

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.
