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Original communication

A comparison of ABAcard® p30 and RSID™-Semen test kits for forensic semen identification

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ABSTRACT

The screening and confirmatory tests available to a forensic laboratory allow evidence to be examined for the presence of bodily fluids. With the majority of evidence being submitted involving sexual assaults, it is important to have confirmatory tests for the identification of semen that are straightforward, quick, and reliable. The purpose of this study was to compare two commonly used semen identification kits utilized by forensic laboratories: ABAcard® p30 and Rapid Stain Identification of Human Semen (RSID™-Semen). These kits were assessed with aged semen stains, fresh and frozen post-vasectomy semen, post-coital samples collected on different substrates, post-vasectomy semen mixed with blood, saliva, and urine, a series of swabs collected at increasing time intervals after sexual intercourse, and multiple non-semen samples. The test kits were compared on the basis of sensitivity, specificity, and the cost and time effectiveness of each protocol. Overall, both semen identification tests performed well in the studies. Both kits proved specificity for identifying semen, however the ABAcard® p30 test surpassed the RSID™-Semen test in sensitivity, cost per test, and simplified test protocol.

1. Introduction

A majority of the evidence encountered in a forensic biology department involves sexual assaults, and the ability to accurately identify semen in such evidence is an important component of the serological examination. A suitable confirmatory method is needed when submitted evidence has yielded a positive screening test for semen and a negative microscopic examination (spermatozoa are not identified in the sample). These types of results may be seen when an individual has an extremely low amount (oligospermatic) or zero (aspermatic) sperm cells.

This study compares two confirmatory methods utilized by forensic laboratories for the identification of semen, ABAcard® p30 (Abacus Diagnostics, Inc., West Hills, CA) and RSID™-Semen (Independent Forensics, Hillside, IL). Both assays utilize an immunochromatographic test strip; the ABAcard® p30 test detects human antigen p30, and the RSID™-Semen test detects human semenogelin.[1,2]

Prostate-specific antigen (PSA) or antigen p30, a glycoprotein, is secreted by the epithelial cells of the prostate gland.[3,4] Semenogelin is a protein that is produced by the seminal vesicles and is responsible for the clotting which occurs with ejaculation.[2,5] Both of these proteins are used as forensic markers for the identification of semen.[1,2,4]

Each semen identification test kit was compared in terms of sensitivity and specificity as related to forensic serological analysis in addition to the cost and time effectiveness of each protocol.

2. Materials and methods

2.1. Study parameters

2.1.1. Sensitivity

The study was independently performed on fresh semen (stored refrigerated at approximately 4 °C) and frozen semen (stored at approximately −15 °C). The semen samples consisted of post-vasectomy samples obtained from a medical laboratory. A 1:2 serial dilution was prepared with reagent grade water up to 1:1024. Fifty (50) μL of each semen dilution was placed onto a sterile cotton swab. Two swabs were made for each dilution. The swabs were allowed to dry completely at room temperature prior to analysis.

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2.1.2. Specificity

The samples consisted of: underwear worn after sexual intercourse, feminine hygiene pads worn after sexual intercourse, a feminine hygiene pad from a menstrual cycle, post-coital vaginal swabs, fecal swabs, skin swabs, ear wax, nasal swabs, breast milk on fabric and swabs, post-vasectomy semen on fabric and swabs, female urine, male urine, olive oil, and body lubricant swabs. Aged semen stains deposited on a brown towel, blue fabric, and white fabric were also used. All samples were allowed to dry completely at room temperature and stored at room temperature prior to analysis. All of these samples were obtained from one female donor excluding the breast milk samples, post-vasectomy semen, and male urine, which were donated from other persons. The olive oil and body lubricants were obtained from the residence of the female donor. The aged semen stains had been created from a single male donor.

2.1.3. Body fluid mixtures

A 1:1 ratio of semen:blood, semen:saliva and semen:urine mixtures were created. Dilutions of the mixtures were prepared with reagent grade water at 1:5 and 1:25. Fifty (50) μL of each mixture was placed onto a sterile cotton swab. Two swabs were prepared for each mixture. The swabs were allowed to dry completely at room temperature and stored at room temperature prior to analysis. The semen samples consisted of post-vasectomy samples obtained from a medical laboratory. The saliva sample was obtained from a female donor. The urine and blood samples were obtained from a male donor.

