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Detection of prostate specific antigen and semenogelin in specimens from female rape victims

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ABSTRACT

The presence of semen is generally accepted as evidence in sexual assault cases prosecution. Detection of sperm is confirmation of semen; however, sperm cannot always be detected. Prostate specific antigen (PSA) and semenogelin (Sg) are used as semen biomarkers. We compared the detection rate and persistence of sperm, PSA and Sg over a range of time intervals from the time of assault to specimen collection.

The results show that sperm had the longest persistence and highest detection rate. The detection rate of the Sg test was significantly better than that of the PSA test overall, whether the sperm test was negative or positive.

In conclusion, the detection of sperm should be the first test executed: if sperm is not detected, the Sg test is more suitable than the PSA test and could be used up to 72 h after assault.

Sexual assault or rape is a criminal act, and the detection of the assailants' body fluid on the victim's genitalia is crucial court evidence. The detection of sperm is pivotal evidence[1,2] for prosecution and usually relies on the preliminary detection of semen. Sperm detection on female genitalia might not be possible in all alleged rape cases because of differing individual and environmental reasons,[3] but this does not mean that no semen is present. DNA or RNA markers are the new trend for semen detection and personal identification as these also provide simultaneous confirmation of the assailant's profile. The Y-chromosome haplotype analysis has good sensitivity and persistence, yet it is not always detected in alleged rape cases. Male DNA may come from sperm, semen epithelium cells and leukocytes containing B and T cells,[4] but the detection rate is never 100%. Previous work from Martinez et al. demonstrated a male DNA (Y-STR) detection rate of 91.4%,[5] and Hall et al. showed that persistence of Y-STR haplotype in cervicovaginal samples could be recovered up to only 4 days post-coitus.[6]

Without sperm or DNA/RNA detection, other factors that can indicate the detection of semen may still be helpful in court. Biomarkers are considered valuable despite the presumptive nature of their tests. The detection of biomarkers can also be used to indicate what test should be done next.[5] Seminal biomarkers have long been utilized in presumptive testing for forensic detection of semen in alleged rape cases, both in the laboratory and at the crime scene.[7–10] Some seminal biomarkers can now be detected with commercial test kits, and these tests are very convenient, fast and cheap when compared to the sperm test, or DNA or RNA analysis.

Prostate specific antigen (PSA) and semenogelin (Sg) have been used as seminal biomarkers in semen detection for over 30 years.[7,8] PSA is a glycoprotein produced by prostatic epithelial cells and often applied as a marker for semen in forensic casework.[10,11] SgI and SgII are the main protein components in semen and are produced in the seminal vesicle.[10,12–14]

Previous studies on PSA and Sg focused on detection comparisons in different human body fluids,[10] detecting semen in body fluid mixtures,[10] the effects of freezing and thawing,[15] sensitivity in fresh semen samples,[5,10,11,16] and aspects of persistence in both experimental consensual postcoital specimens[10,16] and forensic casework.[5,11,15,16] However, to the best of our knowledge, no one has directly compared the detection of PSA with that of Sg, or the detection of PSA and Sg with that of sperm, in specimens from consensual or non-consensual sexual intercourse, over a range of time frames.

It is necessary to understand the duration limitations of detection and persistence of these biomarkers, because if the time of the alleged rape event is known, this will assist forensic scientists in selecting the most appropriate test for semen. Because no one has unlimited resources, human labor, time, or money, it is important to effectively decide which test should be done first, second, last or never be used.

The purpose of this study was to conduct the sperm test, PSA test and Sg test on forensic casework specimens to 1) determine the persistence of each marker, 2) determine their detection rates, comparing all three over specific time intervals, 3) compare the PSA test and the Sg test performance when the sperm test is positive or negative over time, and 4) determine the order of priority for running these screening tests.

