One-Step Acid Phosphatase Spot Test

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1 INTRODUCTION

These procedures describe the procedures for screening evidentiary items for the possible presence of semen by testing for acid phosphatase (AP) using the One-Step AP Spot Test.

2 SCOPE

These procedures apply to DNA personnel that screen evidentiary items for the possible presence of semen.

3 EQUIPMENT

- General laboratory equipment and supplies (e.g., scissors, forceps, scalpel blades, gloves, etc.)
- Acid Phosphatase Spot Test (Serological Research Institute (SERI) Item # R558, or equivalent)
- Culture tubes, 12 x 75 mm (Kimble Glass, Inc., # 73500 1275, or equivalent)
- Transfer pipettes (Samco Scientific Corporation, Cat. No. 232-15, or equivalent)
- Water (Reagent Grade, VWR, Catalog # 48218-710, or equivalent)

Refer to the appropriate DNA QA procedure (i.e., BIO-103) for reagent and control preparation information.

4 STANDARDS AND CONTROLS

The AP Spot Test solution must be tested prior to its first daily use on evidentiary items to verify its continued detection efficacy.

A known positive (KP) semen control is a dried human semen sample. A known negative (KN) semen control is a clean swab. A KP and KN must be tested by each biologist, each day, prior to using an aliquot of AP Spot Test solution for casework. Aliquot(s) of AP Spot Test solution that do not yield a positive reaction (i.e., a pink to purple color) with a KP semen control must not be used for casework. Aliquot(s) of AP Spot Test solution that yield a positive reaction (i.e., a pink to purple color) with a KN semen control must not be used for casework.

5 SAMPLING

Areas with potential semen staining will be selected by the biologist for swabbing and testing using these procedures. The visualization of staining may be aided using an alternate light source (ALS). Generally, a 455 nm wavelength with orange goggles is used for visualizing potential semen stains. At minimum, areas/stains that test positive will be described in the case notes.

6 PROCEDURE

Refer to DNA Procedure Introduction (i.e., BIO-100) for applicable laboratory quality assurance and cleaning instructions.

Ensure the appropriate fields (i.e., reagents, KP) in STACS are completed, as necessary.

1.	Using a new, clean swab moistened to dampness with reagent grade water, rub the stained area until a visible amount of stain has been transferred to the swab, or the swab appears matted.

A liquid sample (e.g., vaginal aspirate, vaginal wash, etc.) may be AP tested by applying 100µL onto a clean, dry swab following centrifugation.

Swabbings from multiple stains may be collected prior to testing. The biologist may collect the swabs by placing each swab into its own culture tube. The culture tubes must be labeled and/or positioned in a rack to allow for identification of each swab.

Generally, samples from a single item may be collected together. Additionally, samples from an individual's sexual assault evidence collection kit may be processed together, provided that only a single evidence item is out at a time for the collection of swabbings.

2.	Add ~3-4 drops of AP Spot Test solution to each swab tip over a receptacle.

Excess AP Spot Test solution should be captured in a weigh boat or other receptacle and transferred to an appropriate chemical waste container for disposal.

Multiple swabs may be processed concurrently in succession.

3.	Observe the swab tip for any color change within approximately 2 minutes and record the result as indicated below: The observation of a pink to purple color ……. POS The observation of no color …….. NEG The observation of a non-pink/purple color … INC

The observation of a non-pink/purple color could mask any potential pink/purple color that would result from the presence of semen. If such an observation is made, an Examiner should be consulted prior to conducting any additional testing.

The language an examiner should use to report the results from this testing and others is contained within the procedure for reporting serological testing results (i.e., BIO-400).

7 LIMITATIONS

- A positive result (i.e., a pink to purple color) with the AP Spot Test solution provides a presumptive indication that semen may be present on an item but it does not constitute an identification of semen. A confirmatory testing procedure is required to identify the presence of semen in a questioned stain.
- While a negative AP Spot Test indicates that no semen was detected in a stain, the failure to detect semen in biological material is not the basis for an absolute determination that semen was not present. False-negative test results (i.e., no pink to purple color) may be obtained when semen is present in a quantity below the detection limit of the AP Spot Test (e.g., insufficient quantity and/or poor quality). The sensitivity (i.e., detection limit) of the AP Spot Test procedure described in this document has been empirically determined in the Laboratory to generally yield a positive result from a dried stain of semen diluted 1/50.
- The utility of the AP Spot Test lies in its ability to provide information that aids in the differentiation between those stains that most certainly do not contain semen and those that may so that any further testing (i.e., confirmatory testing for semen, DNA testing, etc.) can be focused on those stains most likely to yield additional information.
- Presumptive semen testing may not be conducted on items of evidence of potential value for latent fingerprint examination.

8 SAFETY

- All evidence containing or contaminated with blood or other potentially infectious materials will be considered infectious regardless of the perceived status of the source individual or the age of the material.
- Refer to the FBI Laboratory Safety Manual for information on personal protection, the proper disposal of the chemicals used in these procedures, as well as the biohazardous wastes generated.
- Procedural Specific Chemical Hazard:
    - The AP Spot Test Powder can be hazardous and may cause cancer. It may be irritating to eyes, respiratory system, and skin; it is corrosive and may cause burns.

9 REFERENCES

Laux, D.S., Forensic Detection of Semen I. The Acid Phosphatase Test

10 REVISION HISTORY

Revision	Issued	Changes
00	09/30/2022	Reformatted DNA 117-1 into new template and assigned new Doc ID. Added guidance for sampling including ALS. Removed faint POS result.

11 APPENDIX A: REAGENT QUALITY CONTROL

Each new batch of AP Spot Test solution is tested for efficacy at the time of its preparation using the above procedure on an in-use KP semen control and a KN semen control.

A. A positive test result (i.e., a pink to purple color) for the KP semen control establishes that the new batch of AP Spot Test solution is yielding the expected positive result for semen (i.e., a pink to purple color). A new batch of AP Spot Test solution that does not yield a positive reaction with a KP semen control is not assigned a unique identifier (batch number or barcode) and must not be used for casework.
B. A negative test result (i.e., no pink to purple color) for the KN semen control establishes that the new batch of AP Spot Test solution itself is not yielding a positive result (i.e., a pink to purple color) in the absence of semen. A new batch of AP Spot Test solution that yields a false-positive reaction (i.e., a pink to purple color) with a KN semen control is not assigned a new unique identifier (batch number or barcode) and must not be used for casework.
C. If the expected results for both the KP and KN semen controls are obtained using the new batch of AP Spot Test solution, that preparation of AP Spot Test solution may be assigned a unique identifier (batch number or barcode) and may be used for casework.