TECHNICAL NOTE

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A Rapid Screening Technique for the Detection of Spermatozoa

REFERENCE: Fraysier, H. D., **"A Rapid Screening Technique for the Detection of Spermatozoa,"** *Journal of Forensic Sciences,* JFSCA, Vol. 32, No. 2, March 1987, pp. 527–530.

ABSTRACT: Phase contrast microscopy has been used for some time to search for and identify spermatozoa. An enhancement to the technique using xylene in conjunction with phase contrast microscopy is discussed. The method has been found to save time by allowing identification of spermatozoa with xylene-enhanced phase contrast microscopy in many cases that would have been unsuccessful using the normal "dry" phase contrast microscopy techniques. This eliminates a staining and reexamination step.

KEYWORDS: criminalistics, microscopy, spermatozoa, phase contrast microscopy, halation, xylene

Anyone who has microscopically examined slides for spermatozoa can attest to the lengthy examination time required when very few or no spermatozoa are present. A common procedure would be to examine a slide "dry" using either a 10 or ×40 objective on a phase contrast microscope. If no spermatozoa are observed, staining of the slide would be done for more intensive study. Although the use of conventional phase contrast microscopy [*1,2*] can be of some benefit, the problem associated with halation of the Becke line [*3*] can cause severe loss of resolution, particularly in areas where there is particulate matter surrounding areas of spermatozoa. In an effort to eliminate this problem, a suitable immersion substance was tried.

Experimental Procedure

A No. 1½ coverslip, 22 by 50 mm, was placed over a slide prepared from vaginal washings and examined, dry, on a Model BH-2 Olympus Phase Contrast Microscope using a ×40, 0.65 N.A. objective and ×10 wide field eyepieces (see Fig. 1 of stage micrometer). An area containing a possible group of spermatozoa was found and photographed. As can be seen in Fig. 2, confirmation of spermatozoa was not possible because of excessive halation of nearby particulate matter.

Next, reagent grade xylene, having an approximate index of refraction of 1.497, was flowed in from the edge of the coverslip allowing complete immersion and the same area was

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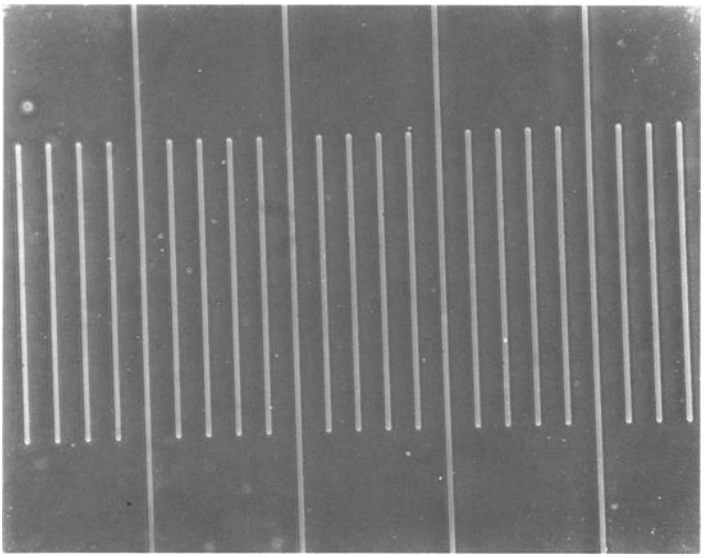

FIG. 1—*Photomicrograph of the stage micrometer, having 0.1-mm divisions, which was photographed on the same microscope, and under the same conditions, as the spermatozoa. The magnifications on the negatives of the stage micrometer and on the spermatozoa were ×500, and the final magnifications, after enlargement were ×600. Therefore, one small division of the stage micrometer should measure 6 mm.*

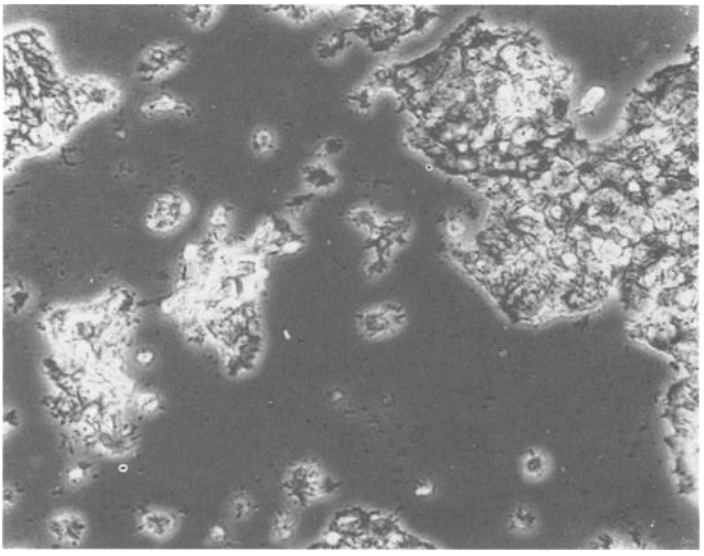

FIG. 2—*Dry mount, phase contrast: obscured Spermatozoa in vaginal washings (×600).*

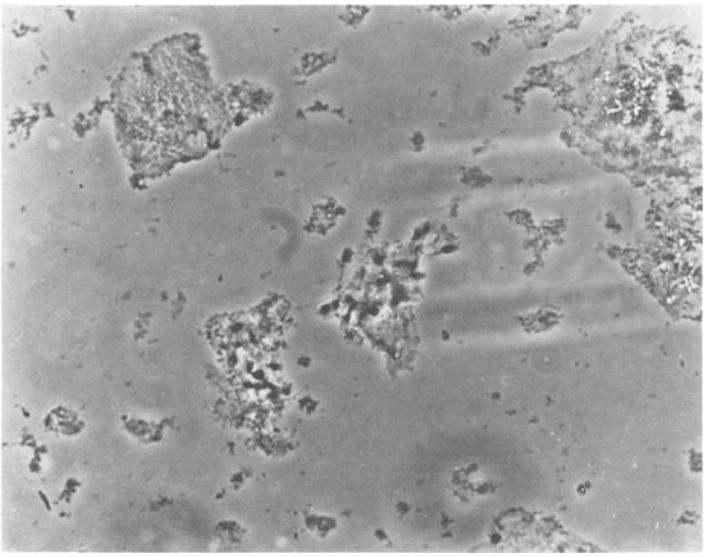

FIG. 3—*Xylene mount, phase contrast: spermatozoa in vaginal washings (×600).*

examined again. It was immediately possible to identify a group of spermatozoa having good tone gradation and resolution (Fig. 3). The net effect was to enhance the spermatozoa and subdue the background particulate matter.

Conclusions

The use of xylene as an immersion medium on prepared slides suspected of containing spermatozoa overcomes the problems associated with "dry" phase contrast microscopy and renders spermatozoa with good resolution and tone gradation in much shorter examination times.

The effects of xylene on the ability to carry out ABO typing or electrophoretic analysis on enzymes has not been examined since it is the practice of our laboratory to carry out such examinations on swabs which are virtually always available in sex crimes evidence kits.

Although the illustrative photographs chosen were made from a case where vaginal washings were examined, the method has been applied successfully in numerous cases to smear slides received in sex crimes evidence kits.

Acknowledgments

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