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1. Materials and methods

We reviewed all cases of female victims raped by men over a period of 5 years (2011–2015) then selected particular cases using six criteria: (1) women examined at Siriraj Hospital; (2) case circumstances indicating that sexual assault may have occurred (i.e. non-consensual sexual contact); (3) women having physical wounds on the body or genitalia, or substantial evidence that these women were under duress, or under the influence conscious-altering drugs (i.e alcohol, ketamine, etc.). Under Thai law, the definition of duress for non-consensual sexual contact may occur under what is considered "reasonable cause/s" and includes use of weapons to coerce, use of physical force (bodily), other forms of coercion such as blackmail (threats) and other reasonable causes as defined by the court/law enforcement, even if there is not clear evidence of physical injuries to the genitalia or other parts of the body. Detection of physical injury by police or doctors is just one criteria of many under this definition; (4) the interval between assault and evidence collection was less than 3 weeks; (5) women had no other sexual intercourse in the intervening 3 weeks, either consensual or not, and no sexual intercourse in the 2 weeks before the alleged rape occurred; and (6) no evidence of condom use during the assault.

The results were recorded as (1) interval of time between assault and evidence collection, and (2) result of the sperm test. For the time period, cases were further divided into 4 subgroups: (1) within 24 h; (2) 24.1 h–48 h; (3) 48.1 h–72 h; and (4) more than 72 h.

1.1. Specimen collection

After specimens were collected from any sites of genitalia from the female victims with or without speculum, they were dried for 24 h at room temperature and transferred to the evidence laboratory in accordance with chain of custody procedures. The specimens were dried in sterile glass test tubes covered by loose sterile cotton to protect from DNA contamination. This was performed at the evidence laboratory of Siriraj Hospital, which is a government laboratory.

1.2. Specimen preparation

When specimens were transferred they were divided into 2 parts for sperm detection, for acid phosphatase testing and for zinc (Zn) testing. The Zn test and acid phosphatase test were performed as described in a previous study.[17] The acid phosphatase test was done according to the method used by the Virginia Department of Forensic Science,[18] and the Zn test was completed using Suzuki's modified method.[19]

1.3. Sperm detection

The specimens were incubated in 1 mL of sterile water about 1 h and then centrifuged to obtain a supernatant and sediment. They were centrifuged at 8000 RPM for 2 min to separate the cotton/swab by using filter caps, then after removal of the filters, all specimens were centrifuged at 14,000 RPM for 2 min to separate the supernatant and the sediment. One portion of the sediment (about 50 μL) was stained with hematoxylin and eosin following the method of Pollak[20] and the presence of sperm detected by observation with light microscopy. Our condition for positive sperm detection was that two sperm without tails, or one sperm with a tail, was found on the whole slide. In our protocol, if sperm is detected and DNA analysis is requested, the remaining sediment and all slides are transferred to the serology laboratory and the technician double-checks all slides for quality control purposes. The supernatant and the rest of sediment are stored at −20 °C for 5 years in case retesting is requested.

1.4. Test kits

The PSA test kit we used was the ABAcard™ (Abacus Diagnostics, 6520 Platt Av. 220, West Hills, CA 91307, USA), evaluated in several studies[21–23] with a reported sensitivity of PSA detection to as low as 4 ng/mL.[23]

The Sg test kit used was the Rapid Stain Identification (RSID™)-Semen Field Test (Independent Forensics, Hillside, IL, USA), evaluated and reported to have a sensitivity of detection for seminal Sg (both SgI and SgII) ranging from 4 to 68 ng/mL.[12,16]

Our positive control for both tests was a 1:10 dilution of fresh semen from a fertility clinic patient who had normospermia. The negative control for both was distilled water mixed with the test kits' respective buffers: 190 μL distilled water and 10 μL buffer for ABAcard™, and 90 μL distilled water and 10 μL buffer for RSID™.

1.5. Test kit procedure

The specimen supernatant described above was used immediately after thawing and was mixed well for each test. The supernatant for the PSA test was prepared with ABAcard™ buffer as described by Pang et al.[11] (supernatant 190 μL and buffer 10 μL); for the Sg test, it was prepared with RSID™ universal buffer following the Martinez et al. method[5] (supernatant 90 μL and buffer 10 μL). Following the manufacturers' instructions, the prepared solutions from each case were tested using the ABAcard™ for detection of PSA or the RSID™ for detection of Sg.

1.6. Control samples, sensitivity and specificity determination

For sensitivity determination, semen from 2 male donors was stained on the cotton swabs and dried at room temperature for 24 h. Then the semen stained swabs were prepared and performed to the serial dilution for sensitivity determination. The supernatant was diluted into 1:100, 1:200, 1:500, 1:1,000, 1:2000 and 1:5000. The diluted supernatant was tested in the same method of the sample specimens.

