ANALYSIS OF SEMINAL FLUID

IDENTIFICATION OF SEMEN-SPECIFIC PROTEIN p30 BY ABAcard® PSA (p30) Test

Because spermatozoa production in males can be affected by aspermia, vasectomy or other conditions, forensic scientists have recognized the need for seminal fluid diagnostic tests which did not rely upon sperm cell presence.

p30 is a 30,000 Dalton semen glycoprotein of prostatic origin that is also known as PSA or prostate specific antigen. The range of PSA is 200,000 to 5.5 million ng/ml of semen. The sensitivity of the ABAcard® PSA test is 4 ng/ml and therefore seminal fluid diluted up to 1 in a million should be detectable. The ABAcard® PSA test has been tested against numerous biological fluids of men and women. Since no cross reactivity has been reported to date, this supports the hypothesis that P30 is a male-specific protein. For this reason, the detection of the P30 antigen in a forensic stain is strong evidence that the stain is seminal in nature.

The determination of the presence of semen may be made by using the ABAcard® PSA (p30) test. In this test 200µl of sample is added to the sample well 'S', and allowed to soak in. If PSA is present in the specimen, it will react with the mobile monoclonal antihuman PSA antibody and a mobile antigen antibody complex is thus formed. This mobile antibody-antigen complex migrates through the absorbent device towards the test area 'T'. In the test area 'T', a polyclonal antihuman PSA antibody is immobilized. This immobilized antibody captures the above complex so that an antibody-antigen-antibody sandwich is formed. The conjugated pink dye particles will form a pink colored band in the test area 'T' indicating a positive test result. As an internal positive control, PSA antibody-dye conjugates cannot bind to the antibody in the test area 'T', but are captured by an immobilized anti immunoglobulin antibody present in the control area 'C' forming a complex. The captured pink dye particles will thus form a band in the control area 'C', indicating that the test worked properly. Presence of two colored lines, one in the test area 'T' and one in the control area 'C' indicates a positive result that identifies the presence of semen.

ABAcard® PSA kits
Note: Each new lot of kits must be validated using a positive control before used in casework. See an example of the validation worksheet in the appendix. Kits should be stored below 28°C and have a shelf life of 18 months.

Preparation

1. Allow sample to warm to room temperature if it has been refrigerated.

2. Cut a small section of the swab or stain, (more may be required if the stain is diffuse), and extract with 3 to 4 drops (approx. 200µl) of deionized water at room temperature for a few minutes to one hour.

3. Label ABAcard® with case and item/exhibit numbers, date, and initials.

4. Add 200µl of sample to the sample well 'S' of the test device.

5. Read result at 10 minutes. Positive results can be seen as early as 1 minute depending on the p30 concentration. For negative results, one must wait for the full 10 minutes.

Conclusions:

Positive: If there are two pink lines, one each in the test area 'T' and in the control area 'C', the test result is positive and indicates that the PSA level is at or above 4ng/ml.

Negative: If there is only one pink line, (in the control area 'C'), the test result is negative. This may indicate that:

- No PSA is present above 4 ng/ml or
- Presence of "High Dose Hook Effect" (Presence of "High Dose Hook Effect" may give false negative result due to the presence of high concentration of PSA in the sample, as for example in undiluted seminal fluid. In such cases the sample may be retested using a 10 to 10,000 fold dilution.

Invalid: If there is no pink line visible in the control area 'C', the test is inconclusive. Repeat the test and reexamine the test procedure carefully.

Limitation:

Positive results may be obtained with male urine. Use of another appropriate test is recommended when male urine is in question.

High Dose Effect: When the PSA concentration is too high it overwhelms this very sensitive test. The mechanism behind the high dose effect is that huge amounts of human PSA bind both to the antibody to form an antigen-antibody complex but also free PSA migrates toward the test area 'T'. The antibody in the test area 'T' is blocked by this free PSA. Therefore the mobile antigen-antibody complex with the pink color cannot bind to the antibody. This results in a false negative.

