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TECHNICAL NOTE

CRIMINALISTICS

Jennifer Old,[1] Ph.D.; Brett A. Schweers,[1] Ph.D.; Pravat W. Boonlayangoor,[1] Ph.D.; Brian Fischer,[2] B.S.; Kevin W. P. Miller,[2,†] Ph.D.; and Karl Reich,[1] Ph.D.

Developmental Validation of RSID™-Semen: A Lateral Flow Immunochromatographic Strip Test for the Forensic Detection of Human Semen*

ABSTRACT: Tests for the identification of semen commonly involve the microscopic visualization of spermatozoa or assays for the presence of seminal markers such as acid phosphatase (AP) or prostate-specific antigen (PSA). Here, we describe the rapid stain identification kit for the identification of semen (RSID™-Semen), a lateral flow immunochromatographic strip test that uses two antihuman semenogelin monoclonal antibodies to detect the presence of semenogelin. The RSID™-Semen strip is specific for human semen, detecting <2.5 nL of semen, and does not cross-react with other human or nonhuman tissues tested. RSID™-Semen is more sensitive with certain forensic evidence samples containing mixtures of vaginal secretions and semen than either of the commercially available PSA-based forensic semen detection tests or tests that measure AP activity that were tested in parallel. The RSID™-Semen kit also allows sampling a fraction of a questioned stain while retaining the majority of the sample for further processing through short tandem repeat analysis.

KEYWORDS: forensic science, forensic serology, semen, RSID™-Semen, sexual assault, developmental validation

Confirming the presence of ejaculate on sexual assault evidence (SAE) both assists in corroborating a victim's allegations (1–5) and provides material for generating the alleged suspect's genetic profile through subsequent DNA analysis (6–8). As the predominant source of genetic material, spermatozoa are generally the biological material of importance in cases involving sexual assault (6–8). However, the production of genetic profiles from the semen of vasectomized men has also been reported (8). The microscopic detection of human spermatozoa in SAE has long been used as a confirmatory method for the presence of ejaculate (1–4,9–14). Nevertheless, the visualization of spermatozoa can be inhibited by the presence of other cells in the sample (e.g., epithelial, bacterial, etc. [3,6,7,15,16]), malformation or degradation of the sperm cells themselves (i.e., detachment of the tail from the head [1,11,12,15–17]), or a low number of spermatozoa in the sample through dilution, or azoospermia of the alleged suspect as a result of vasectomy, or other related condition (1,2,4,5,9,11,12,18–21). In situations such as these, the detection of ejaculate in SAE can be extremely difficult by microscopic visualization alone. As a result, attempts are often made to confirm the presence of seminal fluid markers, especially when it is not possible to visualize spermatozoa in the evidentiary sample.

Examination of SAE for the presence of biological stains can be accomplished through the use of alternative light sources (ALS). However, use of ALS alone does not specifically identify semen because other biological fluids and several other substances have been shown to fluoresce under these types of light (1,11,22–24). Once a biological stain has been identified, acid phosphatase (AP) and prostate-specific antigen (PSA) have been used to indicate that semen is present. However, AP screening tests can yield false positives in the presence of commonly encountered household materials (10,11,25), and AP has been found in other body fluids including vaginal secretions (1,2,5,10,11,13,19,21,23,26–28). Initially believed to be a prostate-specific protein (5,28), PSA has now been identified in many different body fluids and tissues including male and female urine (5,13,15,17,18,20,29–34), albeit in low quantities, as well as at significant levels in breast milk and other tissues associated with pregnancy (13,15,20,35–42). For these reasons, seminal-specific markers have generally been considered in the preliminary screening methods for the detection of semen in SAE.

The development of monoclonal antibody-based systems with predefined specificity to definite antigens has greatly advanced our ability to preferentially discriminate between specific markers of interest (43) and, thus, to attribute source to unknown biological stains in forensic casework (2,13–16,18,19,21,29,44–46). Here, we present the developmental validation of the rapid stain identification test for the identification of human semen (RSID™-Semen). RSID™-Semen is a lateral flow immunochromatographic test strip (Independent Forensics of Illinois, Hillside, IL) designed to detect the presence of human semenogelin by employing two antihuman semenogelin monoclonal antibodies in a lateral flow format.

[1]Independent Forensics of Illinois, 4600 Roosevelt Road, Suite 201, Hillside, IL 60162.
[2]Forensic Biotechnology Institute of California, California State University-Fresno, 2576 East San Ramon Avenue, M/S ST104, Fresno, CA 93740.
*IRB permission was obtained for all samples reported in this study and taken by personnel at the California State University, Fresno, for studies conducted by University personnel at the University. Samples collected by personnel at Independent Forensics of Illinois (IFI) and tested at IFI by IFI personnel were not subject to IRB regulation.
†Present address: Kern Regional Crime Laboratory, 1300 18th Street, 4th Floor, Bakersfield, CA 93001.

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Semenogelin is a unique protein that originates in the seminal vesicles and comprises a major component of human seminal plasma (12). Along with fibronectin, semenogelin gives rise to a gel-like coagulum of fresh ejaculate (12,47). Semenogelin is found predominately in human seminal fluid, but it has also been detected in the serum of individuals diagnosed with certain types of cancer (15,48), and in the ejaculate of some new world primates (49). Although semenogelin has been used as a semen-specific marker before, it has not been as widely used as PSA or AP markers (12).

Here, we assess the RSID™-Semen kit for accuracy, reproducibility, and suitability for use in forensic casework. This includes determining whether the RSID™-Semen kit is sensitive, stable, and species, as well as body fluid, specific. Positive results obtained with the RSID™-Semen test strips were then counted to determine how many RSID-positive stains would subsequently give a proper DNA profile from the remaining sample.

Last, the lateral flow strip test was compared with other semen detection methods to determine whether the kit is more or less sensitive for semen detection when analyzing certain forensic evidence samples.

The Scientific Working Group on DNA Analysis Methods (SWGDAM) has recommended several guidelines for both developmental and internal validations of DNA analysis methods with the intention of establishing the reliability, robustness, and reproducibility of DNA typing procedures (50). However, SWGDAM has not yet established guidelines for the validation of serological techniques. Developmental and internal validations of serological techniques are, nonetheless, warranted. In this study, we attempt to adapt SWGDAM guidelines for the validation of DNA typing methods to serological methods to present a critical evaluation of the RSID™-Semen kit for use in forensic casework. The kit was tested against several scenarios that could potentially be encountered in SAE to demonstrate that the antibody binds reliably to its intended target.

Materials and Methods

Materials

Laminar flow immunochromatographic strip test production equipment was purchased from Biodot (Irvine, CA) and used according to manufacturer's recommendations. Test strip components, including glass–fiber conjugate pads and cellulose wicks were purchased from Millipore (Billerica, MA). Test strip membranes were purchased from Whatman (Florham Park, NJ). Detection antibodies were labeled with colloidal gold (particle size: 40 nm) made by reduction of hydrogen tetrachloroaurate with sodium citrate. Goat antimouse IgG, used at the control line of RSID™-Semen, was purchased from Sigma-Aldrich (St. Louis, MO).

RSID™-Semen Buffer Components

The RSID™-Semen extraction buffer is designed to efficiently extract the protein semenogelin from questioned stains and swabs. RSID™-Semen running buffer is designed to dissolve the antibody–colloidal gold conjugate from the conjugate pad, maintain an extract at the appropriate pH, and facilitate correct running of fluids through the test strip. Components of the extraction and running buffer include buffer and salts (Tris, NaCl, and KCl), as well as a chelating agent (EDTA) for physiological stability, detergents and a surfactant (Tween 20) for extraction efficiency and solubility maintenance, protein (bovine serum albumin) for reducing nonspecific adsorption and loss, and a preservative (sodium azide). Concentrations of the respective solutions are proprietary and are thus not reported here.

