

SER: Seminal Fluid – Acid Phosphatase Spot Test

Principle Acid phosphatase (AP) is an enzyme that is found in seminal fluid, a component of semen. Its concentration in seminal fluid is up to 400 times greater than that found in other body fluids.

Sodium α -naphthyl phosphate is cleaved by acid phosphatase in the sample, releasing sodium phosphate and naphthol. Naphthol couples with brentamine (*o*-dianisidine) to create a purple azo dye. The formation of a purple color indicates the presence of acid phosphatase.

Supplies The following supplies are used in this procedure:

- filter paper
 - cotton swabs
 - glass plates
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Reagents This procedure uses the following reagents:

- AP Spot Test reagent
 - Dissolve 0.26 grams of SERI Acid Phosphatase Spot Test PMR in 10 mL of deionized water. This solution must be made fresh prior to use.
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Quality control The *AP Spot Test reagent* must be quality control tested before each use with

- a positive control (semen or seminal stain)
 - a negative control (water).
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Records Record the lot number and expiration date of the reagent, as well as the results of the quality control tests, in the examination documentation.

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Overlay procedure

Use the following procedure to perform the test using filter paper overlays.

| Step | Action |
|------|--|
| 1 | Moisten a suitably sized piece of filter paper with deionized water. |
| 2 | Lay the filter paper over the suspected stain to transfer it to the filter paper. A glass or plastic plate and weight can be applied to ensure proper contact. Mark seams or other reference points for orientation. |
| 3 | After removing the filter paper from the evidence item, apply the <i>AP Spot Test reagent</i> until the filter paper is saturated. |
| 4 | Record the time of development of any color that occurs within 60 seconds. If no color reaction occurs within 60 seconds, record the result as negative. |

Procedure using swabs or filter paper

Use the following procedure to perform the test using swabs or filter paper.

| Step | Action |
|------|---|
| 1 | Moisten a sterile test swab or filter paper with deionized water. |
| 2 | Apply the test swab or filter paper directly onto the item to be tested. |
| 3 | Add a drop of <i>AP Spot Test reagent</i> to the test swab or filter paper. |
| 4 | Record the time of development of any color that occurs within 60 seconds. If no color reaction occurs within 60 seconds, record the result as negative. |

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Interpretation The development of a purple color within 60 seconds is a positive (+) result for the presence of acid phosphatase.

The absence of a color reaction within 60 seconds is a negative (-) result for the presence of acid phosphatase.

Acid phosphatase is present in seminal fluid, a component of semen, in high concentrations; however, it is also present in other body fluids at lower concentrations and in plants, fungi, and bacteria. Semen stains tend to give a faster and stronger reaction than other sources.

Since acid phosphatase is not unique to seminal fluid, the test is only a presumptive test for the presence of seminal fluid.

A negative reaction does not necessarily mean that other components of semen are not present. If there is a negative reaction for acid phosphatase and no spermatozoa are detected in the sample extract, further testing for p30 or semenogelin must be conducted.
