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Identification of human semenogelin in membrane strip test as an alternative method for the detection of semen

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Abstract

Semenogelin (Sg), a protein originating in the seminal vesicles and a substrate for prostate specific antigen (PSA or p30), is a useful marker for the identification of semen. And detection of Sg has been available commercially in a membrane test recently. PSA is commonly used to detect semen in forensic significant samples taken from sexual assault cases. The strip PSA test has been available commercially from various manufacturers for many years. In this study, we evaluated two immunochromatographic membrane tests, one for Sg and the other for PSA by analyzing human semen, other human bodily fluids/materials including urine, blood, saliva, sweat, breast milk, vaginal secretion and fecal materials, semen from various animals and forensic casework samples. The data demonstrate that both Sg and PSA strip tests provide rapid and sensitive method for identification of seminal plasma. These results show that the immunochromatographic method for Sg detection is useful for the identification of seminal plasma in forensic samples, an alternative to the method for PSA detection.

Keywords: Forensic sciences; Semen; Semenogelin (Sg); Prostate specific antigen (PSA or p30); Immunochromatographic membrane test; Sexual assault

1. Introduction

Confirmatory test for human spermatozoa involves the microscopic identification of spermatozoa. In cases where no spermatozoa is detected from samples taken in sexual assault cases, other methods including acid phosphatase (AP) test, assay for Prostate specific antigen (PSA or p30), or seminal vesicle specific antigen (SVSA), also known as semenogelins (Sg) can be employed to detect the presence of semen. Acid phosphatase test is commonly used only as a screening test for semen because it is not only present in semen and prostate tissue, but also in normal vaginal secretions [1,2].

PSA is a serine protease produced by prostatic epithelial cells and is found in seminal fluid, prostatic fluid, male serum and male and female urine. Low concentration of PSA was detected in breast milk and in some breast tumors [3,4]. Although PSA is found in these fluids, it is very often used as an indicator of the presence of semen in forensic samples. Detection of PSA in genital swabs involves the cross-reactivity of the PSA and anti-PSA antisera in ELISA [1,5,6], in gel electrophoresis or in membrane strip test. The membrane strip test for PSA is easy to use, provides results in a rapid fashion and offers same sensitivity with ELISA. It has been recommended to use in forensic laboratories [7].

SgI and SgII are the major seminal vesicle secreted proteins in human semen. They interact non-covalently and via disulphide bridges to instantly form a coagulum upon ejaculation. The coagulum is liquefied after a few minutes owing to the action of a prostatic serine protease, PSA, which break Sg down to fragments [8–11]. SgI/II were demonstrated in several tissues including seminal vesicles, vas deferens, prostate, epididymis, skeletal muscle, kidney, colon, trachea and lung tumor [9,11–14]. Identification of Sg for semen detection was done in ELISA for years [15,16] while detection of Sg in dot-blot-immunoassay and in one-step immunochromatographic assay was described relatively recently [17,18]. The identification of human Sg in strip membrane format has recently been launched commercially.

The purpose of this study was to evaluate the feasibility of the identification of human Sg using membrane strip device provided in RSID-Semen Test in semen stains as an alternative

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method for the detection of semen. This method was evaluated for its sensitivity and specificity, and compared to the strip membrane detection of PSA using ABAcard p30 for semen identification. Numerous recent and aged casework samples were also employed to verify if the Sg detection could be an alternative method other than the PSA detection for the routine forensic identification of semen.

2. Materials and methods

2.1. Membrane test assays

Two tests, namely rapid stain identification (RSID)-Semen Test (Independent Forensics) [19] and ABAcard p30 Test (Abacus Diagnostics, West Hills, CA, USA) were employed in this study. The RSID-Semen Test was reported to detect Sg as low as its amount in 1 μl of semen while ABAcard p30 Test was reported to detect prostate specific antigen (PSA) as low as 4 ng/ml. The RSID-Semen Test utilizes monoclonal anti-human Sg antibodies whereas the ABAcard p30 Test utilizes monoclonal anti-human PSA antibodies. The two tests use the same immunochromatographic membrane assay technology. The samples were first extracted in buffer for 2 h before being applied to the sample window at one end of the test device. The test results were read after 10 min. Two lines appear for a positive result whereas one control line appears for a negative result. The control line must appear for a valid test (Fig. 1).

For RSID-Semen Test, fabric (25 mm^2) or swab (one-fourth) samples were extracted in 200 μl TE buffer (10 mM Tris–HCl, 0.1 mM EDTA, pH 8.0) in a 1.5 ml microcentrifuge tube for 2 h at room temperature. After extraction, 20 μl was mixed with 80 μl TBS+ running buffer provided by the manufacturer and applied to the sample window of the device.