Configuration of the Semenogelin Lateral Flow Test

The RSID™-Semen test is an immunochromatographic assay that uses two monoclonal antibodies specific for semenogelin. This system consists of overlapping components (conjugate pad, membrane, and wick) assembled such that the tested fluid is transported from the conjugate pad to the membrane and is finally retained on the wick. The conjugate pad and membrane are pretreated before assembly such that the user need only add his/her extract in running buffer to initiate the test. Once the sample is added to the sample window, the running buffer and the sample diffuse through the conjugate pad, which is precharged with colloidal gold-conjugated antihuman semenogelin monoclonal antibodies. The sample redissolves the colloidal gold-labeled antisemenogelin antibodies that bind semenogelin in the sample if it is present. Semenogelin–colloidal gold antibody complexes are transported by bulk flow to the membrane phase of the test strip. These complexes, if present, migrate along the membrane and are bound at the "test line" creating a red line in the presence of human semen. The example strip in Fig. 1 depicts an RSID™-Semen strip test that has already been developed with semen present in the sample, and therefore, the test and control lines are visible on the membrane. Neither the test line nor control line are visible on an unused strip test. Uncomplexed colloidal gold-labeled mouse antibody progresses along the membrane and is bound by antimouse antibody at the "control line," also creating a red line. A red line at the "test" position indicates the presence of human semen, while a red line at the "control" position indicates that the strip test is working as designed. When the test is performed correctly and the test strip is functioning properly, all RSID™-Semen test strips should produce a line at the control position.

The control line is made by "striping" goat antimouse antibody onto the membrane component of the lateral flow strip test; the deposited antibody retains colloidal gold antisemenogelin mouse monoclonal antibody that migrates past the test line. The line closest to the sample well is the test line and indicates that human semenogelin is present in the sample. The test line is made by "striping" a mouse monoclonal antisemenogelin antibody onto the membrane component of the strip test; complexes of colloidal gold-labeled antisemenogelin mouse monoclonal antibody that are formed in solution upon addition of the sample to the sample well and have progressed through the conjugate pad and membrane (or allowed to wick up the conjugate pad when the strip is tested outside of a plastic housing, e.g., in a 12×75-mm test tube) are retained at the test line. A red control line must be visible at 10 min after sample addition to interpret results.

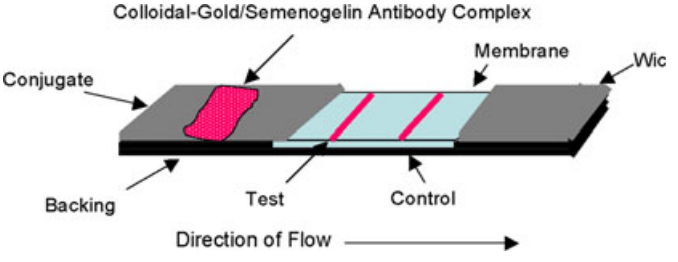

FIG. 1—*Schematic diagram of an assembled RSID™-Semen strip test. The components of the RSID™-Semen strip test encased within a plastic cassette are shown. The test consists of three main components, wick, membrane, and conjugate pad, which are adhered to a backing card. The colloidal gold/semenogelin-antibody conjugate is dispersed on the conjugate pad, and the test and control line antibodies are striped on the membrane. The direction of bulk liquid flow is indicated by the arrow.*

Specimens

All body fluid donors and research participants were healthy volunteers and were informed that they could withdraw from the study at any time in accordance with federal human subjects protection policies. Human saliva, blood, and urine samples were obtained and deposited on sterile cotton swabs or fabric cuttings in aliquots of 50 μL. Urine samples were collected midstream to ensure the cleanest sample possible, and the first elimination from male subjects postejaculation was never collected. Unwashed semen was obtained from a local sperm bank and deposited on sterile cotton swabs or fabric cuttings in aliquots of 50 μL. Human breast milk samples were obtained from SRI (Richmond, CA). Briefly, human breast milk was collected from lactating mothers in a manner that would preclude contamination and deposited on sterile cotton swabs or fabric cuttings and air-dried. Postcoital vaginal swabs were obtained from healthy volunteers. Animal semen samples were donated from the CSU Fresno Jordan College of Agriculture and deposited on sterile cotton swabs in aliquots of 50 μL. Sample cotton swabs or fabric cuttings were air-dried under standard laboratory clean conditions prior to further analysis using the RSID™-Semen kit. Numerous replicate samples from several different subjects were tested to ensure the reproducibility of our results. The numbers of individuals from whom samples were collected are listed in Table 1. The total number of replicate tests performed from the samples is also listed. In instances where whole samples were tested, each sample was tested three or more times. In instances where sample dilutions were tested, a single sample dilution was made and three or more aliquots were subsequently tested. To ensure that high-dose hook effects did not affect tabulated results, each sample was reanalyzed at a 1:20 dilution of the original extract.

Alternate Semen Identification Protocols

A modified version of the sodium α-napthylphosphate AP test (10) was used which required two reagents: Reagent A, which contained 0.25% (w/v) sodium α-naphthylphosphate in acetate buffer (sodium α-naphthylphosphate; Fischer Scientific, Suwanee, GA), and Reagent B, which contained 0.5% (w/v) naphthanil diazo blue B (Fast Blue B Salt; Sigma-Aldrich) in acetate buffer. The acetate buffer was comprised of 1% glacial acetic acid (glacial acetic acid; Fischer Scientific) and 0.24 M sodium acetate in distilled water. To perform the AP test on swab heads, two drops of Reagent A were deposited on the swab and incubated at room temperature for 30 sec. One drop of Reagent B was then deposited on the swab head, and the results were recorded after 10 sec. A positive result for AP was indicated by the rapid development of an intense purple color on the swab head. A negative result for AP was indicated by a lack of color development and, therefore, characterized as a "null" reaction.

The Seratec® PSA Semiquant (Seratec PSA, Goettingen, Germany) test was performed according to the manufacturer's protocol. Briefly, the tests were performed at room temperature, and positive and negative controls were included with every assay. Samples were brought to a final volume of 200 μL with Tris-buffered saline, typically in a disposable 0.6-mL microcentrifuge tube. The sample plus running buffer were then added to the sample window of a Seratec® PSA test strip. The strip was incubated at room temperature for 10 min, and the results were read immediately thereafter.

