

DNA Analyst Training Laboratory Training Manual

Protocol 2.06
Semen Stain Identification:
P30 Analysis by ABACard



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 procedure allows identification of human semen.

SAFETY CONSIDERATIONS

Na₂HPO₄ Caution! Irritant!

PREPARATIONS

PBS (phosphate buffered saline) pH 7.4

3.18 g. NaH₂PO₄ (0.0023M)

14.45 g. Na₂HPO₄ (0.01M)

85 g. NaCl (1.145M)

10 L H₂O

MINIMUM STANDARDS & CONTROLS

Each lot number of ABACards must be tested with a 4 ng/ml and a 10 ng/ml dilution of a P30 standard in PBS, and a negative control (blank) of PBS. Both P30 standards must be positive and the negative control must be negative before the lot can be used in casework. Each card has an embedded control which must be positive for the test to be considered valid (the control "C" area of the card). A negative control (blank) must be run with each daily batch of samples and the result recorded in the case file.

PROCEDURE OR ANALYSIS

1. Place a small piece of sample material (5mm x 5mm) in a 1.5 ml microcentrifuge tube.
2. Add 250 ul of 1X PBS to the tube.
3. Incubate at room temperature for 2 hours with gentle agitation on a shaker.
4. Pipette 200 ul of extract to the sample well "S" of the test strip.
5. Read results at 10 minutes:
 - Valid results will occur only if a pink line is present in the control area "C." If no pink line is visible, the test should be repeated.
 - A positive result is indicated by the presence of one pink line in the test area "T," at or before the 10 minute reading.

- A negative result is indicated by the absence of one pink line in the test area. This may result from either the absence of PSA in the sample or it may be due to “high dose hook effect,” which may give false negatives in the presence of high concentrations of PSA. If a high concentration of PSA is suspected, a 1:100 dilution of the sample may be made to ensure that a false negative does not occur.

- An invalid result will occur if no pink line is present in the control area “C.” In this case the test should be repeated.

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