2.1.4. Post-coital samples

Vaginal swabs were collected from a single donor within an hour after sexual intercourse and then approximately every 10–12 h after the initial collection up to approximately 118 h. Two swabs were used for each collection for a total of twenty-two swabs. The swabs were allowed to dry completely at room temperature and stored at room temperature prior to analysis.

2.2. Test kit procedures

ABAcard® p30: Abacus Diagnostics, Inc. (Catalog Number: 308332).

2.2.1. Sample and test preparation

A cutting from a swab or fabric sample was placed into a test tube. The cutting was extracted in 300 μL of reagent grade water. The sample was incubated for two hours at 4 °C, and then reacclimated to room temperature. The sample was vortexed rapidly for 10 s prior to and after the extraction period. Two hundred (200) μL of the extract solution was added to the sample well of the test strip.[3]

2.2.2. Quality control

The controls were performed in conjunction with the samples to be analyzed. The positive control consisted of a 1:10 dilution of Serological Research Institute (SERI) semen standard (Catalog Number: R563). The negative control consisted of reagent grade water.[3]

2.3. Interpretation

After 10 min, the result was recorded. A positive result was indicated by a pink line at both the control (C) and test (T) positions on the test strip. A negative result was indicated by a pink line at the control (C) position on the test strip. An inconclusive result was indicated by a partially developed pink line at the test (T) position only or a partially developed or no pink line at the control (C) position on the test strip.[1,3]

RSID™-Semen: Independent Forensics (Catalog Number 0200).

2.4. Sample and test preparation

A cutting from a swab or fabric sample was placed into a test tube. A swab cutting was extracted in 200 μL of RSID™-Semen extraction buffer. A fabric cutting was extracted in 100 μL of extraction buffer. The sample was incubated at room temperature for one hour. The sample was vortexed for 5–10 s prior to and after the extraction period. Eighty (80) μL of RSID™-Semen running buffer and 20 μL of the extracted sample solution were placed into a new test tube. The sample was vortexed for 5–10 s. Approximately 100 μL of the sample/running buffer mixture was placed into the sample well of the test strip.[2,6]

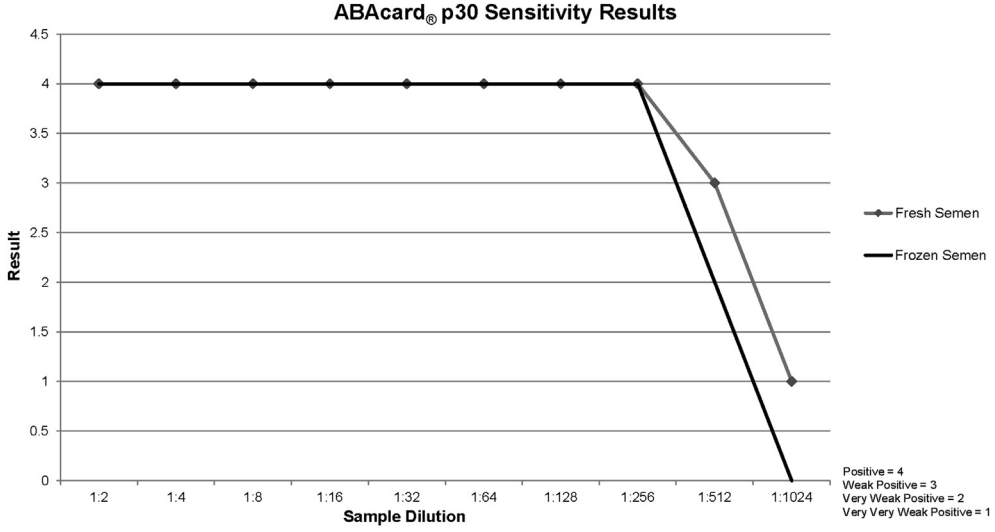

Fig. 1. ABAcard® p30 sensitivity to diluted fresh and frozen semen samples. A weak, very weak, and very very weak positive is based on the faintness of the pink color visualized at the test (T) position on the test strip when compared to the pink color of the control (C) position. The less apparent the pink color then the weaker the result was considered and recorded as such. Positive = 4, weak positive = 3, very weak positive = 2, very very weak positive = 1, negative = 0.