Preparation of Body Fluid Extracts

Human saliva, semen, blood, urine, and breast milk, and animal semen samples were extracted from cotton swabs to which 50 μL

TABLE 1—*Sample design for the developmental validation of RSID™-Semen test strips.*

	No. of Subjects Sampled	Total No. of Sample Replicates Performed
Species study	–	**114**
Animal semen	–	–
Bull	2	12
Cat	2	12
Dog	2	12
Goat	2	12
Horse	2	12
Mouse	2	12
Pig	2	12
Sheep	2	12
Human semen w/extender	3	18
Sensitivity	–	**600**
Volume of semen extract	–	–
1 μL at 1:20 dilution	25 Semen samples	75
1 μL at 1:10 dilution		75
1 μL at 1:5 dilution		75
1 μL at 1:2 dilution		75
2 μL		75
5 μL		75
10 μL		75
25 μL		75
High-dose hook effects	–	**450**
Volume of semen extract (extracted into)	–	–
1 μL (400 μL)	25 Semen samples	75
5 μL (400 μL)		75
25 μL (400 μL)		75
50 μL (400 μL)		75
100 μL (400 μL)		75
50 μL (200 μL)		75
Specificity		**180**
Human	–	–
Blood	15	45
Breast milk	15	45
Buccal (saliva)	15	45
Urine	15	45
Stability testing	–	–
Increased temp. storage (37°C)	–	–
For 7 days	–	**27**
0 μL	3	9
1 μL	3	9
5 μL	3	9
For 14 days	–	**36**
0 μL	3	9
1 μL	3	9
5 μL	3	9
25 μL	3	9
Extended extract storage (room temp.)	–	**24**
4 h	4	12
16.5 h (overnight)	4	12
Casework/mock casework samples	–	–
Postcoital vaginal swabs	–	–
With a condom	–	**18**
Day 0	3	9
Day 14	3	9
Without a condom	–	**75**
Day 0	5	15
Day 1	3	9
Day 2	3	9
Day 3	2	6
Day 4	1	3
Day 5	1	3
Day 6	2	6
Day 7	1	3
Day 8	1	3
Day 9	1	3
Day 10	1	3
Day 14	2	6
Day 17	1	3

Continued.

TABLE 1—*Continued.*

	No. of Subjects Sampled	Total No. of Sample Replicates Performed
Day 19	1	3
Woman's black undergarment	1	**3**
Sampling procedure	–	–
ddH_2O moistened swab	–	**84**
Cotton chambray	4	12
Cotton twill	4	12
Cotton blue denim	4	12
Nylon lace	4	12
Nylon-knit jersey	4	12
Cotton flannel sheet	4	12
Cotton sheet (conventional weave)	4	12
Sample punch (5 mm)	–	**84**
Cotton chambray	4	12
Cotton twill	4	12
Cotton blue denim	4	12
Nylon lace	4	12
Nylon-knit jersey	4	12
Cotton flannel sheet	4	12
Cotton sheet (conventional weave)	4	12
Sexual lubricants/contraceptives	–	**90**
K-Y® Brand Jelly	10	30
VCF® Vaginal Contraceptive Foam	10	30
Conceptrol® Contraceptive Gel	10	30
Condoms (Trojan® Brand)	–	**90**
No lubricant present	10	30
Nonspermicidal lubricant present	10	30
Spermicidal lubricant present	10	30

The right-most column indicates each individual study within the validation.

The middle column shows the number of individual subjects from whom samples were collected for each portion of each study. The right-most column indicates the total number of replicate tests performed from the samples that were collected. In instances where whole samples were tested, each sample was tested three or more times. In instances where sample dilutions were tested, a single sample dilution was made and three or more aliquots were subsequently tested. To ensure that high-dose hook effects did not affect tabulated results, each sample was reanalyzed at a 1:20 dilution of the original extract. Numbers in bold indicate total sample replicates for each respective study.

of the respective body fluid was previously deposited (described earlier). The stained cotton batting was removed using sterile instruments and placed in a 1.5-mL microcentrifuge tube. The sample material was then removed by incubating the cotton batting in 1 mL of RSID™-Semen extraction buffer for 1–2 h at room temperature. Assuming 100% extraction efficiency, each microliter of the extract would have contained *c.* 50 nL (0.05 μL) of whole fluid. This value was calculated from the dilution of the 50 μL of semen deposited on the cotton swab with 1 mL of extraction buffer.

Standard positive control extracts were prepared from 50 μL of human semen that was deposited on a sterile cotton swab. The swab batting was removed and incubated in 1 mL of extraction buffer contained within a 1.5-mL microcentrifuge tube for 1–2 h at room temperature. Negative control extracts were made in an identical manner, substituting human semen with 50 μL of double distilled water (ddH_2O). All sample batches were performed with a positive and negative control prepared in this manner. However, additional experimental controls may be noted within individual studies.

Unless otherwise specified, experimental samples were prepared by combining the noted volume of extracted solution with sufficient running buffer to produce a final volume of 100 μL (e.g., 10 μL of extract + 90 μL of RSID™-Semen running buffer = 100 μL total). The 100 μL of sample extract in running buffer was then placed on the sample well of an RSID™-Semen test strip. While performing the sensitivity experiments, the desired semen concentration often required a volume <1 μL of extract to be added to running buffer. In this situation, a portion of the stock sample extract was first diluted to produce a transferable volume of extract to be added to the running buffer as described earlier (e.g., 1 μL of stock extract diluted 1:20 with running buffer, then 1 μL of diluted extract + 99 μL of running buffer = 100 μL total). Most samples were tested on strips placed in cassettes, but for photographic clarity, some tests were performed in 12 × 75-mm test tubes. All case results were recorded 10 min after sample addition. In immunochromatographic strip tests, false negative results, called high-dose hook effects, may sometimes be seen when too much semen is added to the strip. In this instance, there is too much semenogelin in the system and it is not all bound to antibody. Therefore, free semenogelin saturates the test region of the strip, preventing antibody-bound semenogelin from forming a positive test line. To rule out any chance of high-dose hook effect, all experimental sample extracts presenting a negative result were diluted and reanalyzed at 1:20 of the initial amount of sample extract tested. This was carried out by either using less of the sample extract or prediluting the sample extract accordingly with running buffer. Once diluted, 100 μL of the sample extract in running buffer was run on a strip test, and the results were analyzed after 10 min.

Preparation of Casework/Mock Casework Extracts

A pair of women's black undergarments with a visible unknown stain was obtained by Independent Forensics with a request for semen detection. The stained area was swabbed with a sterile cotton swab that was moistened with ddH_2O, and the swab was allowed to air-dry. Positive and negative control sample swabs were also prepared as described earlier. The cotton batting from the unknown sample, positive control, and negative control cotton swabs were each extracted into 300 μL of RSID™-Semen extraction buffer and incubated at room temperature for 2 h. Ten microliter of the unknown sample extract and 1 μL of the positive and negative control extracts were removed for testing with the RSID™-Semen kit. Equivalent volumes of extract were analyzed using the Seratec® PSA Semiquant and AP screening tests as described earlier.

Postcoital vaginal swabs were collected at various intervals from individuals who had vaginal intercourse with or without the use of a condom. Vaginal swabs were collected at day 0 (the day of intercourse) and day 14 (the 15th calendar day following intercourse) from individuals who had used a condom. Vaginal swabs from individuals who did not use a condom were collected at days 0–10, 14, 17, and 19. In some instances, sample swabs contained menstrual blood, and samples from individual volunteers were not necessarily collected on each day of the collection schedule. Swabs were extracted into either 100 or 200 μL of extraction buffer and prepared for analysis, according to the above-mentioned sample protocol using 10 or 20 μL of sample extract, respectively. A portion of the remaining extracts from the unprotected postcoital vaginal swabs (days 0–7 and 9) were processed for multiplex Y-short tandem repeat (STR) analysis. Menstrual blood was present in some of the samples subsequently processed for DNA analysis. Positive and negative control sample swabs (prepared as described earlier) were processed alongside postcoital samples in an identical manner using 1 μL of sample extract for comparison purposes. Additionally, postcoital swab extracts from both protected and

unprotected intercourse were tested for high-dose hook effects by reanalyzing 20 μL of a 1:20 dilution of the sample extract on an RSID™-Semen test strip. We did not test any procedure other than the RSID-Semen test for high-dose hook effects.

