**Forensic Detection of Semen II. Comparison of the Abacus Diagnostics *OneStep ABAcard p30 Test* and the Seratec *PSA Semiquant* Kit for the Determination of the Presence of Semen in Forensic Cases**

Dale L. Laux, M.S. [1], Anthony J. Tambasco [2] and Elizabeth A. Benzinger, Ph.D. [3]

## Introduction

Prostatic specific antigen (PSA) or p30, was first described in 1971 by Hara, et.al. (1) which they called γ-seminoprotein. Li and Beling (2) described what most likely was the same protein that they called E1 in 1973. A thorough biochemical analysis of a protein isolated from semen utilizing electrophoretic methods was made by Sensabaugh (3) in 1978. He termed the protein p30 as its molecular weight was approximately 30 kdaltons. Graves, et.al. (4) studied the protein extensively and the work was published as partial fulfillment of his Ph.D. thesis in 1985. Antisera to the protein quickly became utilized in the forensic field for the detection of semen.

Initially believed to be a prostate specific protein (3,4) it is now known to be found in many different fluids and tissues including breast tissue and tumors(5,6), periurethral glands (7,8,9), breast milk (10), amniotic fluid (11), female urine (12) and endometrium (13).

Methodologies utilized for detection of the protein included Ouchterlony diffusion, crossover electrophoresis, Laurel rocket electrophoresis and ELISA. Recently, membrane based detection methods have been utilized and commercial kits have been manufactured for forensic use. The sensitivity of these commercial kits has been listed as low as 2 ng PSA/mL.

Several laboratories have validated these commercial kits, primarily the one manufactured by Abacus Diagnostics (14,15,16,17,18). Validation has examined sensitivity and specificity issues and the kits have been used extensively throughout the United States. To our knowledge, no one has studied the issue of PSA degradation as it relates to these kits, to which this research is aimed.

Both the Abacus Diagnostics *OneStep ABAcard p30 Test* and the Seratec *PSA Semiquant* Kit are immunochromatographic one-step tests for the detection of p30. If p30 is present in a sample, the p30 reacts with a mobile monoclonal antihuman PSA antibody in the

[1] Attorney General Jim Petro's Office, Ohio Bureau of Criminal Identification, Richfield, Ohio
[2]Mansfield Police Crime Laboratory, Mansfield, Ohio
[3] Attorney General Jim Petro's Office, Ohio Bureau of Criminal Identification, Director of DNA Laboratory, London, Ohio

strip forming a mobile antigen-antibody complex. This antigen-antibody complex then migrates through the absorbent device toward the test area. A monoclonal antihuman PSA antibody is attached to the membrane in the test area. This immobilized antibody captures the above complex resulting in an antibody-antigen-antibody complex. When the p30 concentration is greater than 2 - 4 ng/mL, the dye particles will form a pink colored band in the test area indicating a positive result (Figure 1). Both tests also have an internal control to ensure that the test is properly working and that proper procedures have been followed. This internal control contains a polyclonal anti-mouse-antibody that captures the monoclonal antibody and forms a complex.

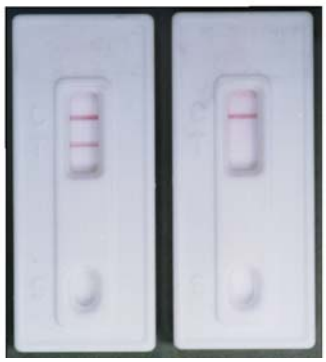


**Figure 1. ABAcard Results (positive and negative results)**

The Seratec *PSA Semiquant* Kit was developed as a screening test for the detection of human prostate cancer. Therefore it was designed as semiquantitative test. In this regard, it contains a 4ng PSA/mL internal standard (Figure 2). The company literature states that for the detection of seminal fluid, this test is used as a qualitative test and the internal standard is insignificant. However, in this analysts study, this internal standard can be quite helpful in estimating the concentration of PSA present in an unknown.


**Figure 2. A Positive Reaction for PSA with the Seratec Kit** (From Seratec's website, www.seratec.com.)

As proteins degrade, they lose their three dimensional conformation. It is possible that the monoclonal antibodies used in the two kits differ in their ability to bind to partially degraded psa. This experiment was designed to compare these two kits in detecting degraded psa.

## Methods

Sexual assault samples were simulated by applying psa to vaginal, anal and oral samples and incubating them at 37°C.

Eleven swabs were collected from vaginal, anal and oral regions from 3 volunteers who had no sexual contact for 5 days (a total of 33 swabs per person) using sterile cotton-tipped swabs (Pur-Wraps Hardwood Products Company). 40 µL of 500 ng/mL PSA standard (Stanford PSA standard, Catalog number L-F 500) was immediately added to each swab (**20 ng PSA added to each swab)**. Swabs were placed in 2.0 mL microfuge tubes (Costar, Catalog number 3213), capped, and incubated at 37 °C in dry bath incubators (Fisher Scientific) and collected at 0, 2, 4, 8, 12, 16, 24, 32, 48 and 72 hours. Swabs were extracted in 1 mL HEPES (0.24 %, pH 7.2) for 2 hours. The tubes were gently shaken, opened and the swabs were removed and placed in spin baskets (Costar, Catalog number 9301), recapped and centrifuged at 13 K rpm for 3 minutes. 200 µL of supernatant was added to each test chamber and results were recorded after 10 minutes. Any visible line or band (sometimes a dot) was recorded as a positive result.