For specificity determination, body fluids include 1) female urine (donor had no sexual intercourse for 1 week and did not take oral contraceptive pill), 2) female urine (donor had no sexual intercourse for 1 week and took oral contraceptive pill (Meliane™ (Bayer Thai Co Ltd. 28/19, Bantalad, Pakkred, Nontahburi, Thailand)), 3) female median cubital blood, 4) menstrual blood (donor had no sexual intercourse for 1 week) and 5) male urine (donor had no ejaculation for 1 week) were stained to the cotton swabs and tested in the same method of the sample specimens.

1.7. Recording of results

The result was recorded as positive or negative at 10 min of incubation time after specimen addition at room temperature, only if the test line was weaker, equal or stronger than the controlled line. If the result was negative or weakly positive, a dilution for a high-dose hook effect retest was done and the result corrected if the retest was positive. Old et al. stated that "… false negative results, called high-dose hook effects, may sometimes be seen when too much semen is added to the strip."[16] Too much Sg or PSA in the system means it is not all bound to the antibody, so free Sg or PSA saturates the test region of the strip, preventing antibody-bound Sg or PSA from forming a positive test line.[16] In this study, we used a 200-fold dilution for the PSA test and a 20-fold dilution for the Sg test.

The cases that gave negative results for all Sg, PSA and sperm tests were not used for any statistical data analysis as we did not perform any further confirmatory tests on these specimens.

1.8. Statistical data analysis

The results of the PSA and Sg tests were compared with each other and with the sperm test using four methods: (1) the persistence of PSA, Sg and sperm at specific intervals after the alleged intercourse event;

Table 1
Sensitivity for sperm test, semenogelin test (RSID™) and prostate specific antigen test (ABAcard™) in semen detection.

Dilutions	Donor 1			Donor 2		
	Sperm test	Sg test	PSA test	Sperm test	Sg test	PSA test
1:100	+	+	+	+	+	+
1:200	+	+	+	+	+	+
1:500	–	–	+	+	–	+
1:1000	–	–	+	–	–	+
1:2000	–	–	–	–	–	+
1:5000	–	–	–	–	–	–

+ = positive result.
– = negative result.

(2) the comparison of the detection rate of the sperm test, the Sg test and the PSA test, in specific periods of times after alleged rape; (3) the comparison of the Sg and PSA tests at specific intervals after the alleged rape, when positive or negative for sperm detection; and (4) the most suitable test (based on detection rate and persistence) and the order of priority for semen identification testing in forensic casework.

Data were analyzed using McNemar's test (to assess the significance of the difference between two tests in the same population), Pearson Chi-squared test with Bonferroni's multiple comparison method (to evaluate the observed differences within each test type), and Cochran's Q test, as appropriate. The statistical data analysis was done by IBM SPSS Statistics 21 program.

2. Results

The sensitivity of the sperm test, Sg test and PSA test in serial dilution is shown in Table 1. PSA sensitivity was better than Sg when dilution was more than 1:200 and better than the sperm test when dilution was more than 1:1000. All had negative results in female urine, female median cubital blood, menstrual blood and male urine.

Between January 2010 and April 2015, 1764 cases of sexual assault were recorded at the Department of Forensic Medicine, Siriraj Hospital. Only 114 cases met our criteria (6.46%), and of these, only 89 cases gave a positive result in at least one of the sperm test, Sg test or PSA test (5.04%). The longest period of time between alleged rape and evidence collection was 227 h ± 0.44% (negative for sperm) and 169 h ± 0.59% (positive for sperm). The longest marker persistence we found was for sperm, followed by Sg, which was 105 h ± 0.95% (positive for sperm) and 65 h ± 1.56% (negative for sperm). The persistence of PSA was much shorter, being 43 h ± 2.32% (positive for sperm) and 8 h ± 12.5% (negative for sperm).

Sperm were detected in 76 of 89 cases (85.39%), Sg was detected in 51 cases (57.30%), and PSA was detected in 33 cases (37.08%). The detection rate over time for each individual test is summarized in Fig. 1. The detection rate of the sperm test and PSA test was highest in the first 24 h, but the detection rate of the PSA test significantly declined over time. The detection rates of the sperm and Sg tests were not significantly affected by increasing time intervals. Analysis using the Pearson Chi-squared test and Bonferroni's multiple comparison method showed that only the decline in the PSA detection rate was significant at the 24.1–48 h time interval.

