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Carl R. Kempe

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POSSIBLE INTERFERENCE IN THE ACID PHOSPHATASE TESTS FOR SEMINAL FLUID STAINS

CARL R. KEMPE

The author is Police Chemist in the laboratory of the Grand Rapids Police Department and a graduate of the University of Minnesota. After joining the Grand Rapids Police Department Mr. Kempe received civil service appointment as Police Chemist and was the officer active in the establishment of this laboratory.—EDITOR.

In rape cases the police laboratory may be requested to examine the garments of the female and/or the garments of male suspects. Various methods for examination of semen confront the technician. The acid phosphatase method of Dr. Steffen Berg has proved of value in our hands. The discovery of the high concentration of acid phosphatase in prostate secretions is the basis for this method of testing for seminal stains.

There are several advantages to the method. It makes no difference whether spermatozoa are present or absent, so that the test is useful in cases of asperima. Sterility due to operation, senility, or interfering fluids may result in loss of the spermatozoa, but the acid phosphatase will be detectable in many such cases.

METHOD

Reagents recommended by Dr. Berg are¹:

1st Solution

Sodium chloride—23 gms
Sodium acetate: 3 (H₂O)—2 gms
Pure acetic acid—0.5 cc
Distilled water—90 cc

2nd Solution

Dianisyltetrazonium chloride—30 mgm
Calcium alpha naphthyl phosphate—50 mgm
Sodium laurylsulphonate, 10% aqueous solution—1 cc

These two solutions are mixed together to make the testing reagent. The first solution will keep indefinitely. Mix the second solution fresh, and when both are mixed together they will keep for 3 weeks under refrigeration. They are to be mixed immediately before using. Filter before using the mixture, and it should have a clear amber color.

¹ DR. STEFFEN P. BERG, INTERNATIONAL CRIMINAL POLICE REVIEW, No. 85, 53 (1955).

APPLICATION

The cloth to be tested is cut out and placed in a small white porcelain evaporating dish. Two controls are used; one from the garment area least likely to contain seminal fluid and the other from a known control seminal stain. A few drops of the filtered reagent is put on the suspected cloth and on the two controls. The test can be done on a piece of cloth only a quarter or eighth inch in diameter, and if one does not wish to cut the garment, the reagent can be placed directly to the material. If seminal fluid is present, the phosphatase reacts with the phosphoric ester forming alpha naphthol, which combines with the dianisyltetrazonium salt to form a bluish-violet color. The test fluid also becomes colored, a fact which proved to be of importance in a case involving black panties, whereby the fluid became colored and gave a positive reading. Dark colored clothing may not permit reading of the color reaction.

The phosphatase activity in a fresh dry seminal stain is very intense and a distinct color appears within a minute or so. Older stains will take a few more minutes to complete the reaction.

Our experience includes four cases in which microscopic examination disclosed the presence of intact morphological spermatozoa on the victim's clothes. The clothing was then examined at later dates by the acid phosphatase method. Regardless of the age of the seminal stain, the method gave excellent results. These cases involved:

1. Female panties—seminal stain 2 mos. 9 days old
 2. Female skirt—seminal stain 25 days old
 3. White percale sheet—seminal stain 9 mos. 7 days old
 4. White handkerchief cloth—1 yr. 3 mos. old
- All of the above gave positive readings while controls on least likely areas of each garment failed to respond.

The research for this paper was begun after the investigation of a murder of a pregnant girl. While in this case examination of vaginal smears was negative, an apparant close color reaction with the test suggested the need of special research.

SPECIFICITY

This new method was tested on vaginal smears from non-pregnant and pregnant women, blood stains, urine, cotton, and phenol. The only interfering reaction found was the cotton swab smears from pregnant women. Both male and female urine do not interfere with this test. Phenol and blood stains gave orange to brown coloration.

A series of tests was run on vaginal smears from pregnant and non-pregnant women in 1957. The results are similar to the 1958 tests which are reported below. All smears taken by gynecologists in this city. Smears were taken from subjects who did not have sexual intercourse for at least 48 hours prior to examination. Gynecologists obtained urine specimens from pregnant and non-pregnant women. Five smears from pregnant women and five smears from non-pregnant women were tested. Ten secretions from smears (pregnant and non-pregnant) failed to respond to the acid phosphatase test. Urine from both groups failed to respond and even when this was dried on clean white cloth did not give a positive test.

The cotton swab vaginal smears of non-pregnant women when tested in small white porcelain dishes gave negative results. There was a slight reddish coloration which appeared after 15 minutes. After 30 minutes, light red color was observed. The fluid had a slight precipitation in one smear, all others failed to have a precipitation. The precipitation was visible with the naked eye.

Pregnant womens' vaginal smears responded faster and developed darker red color than the smears from non-pregnant women. The smears

from pregnant women were just a little slower in developing than the positive controls. After 7 minutes, all smears of pregnant women had a reddish-purple appearance. Many developed the color well before 7 minutes. This was generally similar to seminal fluid controls. Coloration appeared to match even better after 30 minutes, but under 13 \times magnification appeared more red than bluish. After 30 minutes, red precipitate was observed with the naked eye. The precipitate fragments were smaller than those from the seminal fluid control and were present in all smears from pregnant women.

CONCLUSION

Only one possible interfering substance was found and that was vaginal smears from pregnant women. In such instances study of color under magnification and of size and color of precipitate should prevent error. All other body fluids tested do not contain as much acid phosphatase as seminal fluid. Therefore, this test is nearly specific and is simple to administer. The acid phosphatase method can detect seminal fluid from the sterile person as spermatozoa are no longer a prerequisite to a positive test. This gives law enforcement officers a valuable procedure in dealing with sex crimes. The element of time is of little concern with this method as long as the cloth is dry and the stain has not putrefied.

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