ABAcard® p30

Background

This protocol outlines a confirmatory test for the forensic identification of seminal fluid. ABAcard® p30 is an immunographic test for in vitro qualitative detection of the 30,000 molecular weight glycoprotein p30, otherwise known as PSA. p30 originates in the epithelial cells of the prostate tissue and fluids.

Summary of Procedure

Sample is added to the sample well, "S," and allowed to diffuse along the membrane. If p30 is present it will react and form a complex with the mobile monoclonal antihuman p30 antibody-dye conjugate. The mobile antigen antibody-dye conjugate will migrate through the absorbent device towards test area, "T." In the test area an immobilized monoclonal antihuman p30 antibody will capture the antigen antibody-dye conjugate and the pink dye will form into a narrow test band. The pink band in the test window indicates a positive test result.

Sample Handling

Forensic samples may be in limited supply. Retain sufficient sample for replicate analysis.

Label all samples with complete identifying information.

All biological samples and DNA must be treated as potentially infectious. Appropriate sample handling and disposal techniques should be followed. See:

- **Safety Manual**, *Universal Precautions*
- **Quality Assurance Manual**, *General Sample Control and Forensic Sample Preservation Policy*
- **Analytical Procedures Manual**, **Forensic Evidence Handling.**

Warnings and Precautions

The high dose hook effect occurs when the p30 concentration is too high. The p30 will bind to the p30 antibody-dye conjugate. Additionally, free p30 will migrate towards the test area. The free p30 will block the test area preventing the mobile antigen antibody-dye conjugate complex from binding. This results in the lack of a pink band in the test area (false negative). In this case a new test may be performed with a dilution of the sample.

p30 is an accepted marker for detecting semen in criminal cases including vasectomized or azoospermic individuals. Hemoglobin, bilirubin and lipemic[1] samples, (as indicated by triglyceride), do not interfere with test results. In addition, samples with high protein concentration such as prostatic acid phosphatase, albumin, chorionic gonadotropin, transferin and prolactin do not interfere with test results.

Reagents and Materials

See **Appendix B** for reagent preparation
10% Bleach
MBG Water
Phosphate Buffered Saline (PBS)
100% Ethanol
Disposable bench paper
ABAcard® p30 test device

Lab Coat

[1] Lipemic – Pertaining to blood samples having abnormally high amounts of lipid.

Kimwipes
Gloves
Protective Eyewear
Sample handling tools (scissors, scalpel blades, forceps, etc.)
Nutator
Centrifuge
Refrigerator
20-200µl pipette
40-1000uL pipette
20-200µl pipette tips
40-1000uL pipette tips
Timer

Reagents and Materials – Storage and Handling

All reagents and materials are to be kept under sterile conditions. Store all reagents according to the manufacturers' recommendations.

Do not use reagents beyond the listed expiration dates. Date and initial all reagents when put in use. Record in the **Reagent Log**.

Quality Control

Use of the **Evidence Examination Worksheet** or other appropriate worksheet is required for documentation. All information must be completed.

All results must be verified by a second qualified analyst. If a second qualified analyst is unavailable photo documentation of the result is acceptable

Positive Control

An internal positive control is included in the test. The control area, "C," contains an immobilized anti immunoglobin antibody. The antihuman p30 antibody-dye conjugate is captured by the anti immunoglobin antibody in the control area resulting in a pink band.
If a pink band appears in the control area "C", the test is valid. If a pink band does not appear in the control area "C", the test is not valid and a new test should be performed.

Procedure

1. Allow the sample to warm to room temperature.

2. To extract a specimen from a stain or a swab:
    a. Make a cutting of the stained material.
    b. Soak the cutting for at least 30 minutes in ~300µl of PBS (or Molecular Biology Grade Water can be used with approval) at RT on a nutator.
    c. Twirl the swab or fabric with a sterile applicator stick for several seconds or a light vortex to agitate the cells off the substrate. Transfer the cutting into a spin basket. Centrifuge the sample for 5 minutes at 13,200 rpm.
    d. Remove 200µl of supernatant for testing purposes without disturbing the pellet which can be used for sperm identification. This aliquot may be stored at 2-8°C but must be brought back to RT prior to testing.

3. Remove the device from the sealed pouch and label with the sample ID/date/initials.

4. Add 200µl of extracted sample to sample well “S.”

5. Read result in 10 minutes. Positive results can be seen as early as two minutes. Negative results must be read after the full ten minutes.

Results and Conclusions

The antihuman p30 antibody-dye conjugate is captured by the anti immunoglobin antibody in the control area resulting in a pink band. A pink band in the control area, “C,” indicates the test worked properly. If no pink line is present in the control area “C”, the test failed.

Positive Result: The presence of two lines, one in the test area, “T,” and the control area, “C,” is indicative of a positive test result. This would be reported as seminal fluid is identified on the appropriate report.

Negative Result: If there is only one pink line in the control area “C”, and no pink line in the test area, “T,” the test is negative. This would be reported as no seminal fluid is identified on the appropriate report.

Inconclusive Result: If the line in the test area, “T” is not conclusive or decisive, the test is inconclusive. This would be reported as the test for the identification of seminal fluid was inconclusive.

Comments on Storage

Continue to Differential extraction or store at 4°C.

Reference

Abacus Diagnostics, Inc., Technical Information Sheet, ABAcard® p30
WVSP DNA Analysis Manual- Revision #12