Introduction

The secretion of the prostate is a thin, somewhat milky fluid. It contains a glycoprotein of molecular weight 30,000 Daltons which has been designated PSA. PSA can be readily detected in seminal fluid and has been shown to be absent in other biological fluids commonly encountered in forensic situations[1]. Successful isolation and characterization of a PSA from human seminal plasma in 1978 enabled the scientific community to pursue the detection of semen through immunological techniques[2]. Although certain cases such as azoospermia, vasectomy and various disease states can effect the spermatozoa production in males, the presence of PSA allows the forensic analyst to identify human semen even in the absence of spermatozoa. Methods for detection of PSA include Ouchterlony double diffusion, radial immunodiffusion, crossover electrophoresis, rocket immunoelectrophoresis, and ELISA[2]. These processes, although sensitive, are extremely time-consumming. A more expediant test involves the use of an antigen specific membrane test.

Various antigen specific membrane tests are currently used in the medical field to screen for the presence of PSA[3]. Antigen specific membranes are based on the reaction formed between the antigen and the membrane bound antibody. This complex migrates together through capillary forces to the "test area" where a polyclonal antigen is bound. A final complex of antigen-antibody-antigen if eventually formed and results in a colored line. The Northern Illinois Police Crime Laboratory (NIPCL) undertook a validation study of the *OneStep* ABAcard®PSA (p30) test* (Figure 1) in order to evaluate if this antigen specific membrane test could provide the necessary sensitivity in conjunction with the almost immediate results.

*The *OneStep* ABAcard®PSA (p30) test is distributed by:
Abacus Diagnostics
6520 Platt Avenue, #220
West Hills, California 91307
818.716.4735

Material and Methods

Samples
Seminal fluid and other body fluid samples were obtained from NIPCL employees and non-probative casework.

***OneStep* ABAcard®PSA (p30) Procedure:**
The samples used were allowed to warm to room temperature and then were extracted in deionized water (3-4 drops, approximately 200µl) for a few minutes. 200µl of the extract was then added to the sample well 'S' of the test device. The results were read at 10 minutes. Positive results were indicated by a pink line in both the test ('T') area and in the control area 'C'. This indicates that the PSA level was at or above 4ng/ml. Negative results exhibited only one pink line, (in the control area 'C'). This indicated no PSA present above 4 ng/ml.

Crossover Electrophoresis Procedure:
Reagents:
BUFFER - TANK AND GEL

Boric Acid	1.9 grams (0.03M)
Trizma Base	25.2 grams (0.074M)
EDTA (free acid)	2.5 grams (0.0085M)
Deionized Water	1.0 liter

pH = 9.1; adjust pH with 20% w/v NaOH.
GEL

Buffer	3.5 ml
Agarose E25	0.035 grams

Gel buffer and agarose were boiled until dissolved. This solution was then poured onto the hydrophilic surface of a 3 x 1 inch piece of GelBond. When gel was formed wells were made using a disposable pipette. These well pairs were spaced approximately 2-3 mm apart. A small piece of the sample was then extracted in deionized water (3-4 drops, approximately 200µl) for a few minutes (serial dilutions were also made if necessary). The cuttings or extracts were then inserted into the right well and the p30 antiserum into the left wells, opposite the stains being tested. The samples were electrophoresed at 120V for 26 minutes. When the run was complete the gel was soaked in 1M saline overnight at room temperature or at 37°C for at least 4 hours. Then, the gel was dried and stained in 0.1% Coomassie Blue solution in 5:1:5 methanol:acetic acid:deionized water for a minimum of 5 minutes. The gel was destained in 5:1:5 methanol:acetic acid:deionized water on a rotator until background is clear. The white precipitin bands stained blue, indicating a positive result.

In both the *OneStep* ABAcard®PSA (p30) procedure and the crossover electrophoresis test, negative controls were run along with each test.

Results

Sensitivity Tests

The neat semen was not testable due to its extremely viscous nature. Both the *OneStep* ABAcard®PSA (p30) test and the crossover electrophoresis exhibited positive results at dilutions of 1:100 and 1:1,000. Additionally the *OneStep* ABAcard®PSA (p30) test showed a positive result at the 1:10 dilution. The 1:10,000, 1:100,000, and 1:1,000,000 dilutions in both tests were negative.

