

FBS05- Acid Phosphatase Presumptive Chemical Test for the Presence of Semen

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1. Scope

- 1.1. This procedure is used to determine the possible presence of semen on evidentiary material.

2. Background

- 2.1. Qualitative acid phosphatase (AP) testing is used as a screening test for semen. α -Naphthyl phosphate is acted upon by the enzyme AP to produce α -naphthol, which then combines with diazo blue B dye to form a violet colored complex. In the absence of AP, α -naphthyl phosphate is not able to combine with the diazo blue B compound and the reaction will remain colorless.
- 2.2. AP originates in the prostate gland. Although present in other body fluids, it occurs in seminal fluid at concentrations 20 to 400 times higher than that of other body fluids. Since AP is not exclusive to human semen, it can only be used as a presumptive test for the presence of semen.

3. Safety

- 3.1. Wear personal protective equipment (e.g., lab coat, gloves, mask, eye protection), when carrying out standard operating procedures.

- 3.2. Read Material Safety Data Sheets to determine the safety hazards for chemicals and reagents used in the standard operating procedures.

4. Materials Required

- 4.1. Acid Phosphatase Reagent (FBR22)

- 4.2. Positive Control-Semen (FBR03)

4.2.1. NOTE: Never use solutions directly from the stock bottles. Use Reagent SOPs for preparation and labeling instructions.

4.2.2. NOTE: AP working solution reagent expires at the end of the day.

5. Standards and Controls

- 5.1. The Positive and Negative Controls are tested prior to daily use. These results will be recorded in casework documentation.

5.1.1. A known semen dilution strip is tested as a Positive Control (FBR03). Apply the AP working solution directly to the semen dilution strip. This control should develop a violet/purple color upon addition of the AP working solution in the following time frames:

| Positive Control Sample | Results |
|-------------------------|--|
| Semen - 1:16 dilution | Rapid violet/purple color (~0 to 10 seconds) |
| Semen - 1:32 dilution | Slow violet/purple color (~20 to 30 seconds) |

5.1.2. An unstained area on the known semen dilution strip is tested as a Negative Control **or Blank**. This control should not exhibit a violet/purple color within 60 seconds upon addition of AP working solution.

6. Calibration

- 6.1. Not applicable

7. Procedures

- 7.1. Stain areas may be located by a visual examination and/or with the aid of an ALS.

7.2. Stain areas may be sampled using various methods:

7.2.1. Swab stain area with moistened swab (diH₂O)

7.2.2. Small cutting placed on filter paper or other appropriate clean surface

7.2.3. Press a damp filter paper (diH₂O) on the stain area (overlay)

7.3. Add one drop of the AP working solution to the cutting, swab or filter paper. For large filter paper overlays, the reagent may be sprayed onto the filter paper or multiple drops may be added.

7.4. Using a timer, observe for 1 minute. A rapid color change to purple within 30 seconds is a presumptive positive result for the presence of semen. A color change from 31-59 seconds is an inconclusive result indicating the possible presence of semen. No color change at or after 60 seconds is a presumptive negative result for the presence of semen.

8. Sampling

8.1. Not applicable

9. Calculations

9.1. Not applicable

10. Uncertainty of Measurement

10.1. When quantitative results are obtained, and the significance of the value may impact the report, the uncertainty of measurement must be determined. The method used to determine the estimation of uncertainty can be found in the *FSL Quality Assurance Manual – Estimation of Uncertainty of Measurement (Section 5.4.6)*.

11. Limitations

11.1. The AP test is a presumptive test for the presence of semen. In order to confirm the presence of semen, a microscopic examination for the presence of spermatozoa (FBS07) and/or a p30 test (FBS06) must be performed with a positive result.

- 11.2. A color change must be observed within 30 seconds to be considered a positive test result. An unlimited detection time could lead to a false positive reaction.
- 11.3. This test is dependent upon the amount of AP present in the sample. An inconclusive or negative presumptive test for the presence of AP does not necessarily mean that the stain tested does not contain semen. It is possible to have semen present in such dilute amounts in a stain that a positive reaction is not obtained. Additionally, there could be little or no enzyme activity for older semen stains. Alternatively, a positive presumptive test for AP does not confirm the presence of semen.
- 11.4. Insufficient sample quality and/or quantity could limit the development of a positive reaction.

12. Documentation

- 12.1. FBU Serology Examination Worksheets
- 12.2. FBU Report of Results

13. References

- 13.1. Baechtel, F. S. (1988) "The Identification and Individualization of Semen Stains," In *Forensic Science Handbook Volume II*, R. Saferstein editor, Prentice Hall, Englewood Cliffs, New Jersey, p. 347-392.
- 13.2. Davies, A. and E. Wilson. (1974) "The Persistence of Seminal Constituents in the Human Vagina," *Forensic Science*, 3, p 45-55.
- 13.3. Gaensslen, R. E., *Sourcebook in Forensic Serology, Immunology, and Biochemistry*, U.S. Government Printing Office, Washington, DC, 1983, p. 158-159.
- 13.4. McCloskey, K. L., Muscillo, G. C., and B. Noordewier (1975) "Prostatic Acid Phosphatase Activity in the Postcoital Vagina," *Journal of Forensic Sciences*, 20(4), p. 630-636.
- 13.5. Metropolitan Police Forensic Science Laboratory Biology Methods Manual, London, 1978, p. (3-17)-(3-19).

- 13.6. Owen, G.W. and K.W. Smalldon (1975) "Blood and Semen Stains on Outer Clothing and Shoes Not Related to Crime: Report of a Survey Using Presumptive Tests," *Journal of Forensic Sciences*, 20(2), p. 391-403.
- 13.7. Poyntz, F.M., and P.D. Martin (1984) "Comparison of p30 and Acid Phosphatase Levels in Post-Coital Vaginal Swabs from Donor and Casework Studies," *Forensic Science International*, 24, p. 17-25.
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- 13.10. Sensabaugh, G.F. (1983) "The Acid Phosphatase Test," *Proceedings of a Forensic Science Symposium on the Analysis of Sexual Assault Evidence*, Washington, DC, US Government Printing Office, p. 65-81.
- 13.11. Sensabaugh, G.F., Blake, E.T. and J.S. Bashinski (1983) "Acid Phosphatase Assay of Vaginal Swabs," *Proceedings of a Forensic Science Symposium on the Analysis of Sexual Assault Evidence*, Washington, DC, US Government Printing Office, p. 146-148.
- 13.12. *Forensic Science Laboratory Quality Assurance Manual* (Current Version)
- 13.13. *FSL Departmental Operations Manuals* (Current Versions)
- 13.14. *FSL Laboratory Operations Manuals* (Current Versions)
- 13.15. *FBR22- Acid Phosphatase Reagent* (Current Version)
- 13.16. *FBR03 - Positive Control – Semen* (Current Version)
- 13.17. *FBS04 - Use of Alternate Light Source to Aid in Stain Identification* (Current Version)
- 13.18. *FBS06 - P30 Antigen Test for the Presence of Semen* (Current Version)

13.19. *FBS07 - Microscopic Examination for the Presence of Spermatozoa by Christmas Tree Stain* (Current Version)