Several types of fabrics, namely, cotton chambray, cotton twill, cotton denim, nylon lace, nylon-knit jersey, and cotton sheeting (both flannel and conventional weave) were prepared by adding 50 μL of semen to the fabric and allowing it to air-dry completely before further processing. Each dried stain was then sampled either by using a ddH_2O-moistened cotton swab or by excising a 5-mm-diameter circle using a Harris punch. Sample swabs were extracted in 200 μL of extraction buffer at room temperature for 1 h. Fabric punches were extracted under identical conditions in 100 μL of extraction buffer. RSID™-Semen testing was performed using 20 and 10 μL of sample extract for cotton swab and fabric punch samples, respectively. Standard positive and negative control sample swabs were processed alongside unknown samples as previously described using 1 μL of sample extract. A portion of the remaining sample extracts from each sampling method was used for multiplex Y-STR analysis.

Preparation of Contraceptive and Sexual Lubricant Extracts

One hundred microliters of semen was mixed with 100 μL of commercially available sexual lubricant (K-Y® Brand Jelly; Johnson & Johnson, New Brunswick, NJ) or two vaginal contraceptives (VCF® Vaginal Contraceptive Foam, 12.5% nonoxynol-9; Ortho Options® Conceptrol® Vaginal Contraceptive Gel, 4.0% nonoxynol-9; Revive Personal Products, Madison, NJ) individually. Sample stains were then prepared using the semen and sexual lubricant or vaginal contraceptive mixture samples. To do this, the sample mixture was divided equally and either smeared on a portion of clean cotton sheeting or deposited on a sterile cotton swab. The stains were allowed to air-dry completely prior to processing. Sample punches (5 mm diameter) and cotton swabs of each sample mixture were extracted into 300 μL of extraction buffer at room temperature for 1 h. In addition to the standard positive control (described earlier), 50 μL of human semen was deposited onto the cotton sheeting, extracted, and processed under conditions identical to the sample mixtures to act as a known positive control throughout the analysis. The samples and positive controls were then analyzed as described earlier using 20 μL of sample extract. Both the cotton sheeting and cotton swab sample extracts were reanalyzed on RSID™-Semen test strips using 1 μL of extract as previously described to test for high-dose hook effects.

To test the interference of commercially available contraceptives, 50 μL of human semen was pipetted into three varieties of Trojan® Brand condoms (no lubricant present; nonspermicidal lubricant present; spermicidal lubricant present, 7% nonoxynol-9; Church and Dwight Co., Inc., Princeton, NJ) and allowed to air-dry overnight. Samples were then removed from the condoms using ddH_2O-moistened cotton swabs, which were then air-dried before extraction into 300 μL of RSID™-Semen extraction buffer for 1 h at room temperature. Samples were processed with the RSID™-Semen kit as previously described using 20 μL of sample extracts. For this study, in addition to the standard positive control, a second 50 μL of semen was transferred onto a cotton swab and extracted into 300 μL of extraction buffer to more closely mimic a positive control of the condom samples for interference comparison. Both of the positive controls and the negative controls were analyzed using 20 μL of the sample extracts. Each sample was reanalyzed with RSID™-Semen as previously described using 1 μL of sample extract to test for high-dose hook effects.

DNA Extraction and STR Analysis

DNA was extracted using a Chelex extraction protocol (51) from samples on various substrates, as well as from postcoital vaginal swabs collected at days 0–7 and day 9 from individuals who had unprotected vaginal intercourse. The extracted DNA was amplified with the GeneAmp PCR system 9700 (Applied Biosystems, Foster City, CA) using the Ampfℓ STR® Y-filer PCR Amplification Kit (Applied Biosystems). The amplification reaction volume was reduced to 6 μL while maintaining the kit's component ratios. Amplification started with an initial incubation step at 95°C for 11 min followed by 31 cycles of 94°C for 1 min, 61°C for 1 min, 72°C for 1 min, and a final extension step of 60°C for 80 min. The amplification reactions were run on an ABI Prism 310 Genetic Analyzer and analyzed with GENESCAN (v. 3.7) and GENOTYPER (v. 3.7) (Applied Biosystems) using an allele threshold of 75 relative fluorescent units.

Results and Discussion

Sensitivity

The sensitivity of RSID™-Semen was assessed by testing various volumes and dilutions of human semen extract prepared from standard positive control semen swabs (described earlier). The concentration of semen in the sample was varied by lowering the volume of semen extract added to the 100 μL of extract in running buffer that was applied to the test strip, as shown in Table 2. If <1 μL of the sample extract was needed, then a portion of the stock sample extract was first diluted to produce a transferable volume of extract to be added to the running buffer as described earlier (e.g., 1 μL of stock extract diluted 1:25 with running buffer, then 1 μL of diluted extract + 99 μL of running buffer = 100 μL total). Our results with RSID™-Semen using the fluid standard were consistent, reproducible, and independent of the source of the human semen. In some experiments, strips were removed from their plastic casing for photographic clarity. These strips were tested in 12 × 75-mm test tubes using a 100 μL final volume of extract and running buffer. Identical results were obtained from strips that were tested in cassettes or in test tubes. Results of all experiments were scored as positive or negative relative to the test controls 10 min after sample application.

Using human semen extract prepared and tested as previously described, RSID™-Semen test strips detected the presence of

TABLE 2—*Experimental Limit of Detection of RSID™-Semen.*

Sample	Volume of Semen Extract Added	Equivalent Volume of Semen in Sample	Semen Detected
1	0	0	−
2	1 μL at 1:20 dilution	2.5 nL	+
3	1 μL at 1:10 dilution	5 nL	+
4	1 μL at 1:5 dilution	10 nL	+
5	1 μL at 1:2 dilution	25 nL	+
6	2 μL	50 nL	+
7	5 μL	250 nL	+
8	10 μL	500 nL	+
9	25 μL	1.0 μL	+

+: Semen detected with the RSID™-Semen kit; −: Semen was not detected with the RSID™-Semen kit.

Volumes of positive control semen extract (column 2) were brought to 100 μL with RSID™-Semen running buffer and analyzed using the RSID™-Semen test strip. A portion of sample extracts 2, 3, 4, and 5 were prediluted at the noted ratios with running buffer before using 1 μL for testing. The equivalent volume of semen (column 3) assuming 100% extraction efficiency is also listed for added clarity of the amount of semen tested. The lowest level of detection for the RSID™-Semen test strips, as tested here, is 2.5 nL equivalent volume of semen.

semen down to 2.5 nL. Volumes of positive control semen extracts (0–20 μL) were tested corresponding to *c.* 0, 2.5, 5, 10, 25, 50, 250, 500 nL, and 1.0 μL equivalent volume of semen (Table 2). A positive signal at the test line was observed for extract volumes corresponding to 2.5, 5, 10, 25, 50, 250, 500 nL, and 1.0 μL equivalent volume of semen (Fig. 2, strips 2–9, respectively). The intensity of the test line increased as the volumes of semen extract tested increased. However, at equivalent levels of semen above 250 nL, the signal intensity gradually decreased indicating a potential high-dose hook effect. These tests were repeated with different production lots of RSID™-Semen test strips to check for consistency of the limit of detection and appearance of the control line (data not shown). Results from independent production lots demonstrate that the signal intensity at the control line and the limit of detection of the RSID™-Semen test strips remained consistent in their performance (data not shown).