2.5. Quality control

The controls were performed in conjunction with the samples to be analyzed. The positive control was created by pipetting 50 μL of Serological Research Institute (SERI) semen standard (Catalog Number: R563) onto a sterile swab and allowing it to dry completely at room temperature. The entire swab was placed into a test tube and 1 mL of extraction buffer was added. Ninety (90) μL of running buffer and 10 μL of the extracted sample solution was placed into a new test tube. The negative control consisted of a sterile swab placed in a test tube containing 200 μL of extraction buffer. Eighty (80) μL of running buffer and 20 μL of the extracted sample solution were placed into a new test tube.[2,6]

2.6. Interpretation

After 10 min, the result was recorded. A positive result was indicated by a red line at both the control (C) and test (T) positions on the test strip. A negative result was indicated by a red line at the control (C) position on the test strip. A red line at the test (T) position only indicated a failed test.[2]

3. Results and discussion

3.1. Sensitivity

ABAcard® p30 demonstrated a high sensitivity for both fresh and frozen diluted semen samples (Fig. 1). This correlates with other research that found high sensitivity in vasectomy semen samples.[4,7] While RSID™-Semen exhibited a moderately high sensitivity for fresh diluted semen samples (Fig. 2), it revealed very low sensitivity for frozen diluted semen samples (Fig. 2). Another study acknowledged that the sample preparation may have decreased the sensitivity, caused by the two buffers utilized in the RSID™-Semen test.[8] This same study expressed concern that freezing semen samples could compromise the specimen's structural integrity.[8] Repetitive freezing and thawing of semen samples has also been shown to yield weaker positive results for semenogelin.[9] It is presumed that the frozen semen samples used for these sensitivity studies reduced the positive semenogelin response.

Table 1
Specificity results.

Sample description	RSID™-semen	ABAcard® p30
Post-vasectomy sample dried on fabric	Positive	Positive
Post-vasectomy sample dried on fabric	Positive	Positive
Known semen deposited on brown fabric (aged ~ 10 years)	Positive	Positive
Known semen deposited on white fabric (~aged ~ 10 years)	Positive	Positive
Known semen deposited on blue fabric (aged ~ 10 years)	Positive	Positive
Black underwear post-coital	Positive	Positive
Feminine hygiene pad worn post-coital (~1 h)	Positive	Very Weak Positive
Feminine hygiene pad worn post-coital (~30–60 min)	Positive	Positive
Feminine hygiene pad worn post-coital (~24 h)	Positive	Positive
Feminine hygiene pad worn post-coital (~48 h)	Weak Positive	Negative
Vaginal swab ~ 3 h post-coital	Weak Positive	Positive
Vaginal swab ~ 24 h post-coital	Negative	Positive
Vaginal swab ~ 33 h post-coital	Negative	Very weak positive
Forehead skin swab	Negative	Negative
Earwax swab	Negative	Negative
Nasal swab	Negative	Negative
Fecal swab	Negative	Negative
Breast milk dried on fabric	Negative	Negative
Breast milk swab	Negative	Negative
Female urine swab	Negative	Negative
Male urine swab	Negative	Negative
Maxi pad worn menstrual cycle	Negative	Negative
Massage lotion swab	Negative	Negative
Suave lotion swab	Negative	Negative
Neosporin swab	Negative	Negative
Olive oil swab	Negative	Negative
Petroleum jelly swab	Negative	Negative
K–Y plus (nonoxynol-9) swab	Negative	Negative

3.2. Specificity

Both semen identification kits were very specific for the protein they detect (Table 1). No false positive results were observed for any

