Case Study: Analysis of an Anorectal Swab Alleged to Contain Canine Sperm Using a Fluorescently Labeled Human Sperm Head Specific Antibody

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Abstract

Here we present the analysis of an evidentiary anorectal swab that was suspected to contain canine semen. Most methods for identifying sperm from sexual assault evidence utilize a nucleic acid and protein stain (e.g. KPIC or H+E). Due to the non-specificity of these cell stains, another staining technique (SPERM HY-LITER™) was employed to determine whether any sperm cells present in the sample were of human origin. SPERM HY-LITER™ is an antibody based staining technique that employs a general fluorescent stain for nucleic acids and a fluorescently labeled human sperm head specific antibody. A series of experiments were performed to verify the efficacy and specificity of SPERM HY-LITER™ with the evidential anorectal swab. We present experimental data demonstrating the specificity and sensitivity of SPERM HY-LITER™ in the context of an unusual cross-species sexual assault case.

Introduction

A case was presented where sexual assault by a canine was alleged. The evidence submitted was an anorectal swab. The case scenario brought up several questions in how to approach this sample:

- Would the difference between canine and human sperm be discernible under general staining (KPIC, H+E etc)?
- Would the harsh environment affect the morphology of the sperm?

The SPERM HY-LITER™ method was considered as an option as it is not dependent on morphology, rather uses fluorescently tagged antibody to human sperm heads. This method also raised questions:

- Would the oxidative nature of fecal material inhibit the fluorescent staining?
- How could we be sure that different types of samples were staining correctly, as all samples contain unknown contaminants?

Methods

Several experiments were performed to determine what factors were affecting the SPERM HY-LITER™ staining. These included:

- Incubation studies to determine if the fecal material was degrading the sperm head epitope
- Diluting the fecal sample to determine if the fecal material was having an inhibitory effect and at what level
- Several modifications to the manufacturer's protocol to see if any adverse effects could be overcome to achieve proper staining.

Results

The original staining of the evidentiary swab extract with SPERM HY-LITER™ gave inconclusive results (Figure 1).

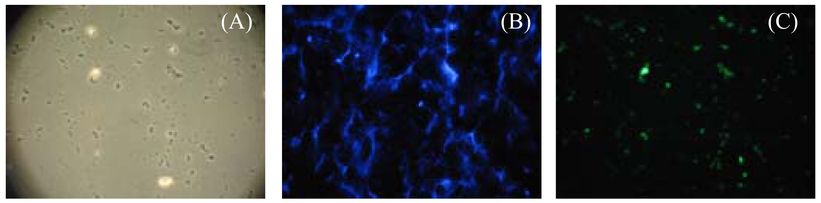

Figure 1: Initial staining of the evidentiary swab extract with SPERM HY-LITER™. Cells observed under phase (A) with cells similar in shape to sperm being clearly stained in the DAPI channel (B) and exhibiting no signal in the FITC channel (C).

Several experimental sets with simulated anorectal swabs demonstrated that the lack of antibody staining was due to inhibition, not epitope degradation. Immediate extraction, as well as extended co-incubation of fecal swabs with semen added, yielded similar sperm head staining results (Figure 2).

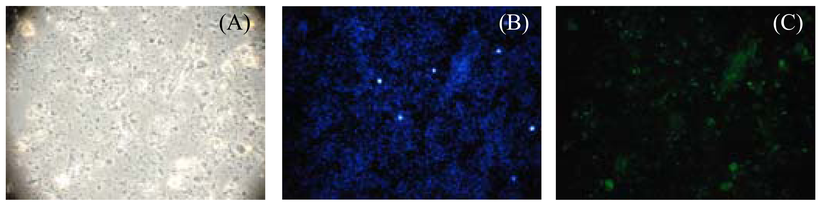

Figure 2: Simulated anorectal swab spiked with human semen and stained with SPERM HY-LITER™. The sperm are visible under phase (A) and are clearly labeled in the DAPI channel (B), but are exhibiting no signal in the FITC channel (C)

Experiments modifying the manufacturer's protocol found that the inhibitory effect of the fecal material in a sample could be overcome with an excess of DTT. This was proven by the proper staining of sperm, added to a fecal sample, using 10X DTT, while an identical sample stained in tandem as per protocol did not fluoresce.

The evidentiary swab extract was re-stained with SPERM HY-LITER™ using 10X DTT. The evidentiary sample with added human semen extract was stained in tandem using 10X DTT as a positive control for the fecal inhibition. The 10X DTT procedure produced identical results to the initial staining (Figure 1) with 1X of DTT, while the positive control stained as expected (Figure 3). This set of tandem experiments gave confidence in the ability of the antibody to stain human sperm, when human sperm is present in anorectal samples.

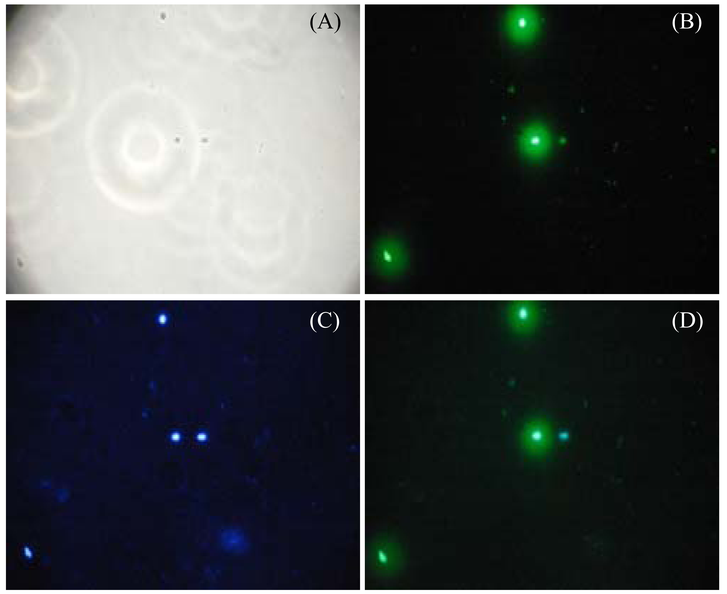

Figure 3: Evidentiary swab extract spiked with semen extract and stained with SPERM HY-LITER™. Cells look similar in the phase (A) and DAPI channel (B), but can be differentiated using the FITC channel (C) and confirmed using a dual cube (D).

Conclusions

After the extensive testing of these conditions the conclusion drawn from the evidence is that the cells present were not human sperm. No determination could be made to support or contradict the presence of canine sperm. The species validation performed by the manufacturer gave confidence that the correctly labeled sperm were human and any cells not labeled were not human sperm.

The cause of dampening effect on the sperm head stain in anorectal samples was not absolutely determined. The use of extra DTT and mixing human sperm in the evidentiary extract were excellent fixes for the initial dilemmas presented in this unusual case.

Acknowledgments

I would like to thank Elizabeth Graffy, Stephanie Novak, and Dr. Jennifer Old for all of their brainstorming and troubleshooting help on this case.

For further information

Please contact *marisa@ifi-test.com* More information on this and related projects can be obtained at *www.ifi-test.com.*

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