



Identification of Human Semen:

A comparison of the ABACard[®] p30 and Rapid Stain Identification (RSID[®]) – Semen test kits.



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As all previous semen testing methods used within PathWest Forensic Biology were considered only presumptive or screening tests, there was a need for a confirmatory semen test for routine use, other than the microscopic detection of spermatozoa. Suspect semen samples with negative microscopy results (i.e. semen stains from vasectomised or aspermic individuals) require further testing to prove without doubt the presence of human semen in criminal cases. Two current commercially available kits for the forensic identification of semen are the Abacus Diagnostics ABACard[®] p30 kit and the Independent Forensics Rapid Stain Identification (RSID[®]) Semen testing kit. The sensitivity and specificity of both kits were compared to assess their suitability as a confirmatory test in the forensic detection of human semen.

TEST KITS

The ABACard[®] p30 test allows qualitative detection of p30 (Prostate Specific Antigen) for forensic identification of human semen, including vasectomised and azoospermic semen samples. The ABACard[®] p30 test kit contains a mobile monoclonal antihuman p30 antibody, which reacts with p30 present in seminal fluid to form a mobile antigen antibody complex. This mobile complex conjugated to pink dye particles, migrates along the test strip until it is bound to an immobilised monoclonal antihuman p30 antibody. A pink coloured band in the test region of the test kit forms with samples containing p30 at a concentration higher than 4ng/mL. The kit also contains an internal positive control, whereby p30 antibody-dye conjugates are bound to an immobilised anti-immunoglobulin antibody present in the control region, forming a pink coloured band^(1,2) (Figure 1).

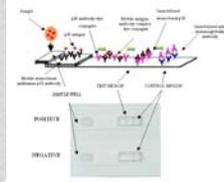


Figure 1: ABACard[®] p30 kit positive and negative results (adapted from⁽⁶⁾)

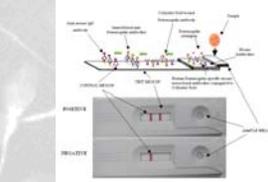


Figure 2: RSID[®]-Semen kit positive and negative results (adapted from⁽⁶⁾)

The Rapid Stain Identification (RSID[®]) test for semen is the first available test kit for the detection of semenogelin, present in human semen. Semenogelin is the major component of human semen, and together with fibronectin accounts for the gel-like coagulum of newly ejaculated semen⁽²⁾. The test comprises immunochromatographic strips that use two mouse monoclonal antibodies specific for human semenogelin; one form of antibody is conjugated to colloidal gold present in the sample pad of the kit, whilst the other form of antibody is present in strips on the test region of the kit. If human semenogelin is present in the semen sample added to the kit, an antigen-antibody conjugated to a colloidal gold complex will form. As this complex migrates down the kit test substrate, the immobilised anti-semenogelin antibodies on the test region bind the semenogelin-antibody-gold complexes, producing a red coloured band. The kit also contains an internal control consisting of anti-mouse IgG antibody. The anti-mouse IgG on the control region binds any mouse antibodies migrating past the control region, producing a red band⁽³⁾ (Figure 2).

Sensitivity was tested using a serial dilution of vasectomised and simulated azoospermic human semen and specificity was tested using a range of bodily fluids other than semen, animal semen and substances and substrates that are likely to be mixed with semen on forensic exhibits.

METHOD

ABACard[®] p30 testing was conducted according to the supplied Technical Information Sheet (Rev 04/05)⁽¹⁾.

RSID[®]-Semen testing was conducted according to the supplied Provided Protocols (Rev. B 2006)⁽³⁾, which was prior to the release of the Extraction Buffer being supplied with the kit. Phosphate Buffered Saline (pH 7.4) was used as the extraction buffer for all testing.

RESULTS

The results from both test kits are read in the same way:

- Positive Result:** presence of 2 bands, one in the test region and one in the control region
- Negative Result:** presence of one band only, in the control region
- Invalid Result:** presence of one band only, in the test region, or no bands present.

SENSITIVITY TESTING

The ABACard[®] p30 tests were more sensitive than the RSID[®]-Semen test kits, with a dilution end point range of 1:2048-8192, as opposed to the RSID[®]-Semen dilution end point of 1:1024-2048. High dose hook effects⁽⁵⁾ were observed using ABACard[®] p30 only, this is an explanation of the negative results observed in the neat samples (Table 1).