For ABAcard p30 Test, fabric or swab samples were extracted in 750 μl sterile ddH_2O for 2 h at 4 °C. Two hundred microliters of an extract were placed in the sample window of the device.

For liquid sample such as semen, it was mixed with TBS+ for RSID-Semen Test or sterile ddH_2O for ABAcard p30 Test before being applied to the test device.

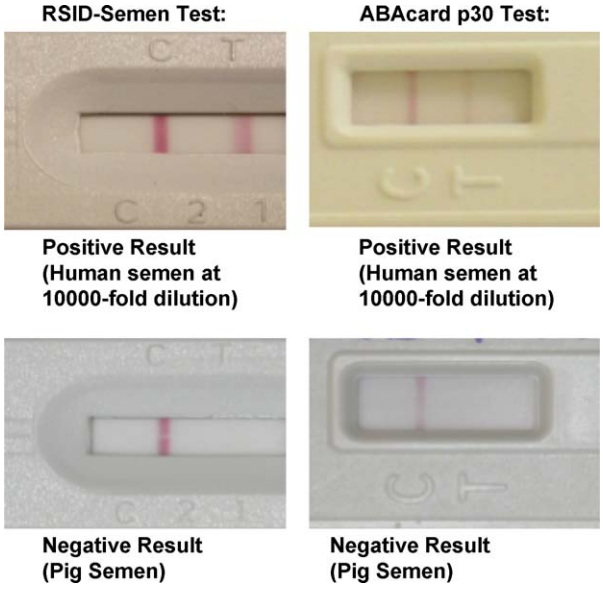

Fig. 1. Positive and negative results obtained from RSID-Semen Test and ABAcard p30 Test. Twenty microlitres of the extract is mixed with 80 μl TBS+ running buffer before being applied to the sample window for RSID-Semen Test whereas 200 μl extracts is added to the window for ABAcard p30 Test. A positive result is revealed by the presence of two lines, one in the test and the other in the control regions. A negative result is indicated by one line in the control area only.

2.2. Specimen

2.2.1. Bodily fluids/materials

Human semen, urine, blood, saliva, sweat, breast milk, vaginal secretion and fecal materials were obtained for this study. Human semen and urine samples were used either in liquid form directly or as dried stains (fabric or swab) after they were placed onto a sterile fabric or cotton swab and air-dried. The other samples were prepared as stains. All these samples were frozen at −20 °C before use.

2.2.2. Contraceptive and lubricant

Liquid water-based lubricant and water-based lubricant from a condom were collected on swab for extraction. Menfegol tablet (60 mg) was dissolved in 10 ml sterile water. Twenty microliters was used for extraction. Condom with nonoxynol-9-lubricant was swabbed by a sterile cotton bud swab for extraction.

2.2.3. Semen from various animals

Semen samples from cock, pig, bull, stallion, cat and dog were used in this study.

2.2.4. Casework samples

Fifty-four forensic casework samples were obtained from a total of 25 cases in this study. The 37 samples from recent sexual assault cases were stored at −20 °C and the 17 samples from aged cases were kept at −80 °C before use. These samples included vaginal swabs, penile swabs, beddings, and clothing. All these casework samples were analyzed for acid phosphatase activity, spermatozoa, PSA and Sg.

2.3. Determination of sensitivity

Semen and seminal fluid standard (SERI) were prepared at the following dilutions: 1/100, 1/1000, 1/10,000, 1/50,000, 1/100,000, 1/200,000 for RSID-Semen Test and for ABAcard p30 Test.

2.4. Determination of specificity

2.4.1. Bodily fluid/materials

All specimens mentioned in Section 2.2 were used to determine the specificity of the two devices.

2.4.2. Bodily fluid interfere

The stains of the bodily fluids, urine, blood, saliva, sweat, breast milk, vaginal secretion and fecal materials, were stained with human semen. The stains were air-dried and tested for Sg and PSA in order to study the possible interference of these bodily fluids/materials with human semen on the detection of Sg in RSID-Semen Test or PSA in ABAcard p30 Test.

2.4.3. Contraceptive interfere

One swab each with contraceptive or lubricant was stained with human semen and air-dried before being tested for Sg and PSA to study the possible interference of the contraceptives or lubricant on the detection of human semen with Sg detection in RSID-Semen Test or PSA detection in ABAcard p30 Test.

2.4.4. Species specificity

Semen samples from cock, pig, bull, stallion, cat and dog were employed to determine the species specificity of the detection of Sg in RSID-Semen Test and PSA in ABAcard p30 Test.

