DNA Analyst Training
Laboratory Training Manual

Protocol 2.03
Semen Stain Identification: Acid Phosphatase
Presumptive Chemical Test (Indication)

This laboratory protocol (or part thereof) has been provided as an example of a laboratory SOP, courtesy of the Illinois State Police. It has been included for training and example purposes only.

PRESIDENT'S DNA INITIATIVE

INTRODUCTION

This test is designed as a preliminary screening test to aid in the identification of semen stains.

SAFETY CONSIDERATIONS

AP Chemical Test

Sodium *a*-naphthyl phosphate: Caution! Irritant!
Naphthanil diazo blue B: Caution! Irritant!
Glacial Acetic Acid: Danger! Corrosive!
Caution! Moderately Toxic!
Caution! Combustible!

PREPARATIONS

Buffer

Glacial acetic acid	5 ml.
Sodium acetate, anhydrous (.24M)	10 g.
Distilled Water	500 ml.

Step 1 Reagent

Buffer	250 ml.
Sodium *a*-naphthyl phosphate, 0.25% (w/v)	0.63 g.

Step 2 Reagent

Buffer	250 ml.
Naphthanil diazo blue B, 0.5% (w/v)	1.25 g.

Step 1 reagent and Step 2 reagent can be made up in bulk and aliquoted into test tubes and frozen. When needed, one tube of each reagent can be thawed under warm running water for use.

INSTRUMENTATION

No Instrumentation Required.

MINIMUM STANDARDS & CONTROLS

A positive and negative control should be run with each test.

PROCEDURE OR ANALYSIS

1. Place a small piece (2 x 2 mm.) of suspected seminal stain material on Whatman No. 1 filter paper or other suitable test paper.

2. Add 1-2 drops of Step 1 reagent and allow to react for 30 seconds. (No color should develop at this stage.)

3. Add 1 drop of Step 2 reagent. Record the results after 10 seconds.

4. A positive reaction is recorded upon rapid development of a purple color, which is indicative of semen. This is not a confirmatory test for semen. The test result is graded on a scale of 1 to 4 using the following guide:

   +1 Slow pink color develops on stain material
   +2 Slight purple color develops on stain material. May develop slowly.
   +3 Intense purple color develops on stain material. Develops quickly.
   +4 Intense purple color develops on stain material and bleeds into test paper.
   Develops rapidly

   A +1 to +2 reaction is considered inconclusive for the indication of semen.
   A +3 to +4 reaction signifies a strong reaction indicative of semen.

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