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TECHNICAL NOTE

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Techniques for Extraction of Spermatozoa from Stained Clothing: A Critical Review

The location of spermatozoa on the clothing of rape victims is of primary concern to the criminalist seeking evidence of sexual assault. The identification of acid phosphatase, spermine, and choline is of secondary importance relative to finding intact spermatozoa. A number of techniques for extraction of spermatozoa are available. In this study these techniques have been compared in an effort to determine their relative merits.

The use of an ultrasonic apparatus to aid in extraction of spermatozoa from cloth has been described [*1–3*] and is apparently applied by a great many analysts. Several techniques employing the ultrasonic apparatus were studied to determine their effects on spermatozoa yield from such extractions.

Although not described in the available literature, it is the understanding of this investigator that mechanical agitation is also being used to free spermatozoa from stained clothing. A form of mechanical agitation was compared with the ultrasonic technique in this study.

Apparatus and Equipment

The following equipment was employed:

1. Ultrasonic Source. A Sears Roebuck & Company Ultrasonic Cleaner was chosen because of its availability and low cost.
2. Mechanical Vibrator. A Scientific Products Vortex Genie Mixer, set on a scale reading of 3, was used for mechanical agitation.
3. Microscope. Slides were examined at ×430 with an American Optical Microstar Microscope.

Procedure and Experimental Details

A cotton cloth was uniformly spotted with known seminal fluid and used in all tests rather than using clothing from alleged rape victims. Spot positions were marked, and the cloth was allowed to dry overnight. Circles of 1-cm diameter were cut from the cloth and placed in centrifuge tubes.

Ultrasonic Separation

Techniques employing ultrasonic separation of spermatozoa from cloth vary primarily in procedures prior to subjecting the fabric in question to ultrasonic vibration.

Kivela [*1*] recommends an aqueous wash of the sample material to obtain "cleaner

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slides." In order to determine how such washing might effect spermatozoa yield, a sample was washed and the washing centrifuged. The residue was removed by capillary pipet, deposited on a slide, and heat-fixed. After a staining with methylene blue, the number of spermatozoa observed were counted. The washed cloth sample was placed in a second centrifuge tube, and 2 ml of water was added. The sample was then subjected to ultrasonic vibration for 15 min. The sample cloth was removed, the solution centrifuged, and a slide prepared as before. The spermatozoa found were counted.

Marcinkowski and Przybylski [*2*] soaked their samples for 10 to 36 h in saline solution before ultrasonic vibration. To determine whether or not such soaking increased spermatozoa yield, three samples were soaked in 0.85% saline solution for 4 h. After soaking the samples were subjected to 1, 3, and 15 min of ultrasonic vibration, respectively. Slides were prepared as previously described, and spermatozoa were counted. Similar extractions were performed with unsoaked samples, using the same ultrasonic exposure times.

Kivela [*1*] subjected his samples to 2 min of ultrasonic vibration, while Marcinkowski and Przybylski [*2*] and Gluckman [*3*] recommend times of 45 and 30 min, respectively. To ascertain the dependence of spermatozoa yield on ultrasonic vibration exposure time, three samples were placed in separate centrifuge tubes and subjected to 1, 15, and 30 min of ultrasonic vibration, respectively. Slides were then prepared as described above, and spermatozoa were counted. A fourth sample was treated as follows: 1 min of ultrasonic vibration was applied, after which the cloth was removed and placed in a second centrifuge tube containing 2 ml of water, and 15 min more of ultrasonic vibration was applied. Finally, the cloth was removed, placed in a third centrifuge tube, and given another 15 min of ultrasonic vibration. Slides were prepared from the three specimens, and spermatozoa were counted.

Mechanical Vibration

Although Marcinkowski and Przybylski [*2*] concluded that ultrasonic exposure caused no injurious influence to spermatozoa, the use of less rigorous means of freeing the spermatozoa from cloth was investigated. Centrifuge tubes containing the samples and 2 ml of water were held on the Vortex Genie Mixer for periods of 1 and 3 min. A third sample was subjected to the mechanical agitation for 3 min, removed and placed in a second centrifuge tube with 2 ml of water, and given 15 min of ultrasonic vibration. Slides were prepared and spermatozoa yields compared.

Results and Discussion

Washing suspect cloth samples before ultrasonic vibration was found to result in removal of a relatively small number of spermatozoa. However, it would seem reasonable to assume that, in some cases, such washing could remove the very evidence being sought.

Soaking of samples in saline solution did not show any significant differences compared to samples placed directly in the ultrasonic bath.

After 1 min of ultrasonic vibration, eight to ten intact spermatozoa were found, along with numerous heads and artifacts. Fifteen minutes of ultrasonic vibration produced 50 to 60 intact spermatozoa. The samples subjected to 30 min of vibration showed a high percentage of tailless heads. The sample that was sequentially exposed to increasing periods of ultrasonic vibration yielded consistent results in that 15 min appeared to be the optimum exposure period. Presumably, ultrasonic exposure beyond 15 min achieved removal of the more tightly held spermatozoa with a subsequent high incidence of de-

capitation. The variance in the optimum exposure time recorded here and those cited in the literature may be attributable to a variance in the ultrasonic devices used.

The mechanical agitation technique was found to produce significantly fewer spermatozoa than the ultrasonic technique. However, the percentage of intact spermatozoa was much greater in the mechanically agitated samples. The 1-min agitation time was sufficient to yield eight intact spermatozoa and three heads. Three minutes of mechanical agitation gave essentially the same results.

The sample that was first mechanically agitated and then immersed in the ultrasonic bath yielded results consistent with those obtained previously. A check of the mechanically agitated residue revealed several intact spermatozoa. Ultrasonic vibration produced a large number of heads and tails, as well as 10 to 20 intact cells. This combination technique would appear to be useful by not only yielding a maximum number of intact spermatozoa but also insuring maximum recovery.

References

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