### Dilution Study

A human PSA standard (Stanford, #060 5/96) was prepared using deionized water resulting in 2 mL of a 500 ng/mL solution. Serial dilutions were prepared resulting in concentrations of 500, 100, 50, 25, 12.5, 6.25, 3.125, 1.56 and 0.78 ng/mL. 200 µL samples were added to each test chamber and the results were recorded after 10 minutes.

### Concordance Study

One of us (AJT) conducted tests using both kits on 70 case samples consisting of a variety of swab types and stains. Stacy Shipman (personal communication) conducted tests using both kits on 31 case samples. 200 µL samples from a single extract were added to each test chamber and results were recorded after 10 minutes.

**Results**

The results in this study are summarized in Table 1. The initial results for the ABAcard using Lot # 23221024 (expires 5/2004) were negative and it was decided to use a different lot to check the results (Lot # 23220621, expires 12/2003). This different lot of cards gave positive results.

Very little difference was noted between the two cards over the time period of the experiment. The Seratec cards were positive to the end of the experiment (96 hours) while the ABA cards were positive to 72 hours.

| Subject 1 | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Hours | 0 | | 2 | | 4 | | 8 | | 12 | | 16 | | 24 | | 32 | | 48 | | 72 | | 96 | |
| Kit | A | S | A | S | A | S | A | S | A | S | A | S | A | S | A | S | A | S | A | S | A | S |
| Vaginal | - | + | + | + | +/+ | +/+ | -/+ | +/+ | -/+ | +/+ | -/+ | +/+ | - | + | + | + | + | + | + | + | - | + |
| Anal | + | + | - | + | +/+ | +/+ | -/+ | +/+ | -/+ | +/+ | -/+ | +/+ | - | + | + | + | + | + | + | + | - | + |
| Oral | + | + | + | + | +/+ | +/+ | +/+ | +/+ | -/+ | +/+ | -/- | +/+ | - | + | + | + | + | + | + | + | - | + |
| Subject 2 | | | | | | | | | | | | | | | | | | | | | | |
| Hours | 0 | | 2 | | 4 | | 8 | | 12 | | 16 | | 24 | | 32 | | 48 | | 72 | | 96 | |
| Kit | A | S | A | S | A | S | A | S | A | S | A | S | A | S | A | S | A | S | A | S | A | S |
| Vaginal | + | + | - | + | -/+ | +/+ | -/+ | +/+ | -/+ | +/+ | -/- | +/+ | - | - | - | + | - | - | nt | nt | nt | nt |
| Anal | - | + | - | + | +/+ | +/+ | -/+ | +/+ | -/+ | +/+ | -/- | +/+ | nt | nt | nt | nt | nt | nt | nt | nt | nt | nt |
| Oral | + | + | - | + | -/+ | +/+ | -/- | +/+ | -/- | +/+ | -/- | +/+ | - | + | - | + | - | + | nt | + | nt | - |
| Subject 3 | | | | | | | | | | | | | | | | | | | | | | |
| Hours | 0 | | 2 | | 4 | | 8 | | 12 | | 16 | | 24 | | 32 | | 48 | | 72 | | 96 | |
| Kit | A | S | A | S | A | S | A | S | A | S | A | S | A | S | A | S | A | S | A | S | A | S |
| Vaginal | - | + | - | + | -/+ | +/+ | -/- | +/+ | -/- | +/+ | -/- | +/+ | - | - | - | - | + | + | - | + | - | + |
| Anal | + | + | - | + | -/+ | +/+ | -/+ | +/+ | -/+ | +/+ | -/+ | +/+ | - | + | + | + | + | + | + | + | + | + |
| Oral | + | + | - | + | -/+ | +/+ | -/- | +/+ | -/- | +/+ | -/- | +/+ | - | + | - | + | + | + | + | + | - | + |

**Table 1.** Results of tests on swabs at designated times. Shaded areas are Lot #23220621 and unshaded areas are Lot#23221024 for ABAcard. Where two symbols appear, the top symbol is Lot # 23221024 and lower symbol is Lot#23220621 for ABAcard and Lot#64803 upper and Lot#60636 lower for Seratec. A = ABAcard S = Seratec
nt = not tested

Results of the PSA dilution study are shown in Table 2. Negative results were obtained with the ABAcards at a concentration of 6.25 ng/mL. Seratec kits were positive down below 1 ng/mL PSA.

The bands in the Seratec kits were tight, well defined and dark. Faint bands appeared at low levels of PSA, below 4 ng/mL. The internal 4 ng standard in the Seratec kit was consistent in intensity between tests and correctly approximated the concentration of PSA in the sample. It is concluded that this internal standard can be used to estimate the concentration of PSA in a sample to some degree, certainly over and under 4 ng/mL. This may aid in determining the size of sample to extract for DNA analysis.