Dilution	Semen				Semen			
	AP Result @ 10 ⁰	AP Result @ 10 ¹	AP Result @ 10 ²	AP Result @ 10 ³	AP Result @ 10 ⁰	AP Result @ 10 ¹	AP Result @ 10 ²	AP Result @ 10 ³
NEAT	+++	++++	++++	++++	NEGO	POS	+++	++++
1:1	+++	++++	++++	++++	NEGO	POS	+++	++++
1:2	+++	++++	++++	++++	NEGO	POS	+++	++++
1:4	+++	++++	++++	++++	NEGO	POS	+++	++++
1:8	+++	++++	++++	++++	NEGO	POS	+++	++++
1:16	+++	++++	++++	++++	NEGO	POS	+++	++++
1:32	+++	++++	++++	++++	NEGO	POS	+++	++++
1:64	+++	++++	++++	++++	NEGO	POS	+++	++++
1:128	+++	++++	++++	++++	NEGO	POS	+++	++++
1:256	+++	++++	++++	++++	NEGO	POS	+++	++++
1:512	+++	++++	++++	++++	NEGO	POS	+++	++++
1:1024	+++	++++	++++	++++	NEGO	POS	+++	++++
1:2048	+++	++++	++++	++++	NEGO	POS	+++	++++
1:4096	+++	++++	++++	++++	NEGO	POS	+++	++++
1:8192	+++	++++	++++	++++	NEGO	POS	+++	++++
1:16384	+++	++++	++++	++++	NEGO	POS	+++	++++
1:32768	+++	++++	++++	++++	NEGO	POS	+++	++++
1:65536	+++	++++	++++	++++	NEGO	POS	+++	++++
1:131072	+++	++++	++++	++++	NEGO	POS	+++	++++

Table 1: Sensitivity results obtained for vasectomised human semen using the ABACard[®] p30 kit and the RSID[®]-Semen kits. Please note that the AP result is based on a reading system according to the intensity of the purple colour produced in the reaction. A result of ++ shows a slight purple colour change, whereas ++++ shows a very intense purple colour change.

HUMAN SPECIFICITY TESTING

False positive results were not observed using the ABACard[®] p30. A negative result was obtained from the post-ejaculatory urine sample when tested with the RSID[®]-Semen test kit. This negative result may be due to the concentration of semen present in the urine being lower than the sensitivity dilution end point observed using the RSID[®]-Semen test kits.

ABACard[®] p30 results showed false negative results for semen mixed with human breast milk, blood, urine and post-ejaculatory urine. These observed false negative results are most likely due to the high dose hook effect (Table 2).

SAMPLE	AP RESULT @ 10 ⁰		AP RESULT @ 10 ¹		ABACard [®] p30 RESULT	RSID [®] SEMEN RESULT
	POS	NEG	POS	NEG		
Vaginal Secretion A	POS	NEG	POS	NEG	POS	POS
Vaginal Secretion B	POS	NEG	POS	NEG	POS	POS
Vaginal Secretion C	POS	NEG	POS	NEG	POS	POS
Menstrual Blood C	POS	NEG	POS	NEG	POS	POS
Menstrual Blood D	POS	NEG	POS	NEG	POS	POS
Menstrual Blood E	POS	NEG	POS	NEG	POS	POS
Urine A	NEG	POS	NEG	POS	NEG	NEG
Urine B	NEG	POS	NEG	POS	NEG	NEG
Urine C	NEG	POS	NEG	POS	NEG	NEG
Urine D	NEG	POS	NEG	POS	NEG	NEG
Urine E	NEG	POS	NEG	POS	NEG	NEG
Urine F	NEG	POS	NEG	POS	NEG	NEG
Urine G	NEG	POS	NEG	POS	NEG	NEG
Urine H	NEG	POS	NEG	POS	NEG	NEG
Urine I	NEG	POS	NEG	POS	NEG	NEG
Urine J	NEG	POS	NEG	POS	NEG	NEG
Urine K	NEG	POS	NEG	POS	NEG	NEG
Urine L	NEG	POS	NEG	POS	NEG	NEG
Urine M	NEG	POS	NEG	POS	NEG	NEG
Urine N	NEG	POS	NEG	POS	NEG	NEG
Urine O	NEG	POS	NEG	POS	NEG	NEG
Urine P	NEG	POS	NEG	POS	NEG	NEG
Urine Q	NEG	POS	NEG	POS	NEG	NEG
Urine R	NEG	POS	NEG	POS	NEG	NEG
Urine S	NEG	POS	NEG	POS	NEG	NEG
Urine T	NEG	POS	NEG	POS	NEG	NEG
Urine U	NEG	POS	NEG	POS	NEG	NEG
Urine V	NEG	POS	NEG	POS	NEG	NEG
Urine W	NEG	POS	NEG	POS	NEG	NEG
Urine X	NEG	POS	NEG	POS	NEG	NEG
Urine Y	NEG	POS	NEG	POS	NEG	NEG
Urine Z	NEG	POS	NEG	POS	NEG	NEG

Table 2: Cross Reaction of human bodily fluids with ABACard[®] p30 and RSID[®] Semen test kits.

ANIMAL SPECIFICITY TESTING

No false positive results were observed in the animal semen samples tested using both test kits. Bovine semen, however, resulted in an invalid result multiple times when tested using the ABACard[®] p30 test kit (Table 3).

ANIMAL SEMEN	AP RESULT @ 10 ⁰		AP RESULT @ 10 ¹		ABACard [®] p30 RESULT	RSID [®] SEMEN RESULT
	POS	NEG	POS	NEG		
Canine (Dog)	NEG	NEG	NEG	NEG	NEG	NEG
Feline (Cat)	NEG	NEG	NEG	NEG	NEG	NEG
Bovine (Bull)	NEG	NEG	NEG	NEG	NEG	NEG
Ovine (Sheep)	NEG	NEG	NEG	NEG	NEG	NEG

Table 3: Cross Reaction of animal semen with ABACard[®] p30 and RSID[®] Semen test kits.