The results of the PSA and Sg tests are shown in Fig. 2, along with sperm detection separated into the four time intervals. The detection rate of PSA declined over time whether sperm was detected or not; the detection rate of the Sg test performed better than the PSA test. There was only one high-dose hook effect observed for the Sg test, a rate of 1.96% (1/51), and no high-dose hook effect was observed for the PSA test.

Table 2 shows the results for each test, with the different combinations divided into eight categories. Category 2 was defined as all tests negative, which may be indicative that no semen was actually present although we did not confirm this. We, therefore, provide our adjusted results with this category excluded.

Detection rates over time for the sperm test, Sg test and PSA test are shown in Table 3. Using McNemar's test, we found that the sperm test detection rate was generally better than that of the other two, and significantly better ($p < .05$) than that of the PSA test in all time periods (PSA was only detected within 24 h and 24.1–48 h). When compared with the Sg test, the sperm test performed significantly better in the first 24 h ($p = .006$) and overall ($p = .001$).

We also analyzed the performance of the Sg test directly with the performance of the PSA test, for each of the four time intervals as well as overall (Table 4). The Sg test's results were significantly better than those of the PSA test ($p = .01$), except in the first 24 h period when sperm was also detected. In this instance, more positive samples were detected by PSA than by Sg (Table 4).

Applying Cochran's Q test to these results showed that the detection rate of sperm was better than that of Sg, the detection rate of Sg was better than that of PSA; the sperm test performed better than the PSA test, with p-values of 0.001, 0.025 and < 0.001 respectively.

3. Discussion

Our analysis revealed several interesting findings. First, Sg persists longer, and the Sg test has a better detection rate when compared with its PSA counterpart, except for the 24 h window when the sperm test is positive. Second, the sperm test performs better than both Sg and PSA tests, and sperm persists longer than Sg or PSA with the tests used and within the timeframes we studied.

Regarding the sensitivity of the sperm test, the Sg test and the PSA test for detection of seminal fluid in control samples, this study's results showed that the PSA test was the most sensitive test whereas the sperm test was more sensitive than the Sg test. Whilst the detection rate in the authentic forensic casework specimens showed that the sperm test had the highest detection rate, the Sg test had a higher detection rate than the PSA test. Previous studies have tested sensitivity of semen detection using both experimental and forensic casework specimens. Pang et al. found that the Sg test (RSID™) was more sensitive than the PSA test (ABAcard™), which correlated with their detection rates in casework specimens.[11] However, Martinez et al. stated that the PSA test (Seratec™) was more sensitive than the Sg test (RSID™), which contradicted their detection rate in casework specimens.[5] However, Sato et al. noted that their PSA test (PSA Check-1™) was more sensitive than their Sg test (Nanotrap Sg™), which correlated with their detection rate in casework specimens.[15] Other research using consensual postcoital specimens found that the PSA test (Seratec™) had higher sensitivity than the Sg test (RSID™), which contrasts with the persistence of these markers in experimental specimens.[10] This could mean that the sensitivity of these tests performed on seminal plasma does not always correlate with the detection rate and persistence of semen in the real postcoital specimens.

Fig. 1 shows that the detection rate of the PSA test declined significantly over time. Our results could imply that PSA is not the most appropriate marker after the first 24 h has passed, because its detection rate declines significantly whether sperm is present or not (Fig. 2). The detection rate for sperm and Sg did not decline as much over time, either because sperm and Sg persist relatively longer than PSA, or simply because the cases that we analyzed had at least one positive test result for them to be included. The Sg test still gave positive results whether the sperm were present or not, over longer time periods than the PSA test, so this may be the most appropriate test for forensic casework where there is a delay between the alleged rape event and specimen collection. The positive correlation between the presence of sperm, concentration of sperm and the persistence of Sg and PSA biomarkers needs to be further investigated in future studies.