Weak positive reactions were obtained with the ABAcard at levels below 100 ng/mL PSA. A spot occurred in one test and faint lines were observed in other tests. These were recorded as positive results but would have to be repeated for case work. Inconsistencies were observed between lots and within a lot. ABAcard Lot 23220621was positive to a dilution of 6.25 ng psa/mL while Lot 23221024 was negative at this dilution. One batch from this lot gave weak results at 50, 25 and 12.5 ng psa/mL.

| PSA | Seratec[1] | Seratec[2] | Seratec[3] | ABA[4] | ABA[5] | ABA[5] |
|---|---|---|---|---|---|---|
| 500 | + | + | + | + | + | + |
| 100 | + | + | + | + | + | + |
| 50 | + | + | + | + | Weak + | + |
| 25 | + | + | + | + | Spot + | + |
| 12.5 | + | + | + | + | Faint + | + |
| 6.25 | + | + | + | Weak + | - | - |
| 2.13 | + | + | + | - | - | - |
| 1.56 | + | + | + | - | - | - |
| 0.78 | - | + | + | - | - | - |

**Table 2.** Results of the PSA dilution study. The values along the y-axis are ng PSA/mL. PSA Lot #060 5/96. The lot numbers of the kits are: [1]50742, [2]60636, [3]64803, [4]23220621, and [5]23221024. The last two columns are tests removed from different boxes from the same lot number of ABAcards. The shaded areas showed either very weak, spotty or negative results.

The ABAcard is not designed to be quantitative. The Technical Information Sheet supplied with the ABAcard states "the intensity of either the control band or the test band should not be compared between tests or to each other for ABAcard p30 test and no quantitative interpretation should be made based upon differences in the intensity".

| Substrate | Seratec | Abacus | n |
|---|---|---|---|
| Stain on clothing | - | - | 7 |
| | + | + | 2 |
| | + | - | 4 |
| Vaginal swab | - | - | 14 |
| | + | + | 12 |
| | + | - | 3 |
| Rectal swab | - | - | 17 |
| | + | + | 3 |
| | + | - | 6 |
| Oral swab | - | - | 18 |
| | + | + | 0 |
| | + | - | 0 |
| Vaginal wash | - | - | 3 |
| | + | + | 3 |
| | + | - | 1 |
| Labia swab | - | - | 2 |
| | + | + | 1 |
| | + | - | 0 |
| Penile swab | - | - | 1 |
| | + | + | 0 |
| | + | - | 0 |
| Dried stain | - | - | 1 |
| | + | + | 0 |
| | + | - | 0 |
| Feminine pad | - | - | 1 |
| | + | + | 0 |
| | + | - | 0 |
| Condom | - | - | 1 |
| | + | + | 0 |
| | + | - | 1 |

**Table 3.** Results of the concordance study. No positive ABAcard negative Seratec results were obtained.

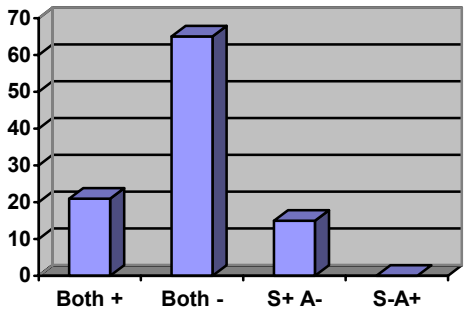


**Figure 3.** Diagram illustrating the results of the concordance study. (S) = Seratec (A) = Abacus; Values along the y-axis are number of cases

Results of the concordance study are shown in Table 3 and in Figure 3. For (85%), the kits were in agreement, i.e., both were positive or both were negative. In 15% of the cases, a difference was noted between the kit results. In all of these cases, a positive result was obtained with the Seratec kit and a negative result was obtained with the ABAcard. There were no occurrences of a positive ABAcard and a negative Seratec.

A large percentage of the differences between kits occurred in extracts prepared from stains and from rectal swabs. Seratec's greater sensitivity may play a role in obtaining more positive reactions from rectal swabs where degradation most likely occurs.

**Discussion**

PSA is now known to occur in a variety of fluids and tissues from both men and women. The term prostatic specific antigen is universally used in the clinical and forensic fields but a more appropriate name may be p30, referring to its molecular weight.

The results in this paper indicate that the Abacus Diagnostics *OneStep ABAcard p30 Test* and Seratec *PSA Semiquant* Kit are quite sensitive in detecting p30. Issues regarding these sensitivity levels and the presence of p30 in other body fluids have been discussed[20] and will be discussed in another paper [21].

The time study, dilution study and concordance study demonstrate that the Seratec *PSA Semiquant* Kit is more sensitive and consistent in detecting p30 from samples that may be encountered by forensic biologists than the Abacus Diagnostics *OneStep ABAcard p30 Test*.

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