

Article

Evaluation of Rapid Stain IDentification (RSID™) Reader System for Analysis and Documentation of RSID™ Tests

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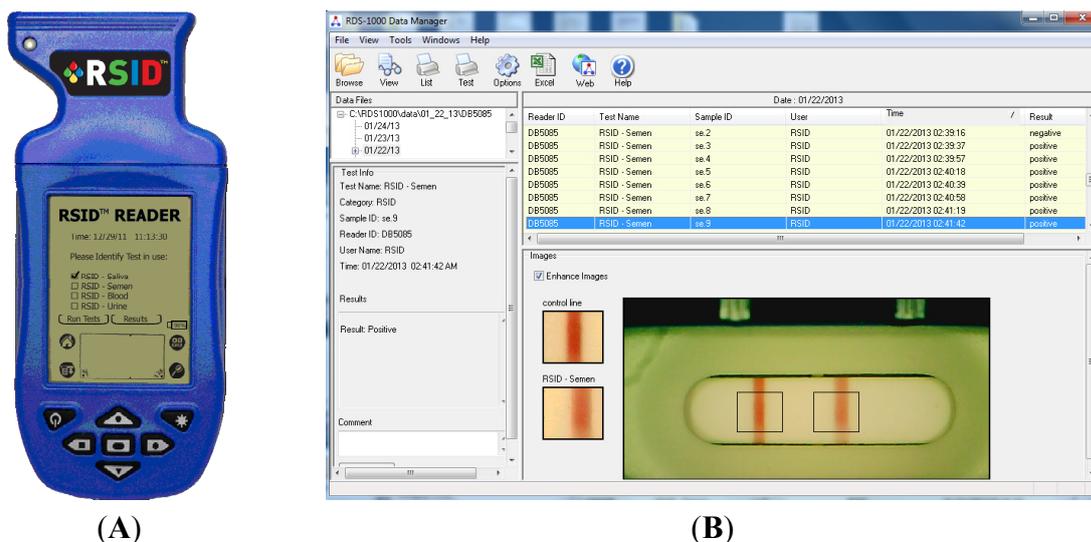
Abstract: The ability to detect the presence of body fluids is a crucial first step in documenting and processing forensic evidence. The Rapid Stain IDentification (RSID™) tests for blood, saliva, semen and urine are lateral flow immunochromatographic strip tests specifically designed for forensic use. Like most lateral flow strips, the membrane components of the test are enclosed in a molded plastic cassette with a sample well and an observation window. No specialized equipment is required to use these tests or to score the results seen in the observation window; however, the utility of these tests can be enhanced if an electronic record of the test results can be obtained, preferably by a small hand-held device that could be used in the field under low light conditions. Such a device should also be able to “read” the lateral flow strips and accurately record the results of the test as either positive, *i.e.*, the body fluid was detected, or negative, *i.e.*, the body fluid was not detected. Here we describe the RSID™ Reader System—a ruggedized strip test reader unit that allows analysis and documentation of RSID™ lateral flow strip tests using pre-configured settings, and show that the RSID™ Reader can accurately and reproducibly report and record correct results from RSID™ blood, saliva, semen, and urine tests.

Keywords: Rapid Stain IDentification (RSID™) Reader System; RSID™ Blood Test; RSID™ Saliva Test; RSID™ Semen Test; RSID™ Urine Test; accuracy; reproducibility