The detection rates summarized in Table 2 categories demonstrate that the sperm test detection rate was generally higher than that of the

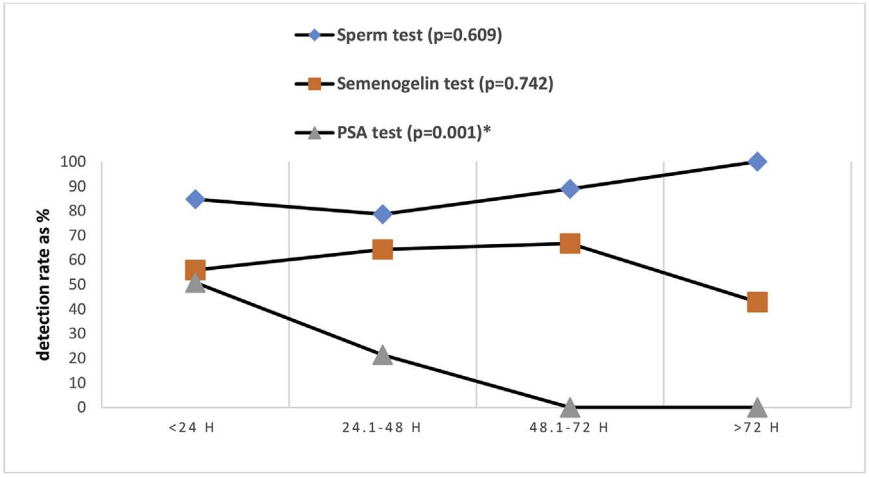

Fig. 1. Detection rates over time for all three semen detection tests.
* = Pearson Chi-squared test and Bonferroni's multiple comparison method show that the PSA test detection rate was significantly different in the periods of ≤24 h and 24.1–48 h (p = .001).

* = Pearson Chi-squared test and Bonferroni's multiple comparison method show that the PSA test detection rate was significantly different in the periods of ≤ 24 h and 24.1–48 h (p=0.001).

Sg test and/or PSA test. When Category 2 was excluded, we determined that only using the sperm test would miss semen detection in 14.61% of forensic casework (Category 6,7 and 8) and using only the Sg test and PSA test would miss 28.09% of positive specimens (Category 1). It is clear that the sperm test should be the first test done, followed by the Sg test. The Sg test was positive in 57.31% of cases (Category 3, 4, 6 and 7), whereas the PSA test was positive in 37.09% of cases (Category 3, 5, 6 and 8). Noticeably, when the sperm test was negative, 13.38% of samples were positive for Sg, with PSA not detected (Category 7); PSA was not detected when Sg was not (Category 8). This implies that the Sg test is more reliable to use as a second test when sperm is not detected. When considering the occurrence of false negative results, we calculated how many times we observed negative results for each test when another test was positive. The sperm test was negative in 14.61% of otherwise positive samples (Category 6, 7 and 8), for the Sg test it was 42.7% (Category 1, 5 and 8), and for the PSA test it was 62.92% (Category 1, 4 and 7). This suggests that using the PSA test alone means there is a higher likelihood of a false negative result than if the Sg test were solely used. However, when using a combination of the sperm test and Sg test, there were no (0%) false negative results (Category 8), compared with 13.48% (Category 7) false negative results using a combination of the sperm test and PSA test.

In the previous research that used forensic casework specimens, Pang et al. found that for recent cases, the detection rate of the Sg test (RSID™) was 40.54%, equal to that of the sperm test, and better than that of the PSA test (ABAcard™), which was 32.43%. However, in older cases, the rate of Sg detection was 81.25%, equal to that of the PSA test and better than that of the sperm test (75%).[11] Martinez et al. also found that the 64.8% detection rate of the Sg test (RSID™) was better than the 61.4% for the PSA test (Seratec®) and the 11.4% for the sperm test.[5] Romero-Montaya et al. compared only the sperm test with the PSA (ABAcard™) test, and found that the detection rate of the sperm test

