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Specification Sheet

InSite® AP Test Strip

Item No. SD5

Diagnostic test for the detection of semen in forensic investigations by the qualitative determination of acid phosphatase (AP)

Intended Use

The InSite® AP test strip is a colorimetric test for the rapid detection of semen in forensic investigations. A POSITIVE test is indicated by the appearance of a purple spot against a white background within 60 seconds of beginning the test. The test is presumptive for semen, and a positive test should be followed by the more specific PSA test for confirmation.

Introduction

AP (acid phosphatase) is an enzyme which is produced by the prostate gland (Ref. 1). It catalyzes the hydrolysis of lysophosphatidic acid (Ref. 2), a breakdown product of membrane phospholipids, and may be involved in the capacitation of spermatozoa (whereby cell membranes are lost) which exposes proteins in the acrosomal head, making possible penetration of an ovum. AP is found in high concentrations in semen (about 1 mg/mL or 300 U/mL) [Ref. 3, 4], making it useful as a marker for the forensic determination of seminal fluid. It is stable when dry, and can be detected in old semen stains.

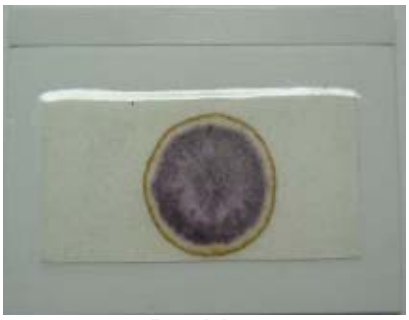
The AP test was first described by Babson in 1959 (Ref. 5). Although not as sensitive as the corresponding PSA test, it gives dramatic results within 15 sec with a strong stain and is very easy to use in the field.

Description of the test

The InSite® AP test strip assembly contains an inner paper element coated with reagents which react in the presence of semen, which element is sandwiched between a transparent cover and an opaque vinyl backing. The paper element contains 1-naphyl phosphate, Fast Blue B Salt and sodium dihydrogencitrate. When acid phosphatase (semen) comes into contact with the test paper, it catalyzes the hydrolysis of 1-naphthyl phosphate to 1-naphthol, which then reacts immediately with Fast Blue B (a diazonium salt) to form an intensely colored blue dyestuff. Citrate acts as a buffer to maintain an optimum pH of 4.

The test strips will detect semen down to a dilution of 1/2,000. Representative test results are shown below:

Negative (control)



Positive

Materials

The InSite® AP test strip comes sealed in pouches of 15 strips with desiccant.

Storage and Stability

Strips should be stored in a cool, dry place and away from sunlight. They may be refrigerated, but not frozen. They must remain in the sealed pouch (and if necessary brought to room temperature) before use. They are stable indefinitely. The pouch should be resealed tightly after use.

Sensitivity

The test is capable of detecting semen at a dilution of 1/2,000. The more concentrated the sample, the more intense the color reaction will be.

Specificity

The AP test is presumptive for semen but not specific in the same sense that an immunochromatographic test is. A positive test should be followed up if possible by a PSA test for confirmation of the presence of semen.

Test procedure

Place 10 drops of distilled or deionized water on the inside (stained) surface of the item to be tested (for example, the crotch of a pair of women's underwear), and let the water soak in for at least one minute. Then, peel apart an AP test strip assembly, and press the inner test paper element against the item for 5 seconds. A color change to purple within the first 60 seconds after that is a POSITIVE test. If the test is POSITIVE, proceed with a PSA test to confirm the presence of semen. If the test is NEGATIVE, but you are suspicious there might be a trace of semen on the garment, do the PSA test anyway.

Alternative procedure: wrap the wetted item around a wet cotton-tipped swab so that the swab becomes saturated with solution. Then, press the swab against an AP test paper element for approximately 10 seconds. A color change to purple within the first 60 seconds after that is a POSITIVE test. This procedure yields an easily visualized, high-contrast spot. It also avoids leaving any stains on the item.

If you suspect there is only a trace of semen on the item, then skip the AP test and proceed to a PSA test instead. The PSA test is 1000x more sensitive, and the AP test paper may absorb all the semen on the item, leaving nothing for the PSA test. The AP test is designed for strong semen stains.

NOTE: latex gloves are recommended for these procedures.

Interpretation of test results

Vaginal fluid contains small amounts of acid phosphatase and may turn a test strip blue after enough time (20 minutes). Therefore, it is important to read the test within 60 sec. A strong semen stain will turn the test paper blue within a few seconds, and a very strong stain will give an almost black color, which can be diluted to purple by placing a drop of water on the test strip. It's important to remember that the AP test is an enzyme-mediated color reaction, and isn't 100% specific for any protein in semen in the same way the PSA test is. Since the implications of a positive test may be serious for personal or legal reasons, it is recommended that a positive result be followed up with a PSA test to confirm the presence of semen. In addition, the combination of two strongly positive test results gives the investigator a more certain conclusion that the item being tested in fact contains semen, than either test taken by itself.

References

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3. Van Etten, R.L. and Risley, J.M., "Phosphate (oxygen)—water exchange reaction catalyzed by human prostatic acid phosphatase," *Proc. Natl. Acad. Sci.* **1978**, *75* (10), 4784-4787.

4. Vaubourdolle, M.; Clavel, J.-P.; Cynober, L.; Piton, A. and Galli, A., "Acid Phosphatase and Zinc in Semen of Subjects with No Clinical Evidence of Prostatic Disease," *Clin. Chem.* **1985**, *31* (6), 878-880.

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