

## SER: Seminal Fluid – Acid Phosphatase Spot Test

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<b>Principle</b>	<p>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.</p> <p>Sodium <math>\alpha</math>-naphthyl phosphate is cleaved by acid phosphatase in the sample, releasing sodium phosphate and naphthol. Naphthol couples with brentamine (<i>o</i>-dianisidine) to create a purple azo dye. The formation of a purple color indicates the presence of acid phosphatase.</p>
<b>Supplies</b>	<p>The following supplies are used in this procedure:</p> <ul style="list-style-type: none"><li>• filter paper</li><li>• cotton swabs</li><li>• glass plates</li><li>• spot plate</li><li>• disposable pipette</li></ul>
<b>Reagents</b>	<p>This procedure uses the following reagents:</p> <ul style="list-style-type: none"><li>• AP Spot Test reagent<ul style="list-style-type: none"><li>– 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.</li></ul></li></ul>
<b>Quality control</b>	<p>The <i>AP Spot Test reagent</i> must be quality control tested before each use with</p> <ul style="list-style-type: none"><li>• a positive control (semen or seminal stain)</li><li>• a negative control (water)</li></ul>
<b>Records</b>	<p>Record the lot number and expiration date of the reagent, as well as the results of the quality control tests, in the examination documentation.</p>

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## SER: Seminal Fluid – Acid Phosphatase Spot Test, Continued

### Procedure selection

Use the following guidelines to select the appropriate Acid Phosphatase procedure:

If	Then...
The evidence will only undergo Acid Phosphatase testing and no DNA analysis will be conducted	Select Procedure 1
Non-swab evidence will be examined for potential DNA analysis	Select Procedure 1
Swab evidence will be examined for potential DNA analysis	Select Procedure 2

### Procedure 1

Step	Action
1	Moisten a sterile test swab or filter paper with deionized water.
2	Apply the test swab or filter paper directly to the item to be tested.  NOTE: A glass or plastic plate and weight can be applied to the filter paper to ensure contact. Mark seams or other reference points to aid in orientation of the filter paper.
3	Add the <i>AP Spot Test Reagent</i> to the test swab or filter paper.
4	Record the time development of any color change that occurs within 60 seconds.  If no color reaction occurs within 60 seconds, record the result as negative.

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## SER: Seminal Fluid – Acid Phosphatase Spot Test, Continued

### Procedure 2

Step	Action
1	Place the entire swab into a sterile microcentrifuge tube.
2	Add 300 µL of TE Buffer and incubate the swab at room temperature for 30-60 minutes on the ThermoMixer.
3	Transfer the swab to a spin basket, place the spin basket into the original tube, and centrifuge for 5 minutes.
4	Using a disposable pipette, add 1 drop (approximately 50 µL) of the supernatant to a spot plate well.
5	Add a drop of <i>AP Spot Test Reagent</i> to the well.
6	Record the time development of any color change that occurs within 60 seconds.  If no color reaction occurs within 60 seconds, record the result as negative.

### 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.