High-Dose Hook Effect

High-dose hook effect is the name given to the observation seen with many lateral flow immunochromatographic strip tests, where the addition of high levels of analyte (i.e., target antigen) can reduce the signal intensity seen at the test line. The reduction in intensity can be such that a false negative result could be obtained. It is thought that the high-dose hook effect can occur when the amount of target antigen in the sample is sufficiently high that a significant amount of target antigen remains unbound by the colloidal gold-labeled antibody in the conjugate pad. Free antigen could then migrate to the membrane ahead of the labeled antibody–antigen complexes, occupying the bound antibody on the test line with unlabeled antigen and thus, leaving no sites for the gold-labeled antibody–antigen complexes to bind. By blocking the test line with unlabeled antigen, the test line could appears negative as no colloidal gold-bound antigen is retained at the test line. Retesting a dilution of the questioned stain extract can be performed to ensure that the observed result is a true negative, and not because of a high-dose effect. We evaluated the RSID™-Semen test strips with increasing amounts of semen extract to determine the kit's high-dose hook effect threshold in response to high levels of target antigen.

The observation of high-dose hook effects during testing of the sensitivity of the RSID™-Semen test strips at levels of semen above 250 nL led us to more stringent testing of the high-dose effect. This involved concentrating the standard positive control extract by decreasing the extract volume from 1 mL to 400 μL (2.5× more concentrated) and, additionally, to 200 μL to produce a more concentrated extract (5× more concentrated than standard positive control). Various sample extract volumes, listed in Table 3, were then added to RSID™-Semen running buffer and run on a test strip as previously described. These experimental approaches for preparing highly concentrated semen extracts were designed to demonstrate the functional upper limit of semen detection by the RSID™-Semen test strips. For these experiments, test strips were run in 12 × 75-mm test tubes. A standard positive control swab with 50 μL of semen was extracted in 400 μL of extraction buffer, and 1, 5, 25, 50, and 100 μL of this extract was run on an RSID™-Semen test strip (Fig. 3*A*, strips 3–7, respectively) as described earlier. For comparison, 20 μL of a ddH_2O cotton swab extract was included as a negative control (Fig. 3*A*, strip 1). A fresh positive control swab was extracted in 200 μL of extraction buffer, and then, 50 μL of the extract was tested with RSID™-Semen. The signal intensity from 5 μL of a standard positive

TABLE 3—*Extract volumes used to test high-dose hook effect of RSID™-Semen.*

Sample	Equivalent Volume of Semen in Sample	Volume of Extraction Buffer Used	Volume of Semen Extract Added	Semen Detected	Semen Detected (1:20)
1	0	1 mL	0	−	−
2	125 nL	1 mL	5 μL	+	+
3	250 nL	400 μL	1 μL	+	n/a
4	625 nL	400 μL	5 μL	+	+
5	3.125 μL	400 μL	25 μL	−	+
6	6.25 μL	400 μL	50 μL	−	+
7	12.5 μL	400 μL	100 μL	−	+
8	50 μL	200 μL	50 μL	−	+

+: semen detected with the RSID™-Semen kit; −: semen was not detected with the RSID™-Semen kit.

To vary the concentration of semen (column 2), positive control semen extracts were extracted in different volumes of RSID™-Semen extraction buffer (column 3) and portions of the sample extracts (column 4) were brought to 100 μL with RSID™-Semen running buffer and analyzed with the test strip. High-dose hook effects were observed from samples containing more than 3 μL of human semen resulting in false negative results (column 5). Testing the same volume of semen after a 1:20 dilution of the extract prevented the high-dose hook effect (column 6).

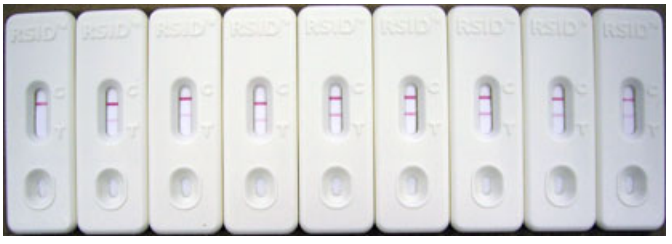

FIG. 2—*Sensitivity of RSID™-Semen, semen extract. A series of dilutions of semen extract were tested with the RSID™-Semen test strips. The following equivalent volumes of semen were tested: lanes (1) 0 nL; (2) 2.5 nL; (3) 5 nL; (4) 10 nL; (5) 25 nL; (6) 50 nL; (7) 250 nL; (8) 500 nL; (9) 1.0 μL. The experimental limit of detection of RSID™-Semen was determined to be 2.5 nL of human semen. The positions of the control and test lines are indicated. Results were recorded 10 min after sample application.*

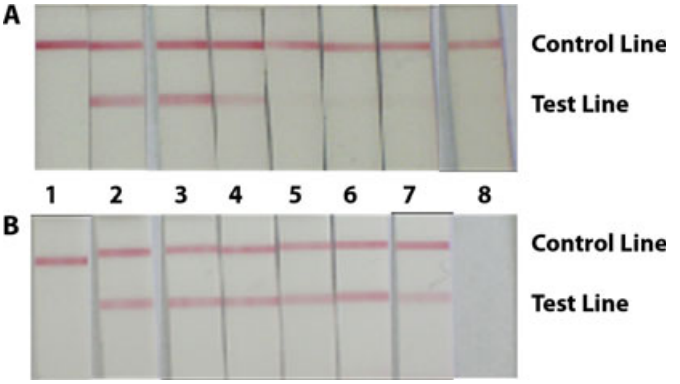

FIG. 3—*The high-dose hook effect encountered with RSID™-Semen. (A) The following equivalent volumes of human semen were tested with RSID™-Semen: lanes (1) 0 nL; (2) 125 nL; (3) 250 nL; (4) 625 nL; (5) 3.125 μL; (6) 6.25 μL; (7) 12.5 μL; (8) 50 μL. False negative results were observed from samples 5–8, as can be seen by lack of banding at the test line, because of high-dose hook effect reduction in signal intensity. (B) Re-analysis after a 1:20 dilution was made of the sample extracts from the original semen concentration. Dilution resulted in strong positive band intensities (lanes 3–7). The standard positive control extracted using 1 mL of extraction buffer (lane 2) was not diluted at 1:20, but displayed consistent band intensities with previous experiments.*

control extracted in 1 mL of extraction buffer was comparable to the signal from 1 μL of the 400 μL positive control extraction (Fig. 3*A*, strips 2 and 3, respectively). As the concentration of semen in the sample increased, the signal intensity gradually decreased (Fig. 3*A*). No discernable signal was observed from samples containing 3.125, 6.25, 12.5, and 50 μL equivalent volume of semen (Fig. 3*A*, strips 5–8, respectively). The high levels of target antigen present in these samples resulted in the presentation of a false negative because of high-dose hook effect. These experiments indicate that a false negative result is possible when analyzing >3 μL of semen in a sample. Dilution of the sample will restore the signal by lowering the concentration of semenogelin in the sample, allowing proper functioning of the lateral flow test strip.

To determine whether a 1:20 dilution of the sample extract was sufficient to overcome the observed high-dose hook effect, both the 400 and 200 μL positive control extracts were diluted accordingly. Diluted extract volumes of 5, 25, 50, and 100 μL of the 400 μL extract and 50 μL of the 200 μL extract (Table 3) were retested with the RSID™-Semen test strips using the previously described protocol (Fig. 3*B*). Positive band signals were observed from each of the diluted test strips, although 50 μL of the 200 μL extract presented the weakest signal of the group, indicating that some high-dose hook effects were still occurring (Fig. 3*B*, strip 7). Similar test line band intensities were observed from 5, 25, 50, and 100 μL of the 400 μL extract (Fig. 3*B*, strips 3–6, respectively). These band signals were comparable to 5 μL of the standard positive control sample (Fig. 3*B*, strip 2). Dilution of the samples displaying high-dose hook effect by a factor of 20 was sufficient to overcome the resulting false negative effect. While it is difficult to predict the levels of semenogelin in an unknown sample, it is recommended that when samples containing high amounts of semenogelin are suspected (i.e., >3 μL) that a 1:20 dilution is performed to reduce the chance of high-dose hook effect confusing the observed result. If the diluted sample tests positive, then the original sample/evidence contains semen and any original negative result was a false negative induced by the high-dose hook effect. If the diluted sample tests negative once again, then the original negative result is confirmed and no semen is indicated on the item being tested.