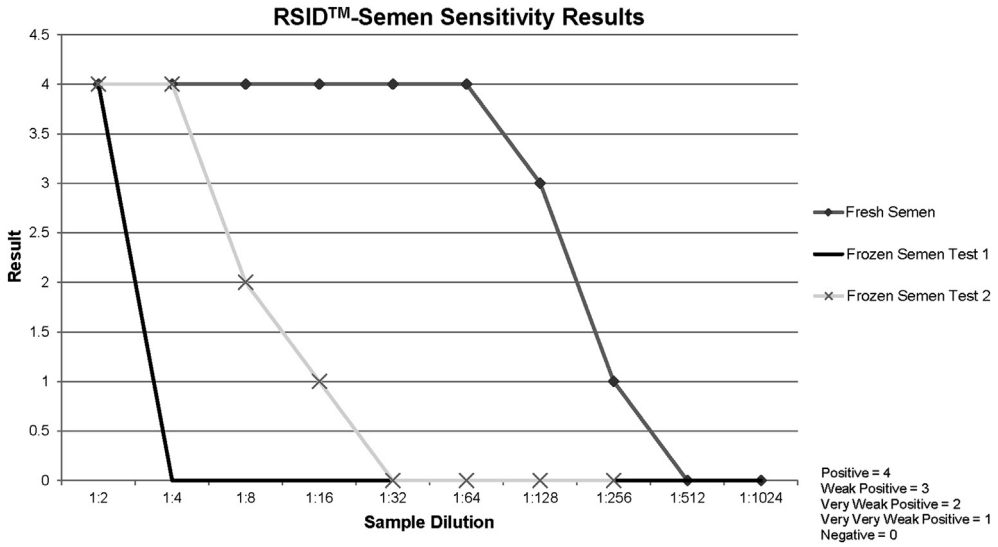

Fig. 2. RSID™-Semen sensitivity to diluted fresh and frozen semen samples. For the second test performed on frozen semen, the 1:512 and 1:1024 dilutions were not tested. A weak, very weak, and very very weak positive is based on the faintness of the red color visualized at the test (T) position on the test strip when compared to the red color of the control (C) position. The less apparent the red color then the weaker the result was considered and recorded as such. Positive = 4, weak positive = 3, very weak positive = 2, very very weak positive = 1, negative = 0.

Table 2
Mixture study results.

Sample description		RSID™-Semen	ABAcard® p30
1:1	Semen:blood	Positive	Positive
1:5	Semen:blood	Positive	Very weak positive
1:25	Semen:blood	Weak Positive	Negative
1:1	Semen:saliva	Positive	Positive
1:5	Semen:saliva	Positive	Positive
1:25	Semen:saliva	Weak Positive	Negative
1:1	Semen:urine	Positive	Positive
1:5	Semen:urine	Positive	Positive
1:25	Semen:urine	Negative	Negative

non-semen sample. Varying results were noted for the majority of the post-coital samples. For the feminine hygiene pad collected approximately 1 h post-coital, a positive result was observed with RSID™-Semen, and a very weak positive result was observed with ABAcard® p30. For the vaginal swabs collected approximately 3 h post-coital, a positive result was observed for ABAcard® p30, and a weak positive result was observed with RSID™-Semen. There were instances where a positive, weak positive, or very weak positive result was observed with one kit and a negative result was observed with the other kit; for example, with the vaginal swabs collected approximately 24 h post-coital, ABAcard® p30 yielded a positive result and the RSID™-Semen yielded a negative result. These differences may have been due to the distribution of sample on the substrate (swab or feminine hygiene pad) and/or the selection of cutting area. A weak and very weak positive is based on the faintness of the red or pink color visualized at the test (T) position on the test strip when compared to the red or pink color of the control (C) position. The less apparent the red or pink color then the weaker the result was considered and recorded as such.

3.3. Body fluid mixtures

No interference of the RSID™-Semen and ABAcard® p30 tests were observed for semen mixed with blood, saliva, or urine. Any weak positive or negative result from either test was attributed to a lower concentration of sample. Overall, RSID™-Semen produced more positive results in the body fluid mixture study (Table 2).

3.4. Post-coital samples

ABAcard® p30 demonstrated a greater sensitivity in the post-coital sample study. It detected the semen component in a 2–3 day post-coital sample (Fig. 3). RSID™-Semen showed very little sensitivity in this study, yielding a positive result only for the earliest collected, zero (0) day post-coital sample (Fig. 3). Some states have increased the time frame for collection of sexual assault evidence kits (i.e. Maryland collects up to 120 h after the alleged assault). While neither semen identification kit provided positive results at approximately 118.75 h, ABAcard® p30 was more sensitive and yielded positive results up to approximately 70.75 h.