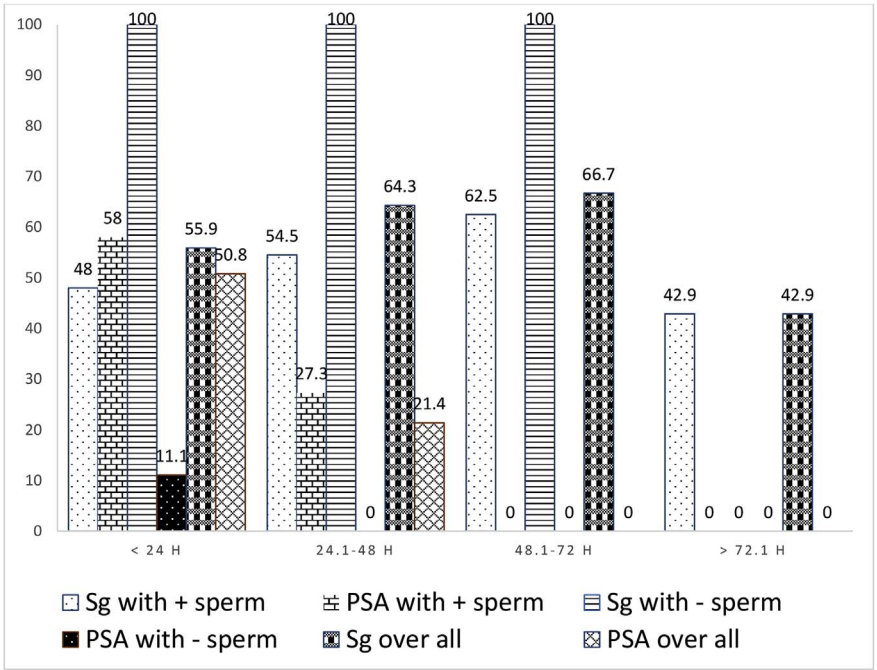

Fig. 2. Detection rates in each time interval for semenogelin and PSA tests when the sperm test was either positive or negative.
- measured as % of total cases.

- measured as % of total cases

Table 2
Detection rates of the sperm test, semenogelin test and PSA test.

Category	Sperm test	Sg test	PSA test	% (N = 114)*	% (N = 89)†
1	+	–	–	21.93	28.09
2	–	–	–	21.93	–
3	+	+	+	16.67	21.35
4	+	+	–	16.67	21.35
5	+	–	+	11.40	14.61
6	–	+	+	0.88	1.13
7	–	+	–	10.52	13.48
8	–	–	+	0	0

* = percentage of total cases examined (114).
† = percentage of total cases where semen was detected (89); 25 specimens negative for all tests (category 2) are not included.
+ = positive result.
– = negative result.

was higher.[24] In contrast, Sato et al. found that the detection rate of the PSA test (Seratec® and PSA-Check 1) at 81% was better than 68.4% for the Sg test (Nanotrap Sg), and 65% for the sperm test.[15] It should be noted that these studies did not include the time intervals between alleged rape and specimen collection; this may have affected detection rates for the tests.

The data in Table 3 shows a direct comparison of detection rates of the sperm test with those of the Sg and PSA tests over a range of time intervals. As far as we have seen, this has not been reported by other authors. The sperm test performed significantly better in all time periods that PSA was detectable and also better than the Sg test in all time periods studied, being significantly better in the first 24 h and overall. This confirms that the sperm test should be the first priority. It is better to choose the sperm test than the PSA test for the time periods we studied if only one test can be done, or choose the sperm test and Sg test if only two tests can be done.

The specificity determination of both PSA and Sg in our study showed no interference with male urine and female urine (with or without use of contraceptives). Nonetheless, PSA was reportedly detected in samples of female urine (from women using contraceptives or after sexual intercourse[25,26]); in sweat glands,[27] endometrium,[28] placenta,[29] breast milk,[30] breast tumor[31] and male urine.[10,11,32] While Sg has previously been found in skeletal muscle, kidney, colon, trachea, lung tissues, lung carcinomas and retina,[33–36] no Sg was detected in the female genital tract.[5] Examples of tissues containing Sg other than the male genital tract are not commonly submitted for semen testing in sexual assault cases.[11] It is clear that the specificity of the Sg test makes it more suitable than the PSA test for forensic purposes.

From Table 4, we can make a direct comparison between the PSA and Sg tests over the four time intervals, taking into account whether the sperm test was positive or negative. Overall, the detection rate of the Sg test was better than that of the PSA test, except within the 24 h timeframe with a positive sperm test. Despite this, the Sg test would be the preferred second choice test, especially when the sperm test is negative or in longer timeframes of up to 72 h. Furthermore, the Sg test still gave positive results more than 72 h later when sperm was present. We have not found any other literature that has made this direct comparison of the two tests over time.