3. Results and discussion

3.1. Sensitivity

The manufacturer of RSID-Semen Test states that Sg in as little as 1 μl human seminal fluid can be detected while that of ABAcard p30 Test claims that PSA as low as 4 ng/ml can be

Table 1
Sensitivity of RSID-Semen Test and ABAcard p30 Test in Sg and PSA detection respectively with semen and seminal fluid standard

Dilutions	RSID-Semen Test[a]	ABAcard p30 Test[a]
Semen and seminal fluid standard		
1:100	+	+
1:1000	+	+
1:10000	+	+
1:50000	+	+
1:100000	+	−
1:200000	−	−

[a] "+" and "−" for positive and negative Sg or PSA detection.

detected. Human semen and a semen standard were diluted to determine the sensitivity of the two tests. While the RSID-Semen Test could detect both human semen and the seminal fluid standard up to 100,000-fold dilution, the ABAcard p30 card could detect both up to 50,000-fold dilution (Table 1). A dilution of 1/50,000 of the seminal fluid standard is equivalent to 2 ng/ml PSA. The sensitivity of the ABAcard p30 Test was tested slightly better in this study than the value claimed by the manufacturer and was comparable to the reported sensitivity of two other PSA detection kits, Seratec PSA Semiquant and PSA-check-1 [7]. The sensitivity of the RSID-Semen Test was equivalent to that of another recently commercialized Sg detection kit called Nanotrap Sg [20]. With both tests capable of detecting the human semen and the seminal fluid standard at 50,000-fold dilution, they are considered offering highly sensitive tests for the detection of semen.

3.2. Bodily fluids/materials

Urine samples from 10 males and 10 females were tested with the two test devices. All neat male urine samples and none of the neat female urine samples were tested positive for PSA. The detection of PSA in the male urine was expected because small amount of prostatic fluid may be present in the male urine [1]. None of the 10 male and the 10 female neat urine samples were tested positive for Sg. The 10 samples of male urine were placed on fabric and air-dried. The samples were extracted and tested for PSA; however, none of the fabric cuttings (25 or 100 mm^2) of the 10 samples were tested positive. Negative results for Sg detection was also obtained from these fabric cuttings.

Other bodily fluid including blood, saliva, sweat and fecal samples each from three males and three females, as well as breast milk and vaginal secretion from three females, were also tested for cross-reactivity with the two test devices. The two tests did not give false positive results with any of these samples. In order to determine if the bodily fluid samples would interfere with the reaction of the human semen with the two test devices, the samples were mixed with the human semen for extraction and analysis. Positive Sg and PSA detections were obtained for all these bodily fluids/materials with the semen. No false negative results due to the interference of these bodily fluids with the semen were observed.

The male and the female urine samples were also mixed with the human semen and extracted after being air-dried. The extracts were tested positive for Sg because the human semen was present in the extract and the male and the female urine did not interfere with the Sg detection.

3.3. Lubricant and spermicides

Lubricant itself or the lubricant recovered from condom was tested negative for both Sg and PSA. In addition, these materials did not interfere with the human semen for the Sg and the PSA tests because the extracts of the stains prepared by lubricant with the human semen gave positive results for both the Sg and the PSA tests.

Spermicides including nonoxynol-9 and menfegol, are commonly used as contraceptive measures. Nonoxynol-9 is often used with condom while menfegol is used as contraceptive tablets for women. Swabs taken from condom supplemented with nonoxynol-9 and 20 μl of dissolved menfegol were extracted separately. The extract of the two spermicides, nonoxynol-9 or menfegol, alone was tested negative for both Sg and PSA.

The stains of human semen/nonoxynol-9 and human semen/menfegol separately were tested positive for Sg and PSA. The specificity and sensitivity of the Sg and the PSA detection were not affected when the human semen was mixed with the two spermicides. Nonoxynol-9 and menfegol are known to kill and immobile sperms. The results indicated that nonoxynol-9 and menfegol did not have any adverse effect on the Sg and PSA structurally.

3.4. Species specificity

Semen samples from animals, including cock, pig, bull, stallion, cat, dog were used in this study. They were all tested positive for spermatozoa. Among these animal semen samples, only cock semen was tested positive for acid phosphatase activity although the signals were very weak compared to that detected for human semen. All these animal semen were tested negative for Sg and PSA in the two membrane tests.