SUBSTRATE SPECIFICITY TESTING

SUBSTRATE	AP RESULT @ 10 ⁰		AP RESULT @ 10 ¹		ABACard [®] p30 RESULT	RSID [®] SEMEN RESULT
	POS	NEG	POS	NEG		
Black denim	NEG	NEG	NEG	NEG	NEG	NEG
Black denim + semen	POS	POS	POS	POS	POS	POS
Blue denim	NEG	NEG	NEG	NEG	NEG	NEG
Blue denim + semen	POS	POS	POS	POS	POS	POS
Condom	NEG	NEG	NEG	NEG	NEG	NEG
Condom + semen	POS	POS	POS	POS	POS	POS
Nappy	NEG	NEG	NEG	NEG	NEG	NEG
Nappy + semen	POS	POS	POS	POS	POS	POS
Tempo	NEG	NEG	NEG	NEG	NEG	NEG
Tempo + semen	POS	POS	POS	POS	POS	POS
Uppie	NEG	NEG	NEG	NEG	NEG	NEG
Uppie + semen	POS	POS	POS	POS	POS	POS
Condom	NEG	NEG	NEG	NEG	NEG	NEG
Condom + semen	POS	POS	POS	POS	POS	POS
Condom - fabricate	NEG	NEG	NEG	NEG	NEG	NEG
Condom - fabricate + semen	POS	POS	POS	POS	POS	POS
Condom - nappy	NEG	NEG	NEG	NEG	NEG	NEG
Condom - nappy + semen	POS	POS	POS	POS	POS	POS
Friskit	NEG	NEG	NEG	NEG	NEG	NEG
Friskit + semen	POS	POS	POS	POS	POS	POS
Uppie	NEG	NEG	NEG	NEG	NEG	NEG
Uppie + semen	POS	POS	POS	POS	POS	POS
Head	NEG	NEG	NEG	NEG	NEG	NEG
Head + semen	POS	POS	POS	POS	POS	POS

Table 4: Cross reaction of substrates with ABACard[®] p30 and RSID[®] Semen test kits.

False positives and false negatives were not observed in the substrate control tests when using RSID[®]-Semen. A false positive result was observed in the tampon substrate control using ABACard[®] p30.

False negatives were observed using ABACard[®] p30 when the substrate controls of black and blue denim, tissue, condom, nappy and a mould sample were tested. These false negatives observed are most likely due to the high dose hook effect (Table 4).

EFFECTS OF WASHING

Negative results were obtained from all washed samples when tested using RSID[®]-Semen. The concentration of semen after washing was most likely past the dilution end point observed for RSID[®]-Semen testing.

Hot and cold washes in Nipasan[®] and a cold wash in detergent gave positive results when tested with ABACard[®] p30. All other tests gave negative results (Tables 5+6).

WASH SUBSTRATE	AP RESULT @ 10 ⁰		AP RESULT @ 10 ¹		ABACard [®] p30 RESULT	RSID [®] SEMEN RESULT
	POS	NEG	POS	NEG		
Black	NEG	NEG	NEG	NEG	NEG	NEG
Black + semen	POS	POS	POS	POS	POS	POS
Detergent + semen	NEG	NEG	NEG	NEG	NEG	NEG
Nipasan + semen	POS	POS	POS	POS	POS	POS

Table 5: Effect on washing in cold water on the detection of human semen using the ABACard[®] p30 and RSID[®] Semen test kits.

WASH SUBSTRATE	AP RESULT @ 10 ⁰		AP RESULT @ 10 ¹		ABACard [®] p30 RESULT	RSID [®] SEMEN RESULT
	POS	NEG	POS	NEG		
Black	NEG	NEG	NEG	NEG	NEG	NEG
Black + semen	POS	POS	POS	POS	POS	POS
Detergent + semen	NEG	NEG	NEG	NEG	NEG	NEG
Nipasan + semen	POS	POS	POS	POS	POS	POS

Table 6: Effect on washing in hot water on the detection of human semen using the ABACard[®] p30 and RSID[®] Semen test kits.

CONCLUSIONS

This validation presents experimental evidence demonstrating that the RSID[®]-Semen test kit is accurate and highly specific for human semen and can identify semen recovered from a variety of substrates, and when diluted with other bodily fluids. The absence of cross reactivity to other substrates and bodily fluids is of great importance in sexual assault cases where substrates are highly variable and contamination involving other bodily fluids is possible.

The high dose hook effect observed using the ABACard[®] p30 test kit may potentially result in registering a false negative result. As the majority of semen stains observed in sexual assault cases are neat samples, RSID[®]-Semen avoids the need for costly and time consuming re-testing of stains.

The ABACard[®] p30 test kit gave greater overall sensitivity than the RSID[®]-Semen kit, however the sensitivity level of ABACard[®] p30 is greatly outweighed by the importance of the high level of specificity offered by the RSID[®]-Semen kit.

REFERENCES

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