Previous literature also does not seem to include studies that use forensic casework specimens to compare the persistence of Sg and PSA using these commercial tests. Some have used consensual postcoital specimens, such as Old et al., who found that the Sg test (RSID™) performed well up to 2 days between event and sampling, while for the PSA test (Seratec®) it was only useful for up to 1 day.[16] Laffan et al., who also used postcoital specimens from consensual intercourse, found that semen persistence on high vaginal swabs detected by the Sg test (RSID™) and PSA test (Seratec®) was 72 h and 48 h respectively.[10] They also found that semen persistence in urine samples from consensual intercourse, when tested by the Sg test or PSA test was 48 h or 24 h, respectively.[10] Laffan et al. also stated that the performance of RSID™ is superior when testing postcoital samples, most likely because there are higher levels of Sg than PSA in semen. Our study used forensic casework specimens and we found the persistence of Sg and PSA to be 105 h and 43 h respectively when samples were also positive for sperm. Persistence of Sg and PSA in our study was much longer than the times given by both Old et al.[16] and Laffan et al..[10] We can postulate this may be due to sample size since Old et al.[16] and Laffan et al.[10] did not state the exact number of samples.

Applying Cochran's Q test made it clear that the sperm test had the best detection rate, with the Sg test being the second choice. At Siriraj Hospital, sperm cytology ($7) is cheaper than the PSA ($9) and Sg tests ($13). Therefore, it is more cost effective to choose the sperm test first, then the Sg test if sperm is not detected. Given our results and the overall expense, the PSA test is the least desirable to use in our particular situation.

Our extraction procedure did not follow the exact manufacturer's instructions because the casework specimens were limited. They were already extracted and no specimen, other than the extract supernatant

Table 3
Detection rates over time for the sperm test, semenogelin test and PSA test.

Sperm test	Semenogelin test		p-value	PSA test		p-value
	negative	positive		negative	positive	
Within 24 h	0 (0)	9 (15.3)	**.006***	8 (13.6)	1 (1.7)	< **0.001**†
negative						
positive	26 (44.1)	24 (40.7)		21 (35.6)	29 (49.2)	
24.1–48 h	0 (0)	3 (21.4)	.727	3 (21.4)	0 (0)	**0.008**†
negative						
positive	5 (35.7)	6 (42.9)		8 (57.1)	3 (21.4)	
48.1–72 h	0 (0)	1 (11.1)	.625	1 (11.1)	0 (0)	N/A
negative						
positive	3 (33.3)	5 (55.6)		8 (88.9)	0 (0)	
More than 72 h	0 (0)	0 (0)	N/A	0 (0)	0 (0)	N/A
negative						
positive	4 (57.1)	3 (42.9)		7 (100)	0 (0)	
Total	0 (0)	13 (14.6)	**.001***	12 (13.5)	1 (1.1)	< **0.001**†
negative						
positive	38 (42.7)	38 (42.7)		44 (49.4)	32 (36)	

Values are presented as n (%) of the total 89 cases.
* = McNemar's test shows the sperm test was significantly better than the Sg test at ≤ 24 h and overall, ($p < .05$).
† = McNemar's test shows the sperm test was significantly better than the PSA test at ≤ 24 h, 24–48 h and overall, ($p < .05$).

Table 4
Comparison of the semenogelin test and PSA test in specific time periods with a positive or negative sperm test result.

Time	Negative sperm test			Positive sperm test			Overall		
	Semenogelin test		p-value	Semenogelin test		p-value	Semenogelin test		p-value
	negative	positive		negative	positive		negative	positive	
Within 24 h	0 (0)	8 (88.9)	N/A	15 (30)	**6 (12)**	0.332	15 (25.4)	14 (23.7)	0.690
PSA test									
negative									
positive	0 (0)	1 (11.1)		**11 (22)**	18 (36)		11 (18.6)	19 (32.2)	
24.1–48 h	0 (0)	3 (100)	N/A	3 (27.3)	5 (45.5)	0.453	3 (21.4)	8 (57.1)	0.109
PSA test									
negative									
positive	0 (0)	0 (0)		2 (18.2)	1 (9.1)		2 (14.3)	1 (7.1)	
48.1–72 h	0 (0)	1 (100)	N/A	3 (37.5)	5 (62.5)	N/A	3 (33.3)	6 (66.7)	N/A
PSA test									
negative									
positive	0 (0)	0		0 (0)	0 (0)		0 (0)	0 (0)	
More than 72 h	0 (0)	0 (0)	N/A	4 (57.1)	3 (42.9)	N/A	4 (57.1)	3 (42.9)	N/A
PSA test									
negative									
positive	0 (0)	0 (0)		0 (0)	0 (0)		0 (0)	0 (0)	
Total	0 (0)	12 (92.3)	N/A	25 (32.9)	19 (25)	0.377	25 (28.1)	**31 (34.8)**	**0.010***
PSA test									
negative									
positive	0 (0)	1 (7.7)		13 (17.1)	19 (25)		**13 (14.6)**	20 (22.5)	