Specificity of RSID™-Semen: Testing Noncognate Body Fluids

To evaluate the potential cross-reaction or inhibition of RSID™-Semen test strips with other body fluids, extracts of human saliva, blood, semen, urine, and breast milk were prepared as described earlier and tested with the RSID™-Semen test strips (Fig. 4). Individual extracts of blood, urine, saliva, and breast milk each produced negative results, with semen extracts producing a positive result (Fig. 4, strips 2–6). Combinations of extracts with or without semen were also tested. Only mixtures containing semen extract produced a positive signal (Fig. 4, strip 8), while mixtures of noncognate body fluids produced only a band at the control line with no visible signal at the test line (Fig. 4, strip 7). Test strips were analyzed in 12 × 75-mm test tubes for photographic clarity, and identical results were obtained with strips held in plastic cassettes (data not shown). Semen samples were run using 5 μL of semen extract to avoid high-dose hook effects. Individual noncognate body fluids were tested using 25 μL of blood, urine, saliva extract, and 20 μL of breast milk extract to ensure that even low levels of cross-reactivity would be observed, if present. For comparison, a negative control was included (Fig. 4, strip 1). Sample mixtures were prepared from 5 μL of semen extract (omitted for mixtures not containing semen) and 20 μL of each of the remaining body fluid extracts (20 μL of extract is equivalent to *c.* 1.0 μL of body fluid). These experiments demonstrate that RSID™-Semen test strips do not cross-react with the other body fluids tested. In addition, the overshadowing presence of saliva, blood, urine, and breast milk in the sample mixture does not interfere with a test strip's ability to detect semen in the sample.

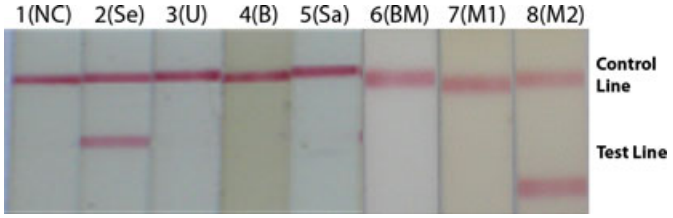

FIG. 4—*Specificity of RSID™-Semen, testing noncognate body fluids. In each case, a specified volume of body fluid extract was brought up to a total volume of 100 μL, which was loaded onto the RSID™-Semen test strip. A negative control containing 100 μL of RSID™ buffer only is pictured in lane 1(NC). Lane 2(Se) contained 5 μL of positive control semen extract. Lanes 3(U), 4(B), and 5(Sa) contained 25 μL of urine extract, blood extract, and saliva extract, respectively. Lane 6(BM) contained 20 μL of breast milk extract. Lane 7(M1) contained 20 μL each of blood, urine, saliva, and breast milk extracts without the addition of semen extract. Lane 8(M2) contained 20 μL each of blood, urine, saliva, and breast milk extracts with the addition of 5 μL of semen extract. Only samples containing human semen extract gave a positive signal from the RSID™-Semen test strips. Images of strips labeled 6(BM), 7(M1), and 8(M2) were taken at a different time and at a slightly higher magnification than strips labeled 1(NC), 2(Se), 3(U), 4(B), and 5(Sa). As a result, the test line of lane 8(M2) appears lower than that of lane 2(Se).*

Species Specificity of RSID™-Semen: Testing of Animal Samples

Semen swabs from various animal species, both livestock and companion animals, were purchased from commercial husbandry sources. Extracts were prepared as described earlier for human samples, and 20 μL of each extract was tested with RSID™-Semen test strips. Sample extracts were also tested using 1 μL of each extract (1:20 dilution) to address potential high-dose hook effects. No cross-reactivity was observed with semen from the following animals using either volume of extract: bull, cat, dog, goat, horse, mouse, pig, and sheep. In addition, because nonhuman semen samples may be stored in an extender mixture of nourishment and antibiotics, human semen was mixed with a common extender and analyzed for interference. These samples were prepared by mixing 50 μL of human semen with 50 μL of the extender and depositing the mixture on a sterile cotton swab. Once air-dried, the sample swab was extracted according to the standard positive control sample protocol described earlier and tested using 5 μL of the sample extract. No difference in signal intensity was observed between human semen positive controls with or without extender present (data not shown).

Stability Testing of RSID™-Semen

We have demonstrated that RSID™-Semen test strips are both specific and sensitive for human semen detection. Here, the stability of the assembled strip tests was investigated by performing semen detection with RSID™-Semen test strips that were stored at 37°C for extended periods of time (i.e., 7 or 14 day periods). These conditions were chosen to test for accelerated degradation of the kit components.

Extracts prepared from positive control swabs were tested on RSID™-Semen test strips (as described previously) that were stored at 37°C for 7 days using 0, 1, and 5 μL of positive control

extract (equivalent to 0, 50, and 250 nL of semen). These extract portions, in addition to a larger 25 μL portion of the positive control extract (equivalent to 1.25 μL of semen), were also tested on strips that were stored at 37°C for 14 days. Test results were compared with strips stored at room temperature for equal lengths of time using an equivalent volume of positive control extract. No difference in band intensity or background was observed at any extract volume tested on the strips stored under high temperature conditions for a period of 7 days when compared to test strips stored at room temperature. Strong band intensities were observed for each volume of sample extract at both room temperature and increased temperature storage conditions. However, after storage for 14 days at 37°C, the sensitivity of the RSID™-Semen test strips were slightly reduced when compared to strips stored at room temperature. When 1 and 5 μL of extract were used, the observed band intensities were slightly weaker when using strips that were stored for 14 days at 37°C. The band intensity was unchanged when using 25 μL of the sample extract. These experiments demonstrate the ability of RSID™-Semen test strips to withstand prolonged storage (data not shown). However, the experiments also show that the test strips do have an unknown limited lifespan. Further testing is required to determine the actual effective lifespan of the test strips.

The stability of semen extracts in RSID™-Semen running buffer for extended periods of time was tested by delaying the analysis of positive control semen swabs already prepared in RSID™-Semen running buffer. Using 5 μL of standard positive control extract, samples were prepared in RSID™-Semen running buffer and incubated at room temperature for 4- and 16.5-h (overnight) intervals before application to a strip test. Comparisons were made against 5-μL extract aliquots of positive and negative control samples that were run immediately after the addition of running buffer. No difference in the signal intensity at the test line was observed for any of the incubation times tested, indicating that prepared sample extracts are stable in running buffer overnight (data not shown).

Detection of Semen from Casework/Mock Casework Samples

We have established that RSID™-Semen test strips can detect semen from laboratory-prepared control samples and from sexual assault-like evidence. Here, we demonstrate the ability of RSID™-Semen test strips to detect semen from samples likely to be encountered in forensic laboratory casework, including postcoital vaginal swabs, stained items of clothing, collections from latex condoms, and samples mixed with commercially available contraceptive fluids or sexual lubricants. In addition, we show that RSID™-Semen testing can be integrated into DNA Y-STR analysis laboratory protocols such that semen detection can be performed prior to DNA Y-STR analysis.

