

Introduction

1. This protocol is used to provide a presumptive indication of the presence of seminal fluid in an unknown stain.
2. This protocol is to be used by all qualified analytical personnel assigned to the Forensic Biology Unit.
3. Seminal fluid is the secretions of the male reproductive organs not containing sperm cells.
4. Semen is defined as the secretions of the male reproductive organs that contain sperm cells.
5. The presumptive test for seminal fluid is based upon the detection of the enzyme acid phosphatase. This enzyme is not specific for seminal fluid.
6. When AP test results are positive the following confirmatory test sequence should be followed:
 - a. Refer to FB Evidence Examination for guidelines for conducting confirmatory testing.
 - b. If microscopic examination is conducted and:
 - i. positive for sperm cells a PSA confirmatory test need not be conducted.
 - ii. negative for sperm cells, a PSA confirmatory test should be conducted.
7. The Acid Phosphatase reagent (AP Spot Test) is purchased by the SERI Company.
8. The AP Spot Test stock reagents are stored at -20°C .
9. It is not required to take photographs or store the AP results.

Safety

1. Personal protection equipment including safety glasses, gloves, face masks, bouffant caps, and laboratory coats must be worn when conducting this procedure.
2. No open toe or open back shoes are to be worn while conducting this procedure.
3. Paper waste must not come in contact with biohazard material if discarded into garbage containers.
4. Before handling any chemicals, refer to the MSDS provided by the manufacturer, and observe all relevant precautions.

Reagent Preparation

1. The AP working solution must be made fresh daily.
2. Add 0.26 grams of the AP Spot Test to 10 ml of deionized water. The reagent volume may be reduced as long as the ratio of AP Spot Test to deionized water remains constant.
3. Mix the solution thoroughly.
4. Place the AP working solution in an amber colored conical to minimize light exposure.
5. Cap the AP working solution when not in use to minimize oxidation.

Verifying the Controls

1. The AP reagent performance must be verified before using on casework evidence.

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2. Test a known semen stain as a positive control and a blank (filter paper) as a negative control (See interpretation of results for determining a positive and negative result).
3. Record the AP chemical tag number and the results of the controls on the FB Evidence Summary Worksheet form by placing a check mark by the "+" and "-" controls.
4. If the controls do not give the appropriate results (purple color change for positive control, no color change for negative control) the AP reagents must be remade and the controls retested.

Test Procedure

1. Cut a small portion of a stain cutting or dampen a sterile swab and swab the stain.
2. Add one to two drops of AP reagent to the stain cutting or swab.
3. If a large area needs to be screened, a technique referred to as "mapping" (dividing the whole into separate areas) may be used. Two examples of mapping include:
 - a. Swab the area of interest. Cut a small portion of the swab and apply AP reagent to cutting.
 - b. Use filter paper moistened with deionized water and gently press down on the desired area(s). Be sure to mark the filter paper for orientation. Deposit the AP reagent over the entire filter paper.
4. The vaginal (or penile), rectal, and oral swabs in a Sexual Battery Evidence Collection Kit will be tested for the presence of AP based on sample size, case scenario, and analyst discretion.
5. If more than one swab is present and a negative result is obtained, each additional swab must be tested for the presence of AP. If a positive AP result is obtained, it will be analyst discretion if the additional swabs are to be tested. The case facts may not support screening for semen, e.g. digital penetration. The analyst may want to confirm the case scenario before performing further testing.
6. The result of each swab tested must be noted on the FB Evidence Summary Worksheet form in the column labeled AP.

Interpretation of Results

Positive Results

1. If acid phosphatase is present, a purple color will appear within a short period of time (30 seconds). Weak stains may take between 1 to 3 minutes.
2. A positive reaction indicates the presumptive presence of seminal fluid.
3. A positive test result is produced by purple color change.
4. A positive result will be reported as "+" on the FB Evidence Summary Worksheet form in the column labeled AP.
5. If the color change is gradual or weak, a positive result may still be reported.
6. The analyst will report this type of positive result as "v.wk +" or "wk +" on the FB Evidence Summary Worksheet form in the column labeled AP.

Negative Results

1. The expected result is no purple color change.
2. The AP test must be read within 3 minutes after the AP reagents are applied to the evidentiary stain.

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3. A purple color may occur immediately or at about 3 minutes. Allowing the reaction to proceed beyond 3 minutes may elicit a purple color in the absence of seminal fluid. This is a normal catalytic reaction that may occur without the presence of seminal fluid. Therefore, any test read after 3 minutes should be interpreted with caution.
4. A negative result will be reported as "-" on the FB Evidence Summary Worksheet form in the column labeled AP.

Inconclusive Results

1. Inconclusive results may be obtained when the color of the stain is the same color as the test or there appears to be interference in reading the test color change.
2. The analyst may indicate this as "INC" in the AP column of the FB Evidence Summary Worksheet form.
3. If discoloration occurs as the test is being conducted, there may be an interfering substance. The analyst should proceed with the test and determine if the test is truly AP presumptive positive, negative, or inconclusive.
4. If an inconclusive AP test result is obtained and enough sample is available, then a confirmatory test for semen should be conducted.

References

1. Saferstein R, Baechtel FS. Forensic Science Handbook, Vol. II. Prentice Hall, Inc.: Englewood Cliffs, NJ, 1988; 347-390.
2. Gaensslen RE. Sourcebook in Forensic Serology, Immunology, and Biochemistry. U.S. Government Printing Office: Washington, D.C., 1983; 155-166.
3. Seri Methods Manual Brentamine Reaction January 14, 2002.
4. Laux, Dale L. Forensic Detection of Semen I. The Acid Phosphatase Test.