Values are presented as n (%) of the total 89 cases.
* = McNemar's test shows that the Sg test performance was significantly better than that of the PSA test at all time intervals with either a negative or a positive sperm test ($p < .05$).

and sediment, is usually preserved in our laboratory. Our laboratory uses distilled water to avoid any crossover buffer effects, as the work of Hobbs et al. demonstrated that using the PSA test buffer for extraction interfered with the performance of the Sg test.[9] Our procedure was close to that of Martinez et al.,[5] and our comparison of detection rates for the Sg test and PSA test in forensic casework specimens yielded similar results. Pang et al., who used distilled water for PSA extraction and used the manufacturer's buffer for Sg extraction, obtained results with their recent forensic casework samples that were similar to ours.[11] Their detection rate was 37.14% for Sg and 34.28% for PSA in recent cases, while our results for within 72 h samples were 58.53% for Sg and 40.24% for PSA. Additionally, Laffan et al. and Old et al. used extraction buffers provided by the manufacturers for samples from consensual sexual intercourse, and also obtained similar results to ours.[10,16] When analyzing forensic casework specimens of clothing collected at the scene, Old et al. concluded that the Sg test was more sensitive than the PSA test.[16]

Jonsson et al. stated that SgI, SgII and PSA play a role in the gel-formation of semen after ejaculation and in releasing trapped sperm. They propose that PSA cleaves SgI and SgII to release sperm.[14] Stability of Sg and PSA in semen or postcoital specimens is still not clearly established and may vary widely between individuals. Sato et al. found that Sg could be detected in semen stains stored at room temperature for 5 years.[37] They also found that the signal obtained from the Sg test (Nanotrap Sg) was diminished by repeatedly freezing and thawing a diluted sample, whereas the PSA test (PSA-check 1) was not affected even after the second round.[15] To avoid the adverse storage effects for both PSA and Sg, our testing was done immediately after the specimen thawed, and there was no repeated freezing and thawing of our samples. However, this is something to consider, as after a long supernatant storage period, the ability to detect Sg and PSA may be decreased, which would affect the accuracy of any results.

We must acknowledge some limitations. We only used specimens from the population in Bangkok Province, which might not represent the whole population of Thailand. We also had to rely on doctors' note-taking to determine whether cases fit our criteria (such as whether it was consensual intercourse or alleged rape, the time of the alleged rape event, other sexual intercourse, usage of condoms, etc.). The sensitivity of the sperm test in semen detection of the sample specimens depends on the criteria of sample selection. In this study, the test showed the detection rate at 85.39% whereas in the previous study it was 68.8%.[38] We also did not perform any confirmatory tests such as DNA(Y-STR) analysis, but we have partially corrected for this by using only specimens giving at least one positive result from our three tests in our subsequent statistical calculations.

4. Conclusion

This study indicates that Sg testing of forensic casework specimens is more reliable than PSA testing, based on its detection rate and persistence across almost all the time periods we investigated. In real-world cases, when considering detection rate, evidence persistence and economic viability, the sperm test should still be the first priority. If this is negative, the next most suitable test is the Sg test, rather than the PSA test, especially for specimens collected between 24 h and 72 h post-event. The PSA test had a higher rate of false negative results because it had a lower rate of detection than the Sg test. Overall, we conclude that the Sg test is more effective than the PSA test for the presumptive detection of semen in forensic casework.

Ethical approval

This study was approved by the Siriraj Institutional Review Board.

Conflict of interest

The authors have no conflicts of interest to declare.

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