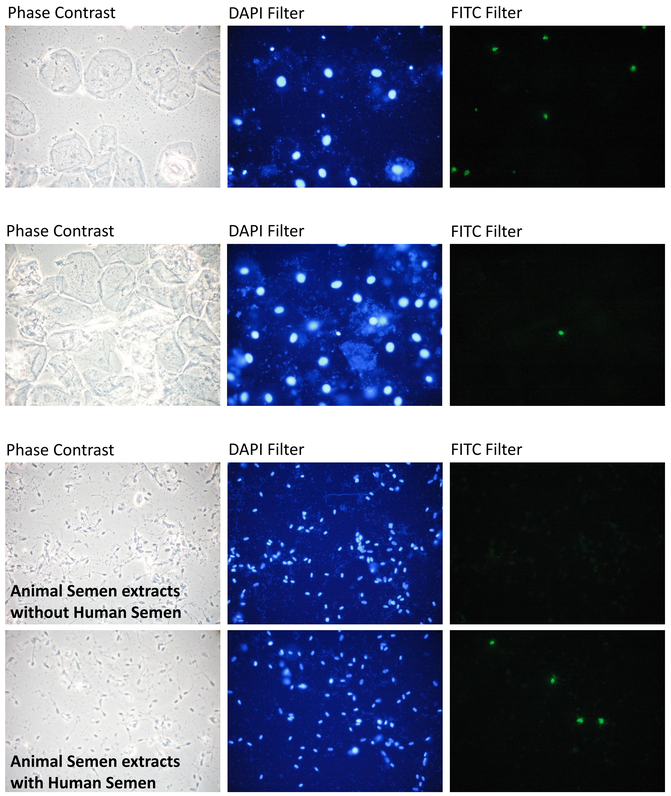
Sample: Buccal, Urine & Blood Extracts with a Single Sperm

Conclusions: SPERM HY-LITER™ has sufficient sensitivity to identify a single sperm head present in an overwhelming background of epithelial cells.

Species Specificity

Sample: Animal Semen (canine, feline, bovine, equine, caprine, ovine, porcine, murine) *without/with* Human Semen

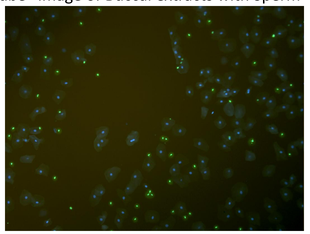
Conclusions: SPERM HY-LITER™ does not cross-react with animal sperm from the tested species: dog, cat, bull, horse, goat, sheep, pig, and mouse. In addition the presence of non-human animal sperm does not interfere with the ability of SPERM HY-LITER™ to specifically detect human sperm.



SPERM HY-LITER™ Advantages

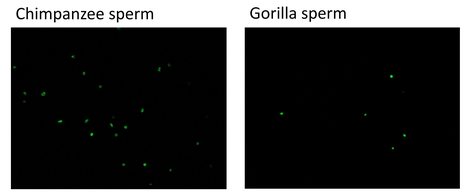
- First *Positive* Identification for Human Sperm
- First *Specific* Identification for Human Sperm
- Sensitivity down to a single sperm
- Fully Compatible with Automated Methods
- Scan slides at lower magnification: 10X or 20X
- **SPERM HY-LITER™ "Dual Cube"**
  - Visualize *both* epithelial cells and sperm simultaneously.
  - McCrone Microscopes Innovation

"Dual Cube" image of Buccal extracts with Sperm

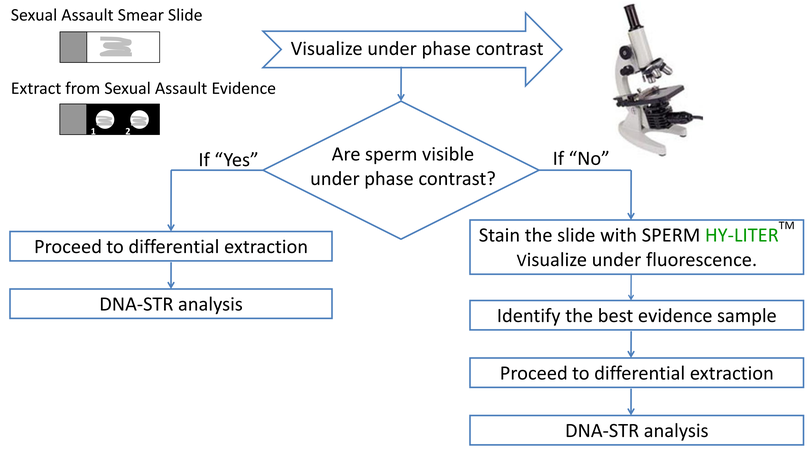


Cross reactions of SPERM HY-LITER™

- Only known cross reaction tested to date are chimpanzee and gorilla sperm
- Samples kindly provided by Winnie Kurowski, Acadiana Criminalistics Laboratory.
- Sperm were extracted in PBS and stained with SPERM HY-LITER™. Chimpanzee and gorilla sperm fluorescence weakly under FITC
- There are no cross reactions of chimpanzee and gorilla sperm with **RSID™-SEMEN**



Proposed Workflow Integration of SPERM HY-LITER™



Automated SPERM HY-LITER™ Microscope System

- Operator independent sperm searches
- Automated screening of up to 8-slides
- Full documentation of all identified sperm
- Review of all positive microscope fields

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