# Assessment of SPERM HY-LITER™ for staining smears related to sexual assault cases

Government of South Australia
Forensic Science SA

Edmund SILENIEKS[1], Kareana TURNER[1], Tegan COLLINS[2]

[1]Forensic Science SA, Adelaide, South Australia, Australia
[2]Flinders University, Adelaide, South Australia, Australia

## Introduction

Sperm Hyliter™, developed by Independent Forensics, is an immunofluorescent stain used for the microscopic detection of human sperm. It incorporates a derivatised fluorophore-conjugated monoclonal mouse antibody, Alexa 488, which specifically binds with an antigen on human sperm heads (see Figure 1). The sperm heads are viewed using a fluorescence microscope and appear bright green when visualised using a FITC compatible filter.

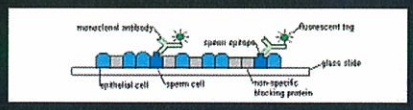


**Figure 1.**
The Sperm Hyliter™ mechanism – a fluorescent tag is attached to a monoclonal antibody, which specifically binds to the sperm epitope.

Sperm Hyliter™ incorporates DAPI as a second (blue) fluorescent dye, which stains all cell nuclei present in the sample. DAPI also stains the sperm and stained smears can be visualised using a DAPI filter (see Figure 2). Independent Forensics recommend the use of a Chroma 51000 Filter Cube, which incorporates both the DAPI and FITC filters to provide a spectacular, fluorescent field of view (see Figure 3).

The visual characteristics, reproducibility and cost of Sperm Hyliter™ were compared to Christmas Tree stain (Kernechtrot-Picroindigocarmine or KPIC), currently used by the Evidence Recovery group at Forensic Science SA to stain smears from sexual assault cases.

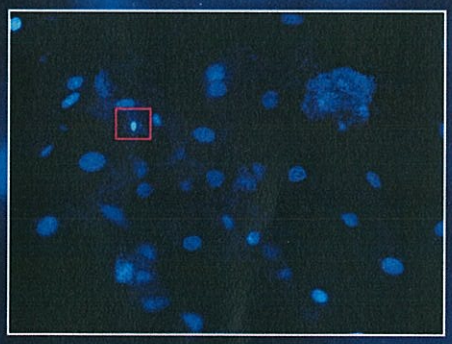

**Figure 2.**
An example of epithelial and sperm cell (marked) stained with Sperm Hyliter™, viewed using a DAPI filter only.

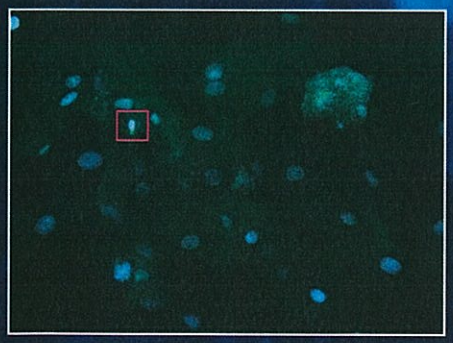

**Figure 3.**
An example of the same epithelial and sperm seen in Figure 2, viewed using the Chroma 51000 DAPI/FITC filter.

## Smear Preparation

Duplicate smears were prepared from human semen, saliva, and vaginal swabs. One was stained with Christmas Tree stain and the other with Sperm Hyliter™.

## Microscopy

Sperm Hyliter™ stained smears were viewed using a Leica DM3000 fluorescence microscope. Sperm heads were visualised using an A5 FITC compatible filter and the DAPI stained nuclear material was visualised using an A4 DAPI filter. A Chroma 51000 DAPI/FITC filter was also used.

Christmas Tree stained smears were viewed using the same Leica DM3000 microscope, using transmitted white light.

## Staining Procedure

**1. CHRISTMAS TREE STAIN**

**Step 1:** Cover the smear with Nuclear Fast Red solution for 15 minutes.
Thoroughly wash the smear with deionised water until no colour remains.

**Step 2:** Cover the smear with Picroindigocarmine solution for 15 seconds.
Thoroughly wash the smear with ethanol until no colour remains.

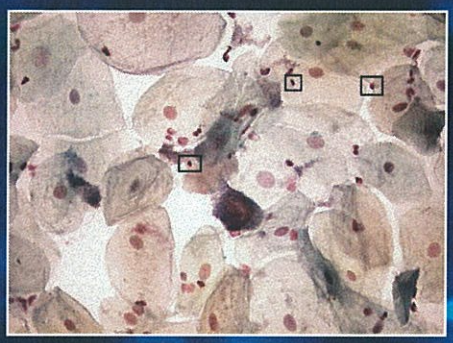

**Figure 4.**
An example of epithelial and sperm cell (marked) stained with Christmas Tree stain.

**2. SPERM HYLITER™**

The staining protocol for Sperm Hyliter™ incorporates four different steps, taking a total 100 minutes to complete. Each step uses a different reagent, which must completely cover the area to be stained for a set period of time. Care must be taken to ensure reagents do not dry out during staining and must be rinsed off with washing solution between each step.