Postcoital vaginal swabs from individuals who did or did not use a condom were prepared as previously described. Postcoital vaginal swabs in which the individual used a condom presented no signal intensity at the test line of swabs collected on day 0 or day 14 using the RSID™-Semen test strips. Dilution and reanalysis of these extracts to test for high-dose hook effect supported the conclusion that no semen was detected. It is difficult to give a definitive period of time during which spermatozoa or semen-specific markers remain within the vaginal vault after intercourse (10,18,45,52), largely because of variations in both the physiology and behavior of different individuals (18,53). However, studies have shown that the detection of spermatozoa on postcoital swabs is generally more likely than the detection of other semen markers over longer periods of time (10,18,52). Still, semen-specific markers have been detected on postcoital sample swabs anywhere from several hours to several days after intercourse and have also been detected when the presence of spermatozoa was not observed (10,18,45,52,54). Our results of the detection of semenogelin on postcoital vaginal swabs are consistent with previously reported values (10,18,45,52,54). With the exception of one sample collected on day 2 in which no signal was observed, semen was consistently detected in our experiments on postcoital vaginal swabs up to 2 days after unprotected vaginal intercourse using the RSID™-Semen test strips. Semen was not detected from unprotected postcoital swabs collected at days 3–10, 14, 17, and 19. Our expectation was that the highest observed band intensity would have been observed from sample extracts that were produced from vaginal swabs collected on the day of intercourse (day 0), because of the likelihood of semen being present at the highest levels during this time. However, band intensities of increasingly positive signal intensity were observed from days 0 to 2 in some of the collection series leading us to believe that some high-dose hook effects were observed. Dilution and reanalysis of unprotected postcoital vaginal swabs with the RSID™-Semen test strips, as described earlier, confirmed that high-dose hook effects were responsible for the reduced signal intensity of some of the postcoital samples tested from a few of the unprotected postcoital vaginal swabs collected at day 0 and day 1. Stronger intensity signals were observed at the test line upon a 1:20 dilution of these samples (data not shown). DNA extraction, multiplex PCR, and Y-STR analysis of the remaining RSID™-Semen sample extracts yielded partial Y-STR profiles (2–16 loci out of 17 loci) for only the sample extracts that tested positive for the presence of semen with the RSID™-Semen test strips. The presence of menstrual blood or vaginal secretions in the sample swab had no interfering effect when the sample was tested using RSID™-Semen or during Y-STR analysis. The number of loci detected varied from sample to sample, and it was observed that fewer loci were obtained from samples with longer time intervals between the time of unprotected vaginal intercourse and sample swab collection. This was consistent with current literature regarding the collection of DNA Y-STR profiles from postcoital swabs, in which at least a partial Y-STR profile was obtained up to 48 h after intercourse without any modified DNA analysis performance parameters (53,55). These results demonstrate the ability to obtain body fluid testing data and Y-STR results without the need for additional evidence sampling. No effort was taken to perform additional concentration steps on the extracted DNA, although it may be possible to improve DNA typing results by using a more focused low copy number analysis protocol. While partial Y-STR profiles were generated from the RSID™-Semen sample extracts that tested positive for semen, there was no observed correlation between the band intensity of the RSID™-Semen test line and the quantity of DNA obtained from the sample extract. However, partial Y-STR profiles were only obtained from samples that gave a positive result using RSID™-Semen. To compare the sensitivity of RSID™-Semen with other common methods of semen detection, the Seratec® PSA lateral flow strip test used to detect the presence of PSA and a presumptive test measuring AP activity were used to analyze the postcoital samples that were previously analyzed with RSID™-Semen. Seratec® PSA test strips were used to analyze the postcoital vaginal swabs from the above-mentioned sample set in accordance with the manufacturer's protocol. To measure AP activity, the same volume of vaginal swab extract tested with RSID™-Semen was deposited on cotton swabs and allowed to air-dry before testing for dye-enhanced AP activity (as described previously). A positive signal was obtained only from postcoital samples collected on day 0 and day 1 after unprotected intercourse using the Seratec® PSA and AP tests. These data

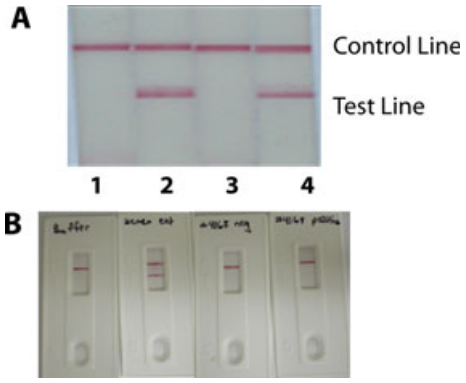

FIG. 5—*Analysis of casework samples using RSID™-Semen. (A) A pair of woman's black undergarments with a visible stain was swabbed at both an unstained portion and a stained portion of the undergarment and analyzed with RSID™-Semen using 10 μL of the sample extracts (lanes 3 and 4, respectively). Lanes 1 and 2 show negative and positive control swabs extracted in 300 μL of RSID™-Semen extraction buffer, 1 μL of extract analyzed. (B) These same samples were analyzed on Seratec® PSA strip tests using the same volumes of sample extract. Unstained and stained portions of the undergarment (strips 3 and 4, respectively) both produced negative results for the presence of semen while controls, both negative and positive, reacted as expected (strips 1 and 2, respectively).*

indicate that RSID™-Semen is more sensitive for detecting semen from postcoital vaginal swabs than the Seratec® PSA or AP activity test used here.

Often in cases of sexual assault, forensic laboratories process items of clothing collected at the scene or from the victim to look for evidence of seminal fluid deposit. Therefore, samples of this type were analyzed using RSID™-Semen. A pair of women's black undergarments with a visible unknown stain was processed as previously described. Standard positive and negative control extracts were produced using 1 μL of ddH_2O extract or human semen, respectively (Fig. 5*A*, strips 1 and 2 respectively). The band signal of the unknown sample swab collected from the visible stain on the garment was of similar intensity to the positive control when using 10 μL of the extract (Fig. 5*A*, strip 4). The negative control sample swab of the undergarment in an area without a visible stain showed no positive signal at the test line of an RSID™-Semen test strip (Fig. 5*A*, strip 3). The same undergarment was analyzed with a Seratec® PSA test strip and assayed for AP activity with a PSA-based semen detection test strip and the presumptive dye-enhanced AP test. To test the stain with the Seratec® PSA test, two additional cuttings (stained and unstained portions) were taken from the undergarment and analyzed in accordance with the manufacturer's suggested protocol (described previously). To test for AP activity, ddH_2O moistened swabs were used to sponge the visible unknown stain as well as an unstained region to act as a negative control. The dye-enhanced AP assay was performed on the swab heads as described in the Materials and Methods. Standard human semen positive and negative control swabs (described earlier) were included for comparison (Fig. 5*B*, strips 3 and 1, respectively). The PSA-based test strip failed to detect semen on the cutting from the stained and unstained portion of the undergarment (Fig. 5*B*, strips 4 and 2, respectively). The Seratec® PSA card produced a negative result from 10 μL of the unknown sample swab of the visible stain after 10 min. Furthermore, the presumptive AP test on the stain was recorded as "weakly positive" (data not shown). Because of the fact that low levels of AP are found in body fluids other than semen (5,19), using the results from both the PSA and AP tests would lead one to a conclusion that is inconsistent with the presence of semen. To determine whether or not positive RSID™-Semen results correlated with the presence of male DNA in the sample, DNA was extracted from a cutting of the stain and processed for Y-STR analysis. No efforts to optimize extraction or vary the cutting size were made. A partial DNA profile (16 out of 17 loci) was obtained from the sample, indicating that the positive RSID™-Semen result was because of a male contribution to the sample. These data demonstrate that RSID™-Semen is more sensitive on certain forensic evidence than PSA-based test strips or AP activity tests.