3.5. Cost-effectiveness

The cost for running an individual test was calculated for each methodology. The cost of ABAcard® p30 was approximately $4.40, while RSID™-Semen was approximately $7.84 per test. ABAcard® p30 has been shown to be more cost effective.

3.6. Simplicity

The RSID™-Semen protocol included a shorter incubation time for sample extraction (1 h) when compared to the ABAcard® p30 protocol (2 h). The RSID™-Semen protocol required varying amounts of the extraction buffer based on the type of sample substrate (fabric versus cotton swab). For ABAcard® p30, the same amount of extraction fluid was used with all sample substrates. Thus, the ABAcard® p30 protocol was more efficient and not as complicated.

4. Conclusion

RSID™-Semen showed low to moderate sensitivity when compared to the high sensitivity observed with ABAcard® p30, represented by the test results from the fresh and frozen semen samples. Based on the findings from the specificity and body-fluid mixtures studies, a high degree of specificity was observed for both ABAcard® p30 and RSID™-Semen. Based on the post-coital study results, the ABAcard® p30 test proved more

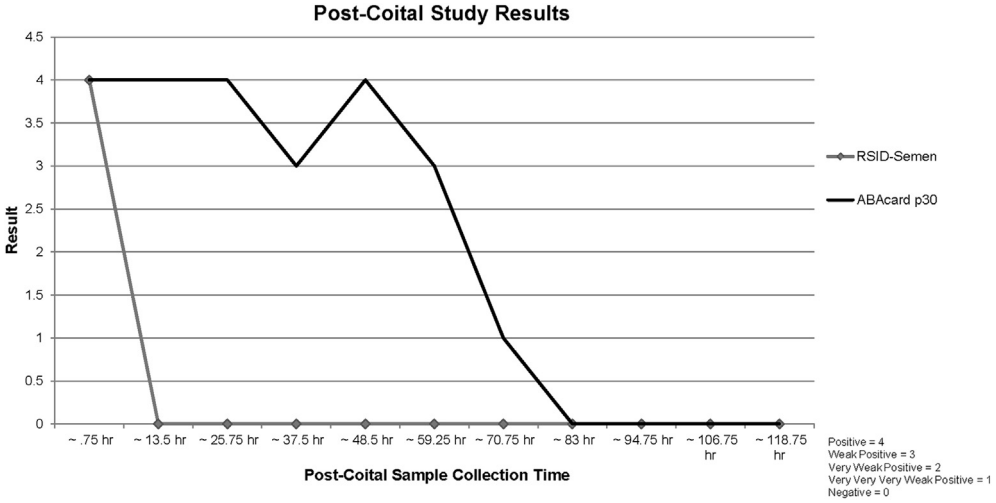

Fig. 3. RSID™-Semen and ABAcard® p30 sensitivity results for post-coital vaginal swabs collected consecutively over time. A weak, very weak, and very very very weak positive is based on the faintness of the red or pink color visualized at the test (T) position on the test strip when compared to the red or pink color of the control (C) position. The less apparent the red or pink color then the weaker the result was considered and recorded as such. Positive = 4, weak positive = 3, very weak positive = 2, very very very weak positive = 1, negative = 0.

efficacy in identifying semen. The overall findings from this comparison study reflect that the ABAcard® p30 test demonstrated a higher sensitivity and reliability for the serological identification of semen on forensic evidence than the RSID™-Semen test. While not investigated in this study, further examination is needed to assess the effects of freezing on simulated evidentiary forensic samples. For example, post-coital samples could be collected, allowed to air dry, and frozen. The samples could then be analyzed with ABAcard® p30 and/or RSID™-Semen tests at specific time intervals after being frozen [i.e. 6 months, 12 months, 18 months, and 24 months].

Disclaimer

The authors and the Western Maryland Regional Crime Laboratory do not endorse any products for the purpose of semen identification.

Ethical approval

None.

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None.

Conflict of interest

The authors have no conflicting interests.

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