

Procedures for the One-Step Acid Phosphatase Spot Test

1 Scope

These procedures describe the method in the DNA Casework Unit (DCU) by which evidentiary items are screened for the possible presence of semen by testing for acid phosphatase (AP) using the One-Step AP Spot Test.

2 Equipment/Materials/Reagents

- 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)
- General laboratory equipment and supplies (e.g., scissors, forceps, scalpel blades, rulers, tape, towels, gloves, etc.)
- Transfer pipettes (Samco Scientific Corporation, Cat. No. 232-15, or equivalent)
- Water (Reagent Grade, VWR, Catalog # 48218-710, or equivalent)

3 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.

4 Procedures

Refer to DNA Procedure Introduction (DNA QA 600) for applicable laboratory quality assurance and cleaning instructions.

The unique identifier [e.g., batch number (and expiration date) or barcode] of the AP Spot Test solution and KP control used for the examination of evidence must be documented in the casework notes.

4.1	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.	
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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 at a given time. The biologist may collect the swabs by placing each swab into its own 12 mm x 75 mm culture tube. The culture tubes should 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 the samples.

4.2	Add ~3-4 drops of AP Spot Test solution to each swab tip over a receptacle.	
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Excess AP Spot Test solution should be captured in a weigh boat or other receptacle. Excess liquid must be collected into an appropriate chemical waste container for disposal.

Multiple swabs may be processed concurrently in succession.

4.3	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 a faint pink color Ft. POS The observation of no pink to purple color ... NEG The observation of a non-pink/purple color ... INC	
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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.

5 Reporting Procedures

Refer to SOP 100 for reporting language to use based on the test results from this analysis and others.

6 Reagent Quality Control

6.1 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.

6.1.2 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.

6.1.2 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.

6.2 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.

7 Sampling

Not applicable.

8 Calculations

Not applicable.

9 Measurement Uncertainty

Not applicable.

10 Limitations

10.1 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.

10.2 A faint positive result indicates that semen may be present on an item but it does not constitute an identification of semen. The faint nature of the positive result is recorded in the case work documentation to capture semi-quantitative information that may be of value in determining what, if any, additional tests are conducted on a stain (e.g., confirmatory testing, DNA testing, etc.) and/or the amount of stain consumed for such a test(s).

10.3 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.

10.4 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.

10.5 Presumptive semen testing may not be conducted on items of evidence of potential value for latent fingerprint examination.

11 Safety

11.1 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. All nDNAU personnel who work with such material will follow the “Bloodborne Pathogen (BBP) Exposure Control Plan (ECP)” found in the most current version of the *FBI Laboratory Safety Manual*.

11.2 Refer to the “Safe Work Practices Procedures,” “Bloodborne Pathogen (BBP) Exposure Control Plan (ECP),” “Personal Protective Equipment Policy,” and “Chemical Hygiene Plan” sections of the *FBI Laboratory Safety Manual* for important personal safety information prior to conducting these procedures.

11.3 Refer to the “Hazardous Waste Disposal” section of the *FBI Laboratory Safety Manual* for important information concerning proper disposal of the chemicals used in these procedures as

well as the biohazardous wastes generated.

11.4 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.

12 References

FBI Laboratory Quality Assurance Manual (QAM)

FBI Laboratory Safety Manual

DNA Procedures Manual

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

Rev. #	Issue Date	History
0	10/02/12	Original document issued.
1	12/01/15	Changed to nDNAU to DCU. Simplified title and text of procedure. Deleted background section. Eliminated specific general laboratory supplies from list. Removed reagent and control preparation now contained in QA SOPs. Moved reagent QC to end. Loosened language to allow ~3-4 drops. Removed interpretation information from procedural section, still described in limitations section. Relocated footnote into 10.3. Updated reference for DNA Procedures Manual

Approval

Redacted - Signatures on File