Item sampling of an unknown stain in forensic laboratories can generally involve two different approaches: removal of a small portion of the stained area from the item or transfer of the stain to a moistened swab. The sampled material is then extracted and processed for analysis. A clear difference was observed from the analysis of several types of fabrics, namely, cotton chambray, cotton twill, blue cotton denim, nylon lace, nylon-knit jersey, and cotton sheeting (both flannel and conventional weave) that were stained with human semen and sampled using either a 5-mm-diameter fabric punch or ddH_2O-moistened sample swab. Strong intensity bands at the test line were observed for each fabric type after 10 min when extracted from a fabric punch (Fig. 6, strips 4, 6, 8, 10, 12, 14, and 16). In contrast, signal intensities at the test line using extracts from the swab sampling method showed a dependence on the type of fabric from which the sample was taken (Fig. 6, strips 3, 5, 7, 9, 11, 13, and 15). Transfer of the stain using a ddH_2O-moistened swab was not as efficient when sampling from the cotton fabrics as opposed to when sampling from the nylon fabrics. Observed band signal intensities of the swab extracts from the nylon lace and nylon-knit jersey were strong for both fabrics after 10 min (Fig. 6, strips 11 and 13, respectively). However, the band signal intensities from swab extracts sampled from cotton fabrics were much lower (Fig. 6, strips 3, 5, 7, 9, and 15). Reanalysis of the sample extracts after a 1:20 dilution did not reveal any high-dose hook effects (data not shown). The extracts from both sampling methods were subsequently processed for Y-STR analysis (see Materials and Methods for details) to determine if a correlation between RSID™-Semen signal intensity and DNA content could

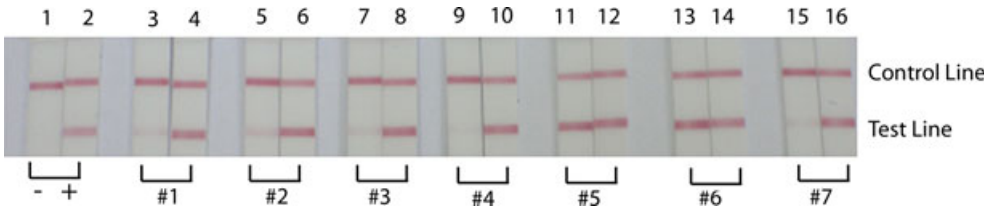

FIG. 6—*Evaluation of fabric substrate on RSID™-Semen. Human semen deposited on cotton chambray (strip pairing no. 1, lanes 3 and 4), flannel cotton sheet (strip pairing no. 2, lanes 5 and 6), cotton twill (strip pairing no. 3, lanes 7 and 8), cotton denim (strip pairing no. 4, lanes 9 and 10), nylon lace (strip pairing no. 5, lanes 11 and 12), nylon-knit jersey (strip pairing no. 6, lanes 13 and 14), and a cotton sheet (strip pairing no. 7, lanes 15 and 16) were sampled by swabbing the stain using a moistened cotton swab (odd lanes) or taking a fabric cutting (even lanes). Control samples (strip pairing no. 1, (−) and (+), negative and positive control swabs, respectively) were extracted from the cotton swab as described in the Materials and Methods.*

be observed. Partial DNA profiles ranging from 10 to 14 loci were obtained when analyzing the 5-mm fabric punch extracts. In contrast, partial profiles of 4–6 loci were obtained from the sample swab extracts (data not shown). While our results showed that more loci were obtained from sample extracts that gave stronger RSID™-Semen results because the efficiency of the sampling procedure could not be taken into account, no correlation between the concentration of DNA obtained and the RSID™-Semen test results could be made. However, partial DNA profiles were obtained from sample extracts that gave positive RSID™-Semen results.

Because of the possibility of encountering contraceptive solutions or sexual lubricants in the presence of semen in SAE, a commercially available sexual lubricant (K-Y® Brand Jelly) and two vaginal contraceptives (VCF® Vaginal Contraceptive Foam, 12.5% nonoxynol-9; Ortho Options® Conceptrol® Vaginal Contraceptive Gel, 4.0% nonoxynol-9) were tested with human semen for possible interference with the RSID™-Semen test strip. Human semen was mixed individually with equal volumes of the respective solutions and processed using RSID™-Semen test strips for the presence of semen (described previously). Visible bands were observed at the test line from each of the sample mixtures at signals of similar intensity to the positive control sample, indicating the presence of semen. No reduction in the signal intensity was observed. Additionally, increases in the background or interference of the test strip did not occur as a result of the presence of sexual lubricant or contraceptive solution. Reanalysis of the sample mixtures using less extract (1:20 dilution) did not reveal any high-dose hook effects. Similar positive band intensities were obtained from using either 20 or 1 μL of the sample extract (data not shown).

In addition to sexual lubricants and contraceptive solutions, several used condoms were tested for the presence of seminal fluid using RSID™-Semen to determine if commercially available condoms would interfere with the test strips. To take into account the selection of condoms available, three varieties of Trojan® Brand condoms (no lubricant present; nonspermicidal lubricant present; spermicidal lubricant present, 7% nonoxynol-9) were tested using RSID™-Semen (previously described). Weak positive signal intensities were observed from 20-μL aliquots of sample extracts produced from each type of the condoms tested and the positive control sample that was extracted in 300 μL of extraction buffer. When retested for signs of high-dose hook effect using only 1 μL of extract (1:20 dilution), stronger signal intensities were recorded for each of the condom types tested and the representative positive control sample. Interference of the test or control bands of the RSID™-Semen test strip or increases in the sample background were not observed for any of the condom types used (data not shown).

This group of experiments demonstrates that RSID™-Semen is a reliable test for semen detection from a wide range of potential forensic evidence. RSID™-Semen can detect as little as 2.5 nL of human semen and does not cross-react with the other human body fluids or animal semen tested. RSID™-Semen is more sensitive with certain forensic evidence samples containing mixtures of vaginal secretions and semen than either of the commercially available PSA-based forensic semen detection tests or tests that measure AP activity. Furthermore, RSID™-Semen can detect human semen from a wide variety of forensic evidence samples without the cross-reactivity concerns associated with PSA and AP activity-based semen detection tests. Additionally, partial Y-STR profiles were obtained from extracts that tested positive using RSID™-Semen, demonstrating the ease of integrating this lateral flow strip test into forensic DNA laboratory procedures and work flow. The correlation of positive RSID™-Semen results with Y-STR analysis from these mock forensic case samples will enable analysts to efficiently triage crime scene evidence and to choose the best sample(s) to process for Y-STR analysis. In conclusion, we suggest that RSID™-Semen is effective and useful for semen detection, will reduce cost and labor for forensic labs, and will become an essential tool to aid forensic scientists in crime scene investigations.

Acknowledgments

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Additional information and reprint requests:
Kevin W. P. Miller, Ph.D.
Kern Regional Crime Laboratory
1300 18th Street, 4th Floor
Bakersfield, CA 93001
E-mail: kmiller@co.kern.ca.us