3.5. Casework samples

The 37 recent casework samples were first analyzed for acid phosphatase activity and then spermatozoa microscopically. Eighteen of them were tested positive for acid phosphatase (Table 2). Spermatozoa were identified in 15 samples with only one of these being tested negative for acid phosphatase (Cat. III, Table 2). All samples with spermatozoa were tested positive in the strip test for Sg; however, only 12 of these samples were tested positive in the strip test for PSA. The three casework samples tested negative for PSA in triplicate but positive for Sg were vaginal swabs collected in the same case (Cat. II, Table 2). Mucus was found on the surface of these three vaginal swabs and microscopic examination of the smears from these swabs revealed the presence of abundant microorganisms. The microbial action on the PSA might account for the negative results. Among the 12 recent casework samples tested positive for Sg and PSA, 11 of them were also tested positive for acid

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Table 2
Results of acid phosphatase activity, spermatozoa, PSA and Sg detection for 37 recent and 17-aged forensic casework samples

Category	No. of samples	AP activity[a]	Spermatozoa[a]	PSA[a]	Sg[a]	Number of samples	
						Recent cases	Old cases
I	21	+	+	+	+	11	10
II	3	+	+	−	+	3[b]	0
III	3	−	+	+	+	1	2
IV	4	+	−	−	−	4	0
V	1	+	−	+	+	0	1
VI	21	−	−	−	−	18	3
VII	1	−	+	−	−	0	1[c]
Total	54					37	17

[a] "+" and "−" for positive and negative AP (acid phosphatase) activity; presence or absence of spermatozoa; positive or negative detection of PSA and Sg.
[b] The three samples were obtained from the same case. Weakly positive results were obtained from two of these samples when the PSA test devices were left for few hours at room temperature.
[c] Dog semen.

phosphatase. Acid phosphatase test remains one indispensable screening test for semen in sexual assault cases.

Another 17 aged casework samples were analyzed for AP activity, spermatozoa, Sg and PSA. Spermatozoa were detected in 13 samples and 10 of these samples were tested positive for AP activity. One of the spermatozoa bearing samples was dog semen (Cat. VII, Table 2), which was tested negative for AP activity, PSA, and Sg. The findings were consistent with those found for the dog semen mentioned in Section 3.4. With the exception of the dog semen sample, all other 12 samples with spermatozoa were tested positive for both Sg and PSA. Two of these 12 samples were tested negative for AP activity. PSA antigen was reported to be relatively more stable than prostatic acid phosphatase in vaginal fluid [21]. These samples were kept in −80 °C freezer for 9–17 years. Positive results obtained in the Sg and the PSA tests for human seminal fluid from these 12 samples indicated that the two test devices were capable of detecting the aged semen stains up to 17 years in this study. Spermatozoa were not detected in one of the samples (Cat. V, Table 2), but it was tested positive for both Sg and PSA. The sample was previously tested positive in cross-over electrophoresis, a less sensitive test for detection of PSA in gel. The semen donor could be azoospermic or vasectomized.

3.6. Comparison of Sg and PSA as a marker for semen identification

Sg and PSA have been used for over 30 years as a marker for semen identification since the two proteins were characterized and anti-Sg and anti-PSA antisera were discovered [1,15]. They are expected to play an important role in the seminal fluid identification in the forensic community in future. The relative concentration of Sg and PSA and the contribution of the seminal vesicles and the prostate in the seminal plasma may be considered as factors for the relative importance of the two proteins in the semen identification. The average levels of Sg and PSA concentration in the seminal plasma were 19 and 1.92 mg/ml, respectively [1,22]. In addition, the prostate contributes from 15 to 30% of the ejaculate volume whereas the seminal vesicles contribute from 50 to 80% [16]. However, the concentration of Sg and PSA in a forensic sample was unknown. For samples tested negative in one test but with strong indication of sexual intercourse in the case, careful consideration of the case history and retest of the sample with the same or the other test should be considered.

Sg was absent in both male and female urine while PSA was always detected in male urine in this study. PSA was also reported previously in other bodily fluids such as breast milk, female urine after sexual intercourse and taking oral contraceptives [23,24] and sweat gland [25]. This gives benefit to Sg detection over PSA detection in the seminal plasma identification. Furthermore, PSA was also detected in breast tumor [3] and Sg was detected in lung carcinomas [12]. Sg was also reported in other non-genital tissue such as skeletal muscle, kidney, colon and retina [11,26]. This should not be a problem because tissue samples are normally not submitted in sexual assault cases for the semen detection. Interpretation of the findings using both tests should be performed carefully and the clinical history of the victim and the suspect may have to be considered.

4. Conclusion

This study demonstrates that detection of Sg in seminal stain is an effective approach to the identification of semen. The RSID-Semen Test offers the same sensitivity towards the detection of semen as the ABAcard p30 Test. Like most PSA detection kit, the Sg detection kit provided in RSID-Semen Test is a membrane strip test, which is easy to use and rapid to get result. Results of the Sg and the PSA detection were obtained in 10 min. Both tests could be performed without consuming the DNA present in the samples since the aliquots can be taken during DNA extraction procedures. Three of the forensic samples were tested positive for Sg, but negative for PSA; however, it is not certain whether Sg might be more stable than PSA. Investigation into the stability and the loss of Sg in vaginal fluid may be required to understand its degradation process. The use of this commercially available kit for Sg detection allows an alternative approach to the forensic identification of seminal plasma.

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