**Step 1: Fixative Reagent**
Fixes the sample to the glass slide.

**Step 2: Sample Preparation Solution (+ DTT)**
Conditions the sample for staining. Freshly thawed DTT acts as a protective agent for SH groups and is a reducing agent for proteins.

**Step 3: Blocking Solution**
Eliminates non-specific antibody interactions by blocking unoccupied sites on the slide with a nonspecific protein. The Blocking Solution contains the DAPI fluorescent dye.

**Step 4: Sperm Head Staining Solution**
Contains the fluorescently labelled antibody that specifically binds with a single epitope on the sperm head.

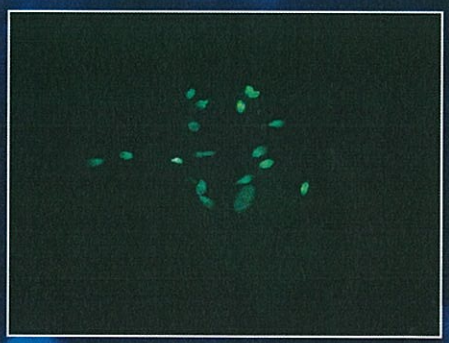

**Figure 5.**
An example of sperm cells stained with Sperm Hyliter™ and viewed using a FITC filter only. An epithelial cell can be seen under the sperm.

## Cost Comparison

At the time of this assessment, the estimated cost of Sperm Hyliter™ was approximately $46 per slide, and Christmas Tree cost approximately $1.00 per slide.
A microscope with fluorescent capability and suitable filters would need to be purchased, which would cost approximately $20K to $35K.

## Discussion

Sperm Hyliter™ was found to be a stain where sperm were easily visualised. Several advantages and disadvantages of this stain were observed over the commonly used Christmas Tree stain.

Generally, the Christmas Tree stain is easier to use and can provide a result within approximately 30 minutes. It provides a good contrast between sperm heads and epithelial cells, generally making sperm easy to locate. However, in samples with high numbers of epithelial and white blood cells sperm can be masked by cells and difficult to locate. The major advantage of Sperm Hyliter™ over Christmas Tree stain is that in these cases, fluorescent sperm can be easily and rapidly located.

The increased searching efficiency of Sperm Hyliter™ is countered by the time taken to prepare the smear. Care must also be taken to prevent the Sperm Hyliter™ reagents from evaporating during the incubation periods.

Sperm Hyliter™ allows for the visualisation of the stained sperm and cell nuclei, thus it can be difficult to see the cytoplasm of cells and gain an understanding of the amount of cellular material present on a smear. This is not an issue with Christmas Tree stain. White blood cells can still be visualised with Sperm Hyliter™ as the nuclei stain bright. (see Figure 6).

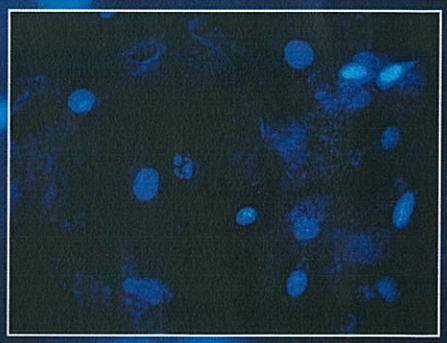

**Figure 6.**
An example of epithelial and white blood cells, viewed using a DAPI filter.

Variation in the fluorescence intensity of sperm heads was observed. In some examples the fluorescence intensity of sperm heads varied from pale to intense across the one smear. Fluorescent halos were observed around some sperm while some exhibited uneven fluorescence, which may have been due to the light refracting in the surrounding mounting media. Some individual sperm were observed to have a fluorescent perimeter, with a dark central portion showing no fluorescence (see Figure 7). To monitor staining efficiency, a positive control may need to be performed with each staining batch.

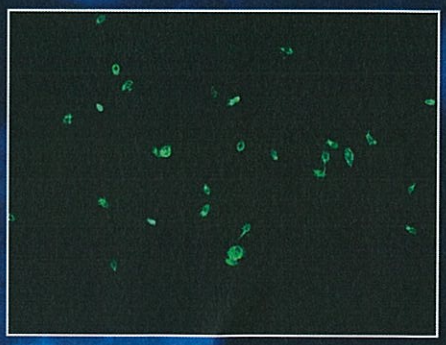

**Figure 7.**
An example of the variability in fluorescence of sperm cells stained with Sperm Hyliter.™

## Conclusions

1. Sperm Hyliter™ allows sperm to be easily located, particularly in smears with high numbers of epithelial or white blood cells
2. Efficiency gained through reduced reading time may be countered by the time taken to stain a smear
3. The fluorescence allows for automated searching of smears
4. The fluorescence intensity of sperm will fade with time
5. The cost of Sperm Hyliter™ is the major drawback to its introduction

Presented at The 20th International ANZFSS Symposium September 5-9, 2010 Sydney Australiaa