

ACID PHOSPHATASE

Background

This protocol outlines a presumptive test for seminal fluid. Acid Phosphatase is the enzyme secreted by the prostate gland, which is present in large amounts in seminal fluid. It is not unique to the prostate gland and can be present in vaginal secretions. Therefore, acid phosphatase testing is a presumptive test for semen.

Summary of Procedure

The liberation of naphthol from sodium a-naphthyl phosphate by the enzyme acid phosphatase and the formation of a purple azo dye by the coupling of naphthol with buffered fast blue B is the mechanism of this reaction. Stains that are positive for semen produce a deep purple color reaction which will be visible in the liquid.

Sample Handling

Forensic samples may be in limited supply. Retain sufficient sample for replicate analysis.

Label all samples with complete identifying information.

All biological samples and DNA must be treated as potentially infectious. Appropriate sample handling and disposal techniques should be followed. See:

- **Safety Manual, Universal Precautions**
- **Quality Assurance Manual, General Sample Control and Forensic Sample Preservation Policy**
- **Analytical Procedures Manual, Forensic Evidence Handling.**

Warnings and Precautions

The enzyme acid phosphatase occurs in other bodily fluids as well as bacteria, fungi and plants. False positive reactions may occur due to the following:

- Vaginal secretions – bacterial infection and pregnancy cause increased levels of Vaginal Acid Phosphatase (VAP). The reaction is slow and faint.
- Fecal stains – The reaction is slow and faint pink.
- Blue dye transfers – from fabric or other objects can cause a false positive.

Reagents and Materials

See **Appendix B** for reagent preparation

10% Bleach
MBG Water
100% Ethanol
AP Spot test
Disposable bench paper

Lab Coat
Kimwipes
Gloves
Protective Eyewear
Swabs
Sample handling tools (scissors, scalpel blades, forceps, etc.)
Clear test tubes (6 x 50mm)
Spray bottle

15ml Dropper bottles

Reagents and Materials – Storage and Handling

All reagents and materials are to be kept under sterile conditions. Store all reagents according to the manufacturers' recommendations.

Do not use reagents beyond the listed expiration dates. Date and initial all reagents when put in use. Record in the **Reagent Log**.

Quality Control

Use of the **Evidence Examination Worksheet** or other appropriate worksheet is required for documentation. All information must be completed.

The AP spot test working solution must test successfully against a positive semen control and a negative control to ensure detection efficacy prior to use.

All results must be verified by a second qualified analyst. If a second qualified analyst is unavailable photo documentation of the result is acceptable

Positive Control

Semen Positive Control

Negative Control

This negative control consists of all reagents used in the procedure but contains no substrate.

Procedure

1. A small cutting of the sample, ~ 2mm², is placed in a small test tube.
2. Add several drops (~5-10, depending on the sampling in step 1) of the AP spot (brentamine) solution.
3. Observe for ~1 minute for a color change in the liquid.
4. Note the results on the **Evidence Examination Worksheet** or other appropriate worksheet.

Results and Conclusions

Positive Result: A pink or purple color change in the liquid indicates the presence of seminal fluid which is a positive result. This would be reported as seminal fluid is indicated on the appropriate report.

Negative Result: No pink or purple color change in the liquid indicates the absence of seminal fluid which is a negative result. This would be reported as no seminal fluid is indicated on the appropriate report.

Inconclusive Result: A color change in the liquid other than pink or purple may indicate the presence or absence of seminal fluid which is not a conclusive or decisive result. The substrate may have a color change after one minute. This would be reported as the test for seminal fluid was inconclusive on the appropriate report.

Technical Assistance

For information and assistance regarding the performance or applications, contact Serological Research Institute
Phone: (510) 223 – 7374 Email seri@serological.com

Reference

Forensic Detection of Semen I. The Acid Phosphatase Test – Laux, D., Ohio Bureau of Investigation.

The Identification and Individualization of Semen Stains – F. Samuel Baechtel, Ph.D., Federal Bureau of Investigation

Brentamine Reaction – Serological Research Institute Methods Manual.