Aspermatic Semen Sensitivity Tests

The *OneStep* ABAcard®PSA (p30) test was positive at the following dilutions: 1:1, 1:10: 1:100, 1:1,000, 1:10,000, 1:100,000. The crossover electrophoresis exhibited positive results at only the 1:100 and 1:1,000 dilutions. (Figure 2).

Miscellaneous Human Body Fluid Samples

Samples of urine, saliva, and both a 1:1,000 and 1:10,000 dilution of blood revealed negative results in both the *OneStep* ABAcard®PSA (p30) and the crossover electrophoresis tests. (Figure 3).

Miscellaneous Fabrics with Seminal Stains

All fabrics tested positive for the presence of semen with the *OneStep* ABAcard®PSA (p30) test. The crossover gel was positive in all fabrics if a cutting was used, however, when an extract was placed in the gel, only fabrics identified as one and two exhibited positive results. (Figure 4)

Non-probative Casework Samples

Case #	ABAcard	Crossover gel extract	Crossover gel cutting	AP	Sperm
84-3045	+	-	+	+	+
84-3262	+	-	+	-	+
84-3160	+	-	+	+	+
84-3043	-*	-	-	-*	+
84-2768	-*	-	-	-*	+

*There was an observable correlation between a negative AP test and a negative the *OneStep* ABAcard®PSA (p30) test.

Conclusions

In conclusion, compared to the time-consuming process of the crossover electrophoretic technique of measuring PSA, rapid membrane tests offer the same sensitivity within 10 minutes using 200 µl of the extract. Testing indicated that the ABAcard test was more sensitive than the crossover gel. The actual cutting of the sample in question was often needed to generate a positive result on the crossover. It is important to note that when the PSA concentration is too high it may overwhelm this very sensitive test. The mechanism behind this 'high dose effect'[4] is that huge amounts of human PSA bind both to the antibody to form an antigen-antibody complex but also free PSA migrates toward the test area 'T'. The antibody in the test area 'T' is blocked by this free PSA. Therefore the mobile antigen-antibody complex with the pink color cannot bind to the antibody. When this false negative occurs, a 1:100 or 1:1,000 dilution of the remaining extract should be retested. This rapid membrane test is easy to implement into routine casework protocols[5] and provides the forensic community with a very sensitive, reliable, and expeditious way of identifying seminal fluid from vasectomized individuals.

References

1. Gaensslen, R.E., *Sourcebook in Forensic Serology, Immunology, and Biochemistry*, U.S. Department of Justice, The National Institute of Justice. U.S. government printing office, Washington, D.C. August 1983.

2. Sensabaugh, G.F., Isolation and characterization of a semen-specific protein from human seminal plasma: a potential new marker for semen identification. *Journal of Forensic Science*, v23(1), p.106-115, 1978.

3. Alfthan, H. and Stenman, U. (1988) Falsely low results obtained with the Hybritech Tandem-R PSA Assay. *Clinical Chemistry*. 31, 2152.

4. Fernando, S.A., and Wilson, G., Studies of the 'hook' effect in the one-step sandwich immunoassay. *Journal of Immunological Methods*, 151 (1992) 47-66.

5. Northern Illinois Police Crime Laboratory, Forensic Biology & DNA Section Standard Operating Procedures, 1998

OneStep ABAcard PSA (p30) Test Sensitivity

Aspermatic Semen	ABAcard Results	Cross-over Results
1:10	+	-
1:100	+	+
1:1,000	+	+
1:10,000	+	-
1:100,000	+	-
1:1,000,000	-	-

Figure 2

OneStep ABAcard PSA (p30) Test

Fabric	ABAcard Results	Cross-over Results (extract)	Cross-over Results (cutting)
1	+	+	+
2	+	+	+
3	+	-	+
4	+	-	+
5	+	-	+
6	+	-	+
7	+	-	+
8	+	-	+
9	+	-	+

Figure 4

	+	-
)	+	-
00	-	-

Figure 2

[redacted]

ard PSA (p30) Test of Other Body Fluids

e	ABAcard Results	Cross-over Results
	-	-
	-	-
,000)	-	-
),000)	-	-

Figure 3

[redacted]