1. Introduction

The Rapid Stain Identification (RSID™) Blood, Saliva, Semen and Urine Kits (Independent Forensics, Lombard, IL, USA) are lateral flow immunochromatographic strip tests designed for identifying one of four body fluids from biological residue on forensic evidence recovered from crime scenes [1–5]. Lateral flow tests, like the RSID™ series are widely used for screening forensic evidence. Like the majority of lateral flow strip tests, RSID™ tests are qualitative and results are recorded as either positive or negative based on the presence or absence of a visible single red or blue line at the “Test” position on the strip, 10, or 15 min following addition of the sample to the sample well (saliva, semen, blood and urine, respectively). The results are determined by visual inspection of the strip test and no image analysis or optical reader is required for scoring the test. For the majority of samples assayed by lateral flow methods, there is little or no ambiguity regarding the results of the analysis; either the Test band is visible or it is not. However, under field conditions, when testing samples at or near the limit of detection for the device, or in the hands of less experienced personnel, a handheld Reader capable of recording and scoring the tests can be a useful tool. The steadily increasing requirements for documenting all steps in forensic testing is another advantage to the Reader as the device records a time and date stamp and can download a recorded image of the test strip. The Reader is not required for correct or proper interpretation of strip test results, but may be useful in resolving potentially ambiguous (*i.e.*, very faint bands) results.

Figure 1. The Rapid Stain Identification (RSID™) Reader. (A) Ruggedized hand-held RSID™ Reader unit with touch screen. Cassettes are inserted into the slot on the upper right hand side, observation window facing down, cassette name first; (B) Screen capture image of the downloaded electronic data from Reader; sample name, time, date and results of individual tests are recorded by the software and shown in a spreadsheet format.



The Rapid Stain Identification (RSID™) Reader System (Independent Forensics, Lombard, IL, USA) is a ruggedized, hand-held strip test reader unit that allows analysis and documentation of RSID™ lateral flow strip tests using pre-configured settings. The unit features a touch screen based on Palm Pilot technology and an imaging system for analyzing RSID™ cassettes (Figure 1A).

The test data, including reader ID, test name, sample ID, date and time of the test, results of the analysis and digital images of control and test lines of RSID™ strips are automatically recorded by, and stored in, the RSID™ Reader memory; these data can be downloaded through a USB cable to a computer and are then displayed in the format shown (Figure 1B).

In this study we tested the accuracy and reproducibility of the RSID™ Reader using image detection parameters and thresholds that were set during developmental validation of this device.

2. Experimental Section

2.1. Samples

Blood, saliva, semen and urine samples were prepared by applying 50 µL of whole blood, saliva, semen, or 100 µL of urine, to a sterile cotton swab and allowing the swab to air-dry. The cotton batting was removed using laboratory clean technique, placed in a 1.5 mL microcentrifuge tube and extracted in 1 mL of RSID™ extraction buffer. Samples were incubated in extraction buffer for 30 min at room temperature. Assuming 100% extraction efficiency, each microliter of extract therefore contained 50 nL of blood, saliva, semen, or 100 nL of urine, respectively.

Extraction negative controls were produced by extracting a clean, unused, sterile swab directly alongside the body fluid swab samples.

2.2. Principles of RSID™ Tests

RSID™ Blood, Saliva and Semen strip tests (Independent Forensics, Lombard, IL, USA) are immunochromatographic assays that each use two mouse monoclonal antibodies specific for human glycophorin A (RSID™ Blood), α -amylase (RSID™ Saliva), or semenogelin (RSID™ Semen). For each test, one of the antigen-specific antibodies is conjugated to colloidal gold particles and is deposited on a conjugate pad located beneath the sample window in the completed strip test. The other antibody is striped onto the “Test line” of a membrane attached to the conjugate pad. The “Control line” on the membrane consists of anti-mouse IgG antibody and is used as an internal positive control. RSID™ Urine test (Independent Forensics, Lombard, IL, USA) is an immunochromatographic assay that uses two rabbit polyclonal antibodies specific for Tamm-Horsfall glycoprotein. One of these antibodies is conjugated to blue latex beads and is deposited on a conjugate pad beneath the sample window. Polyclonal anti-Tamm Horsfall antibodies are striped onto the “Test line” on a membrane visible through the test window, and attached to the conjugate pad. The “Control line” on the membrane consists of anti-rabbit IgG antibody and is used as an internal positive control.

2.3. RSID™ Reader

The Rapid Stain IDentification (RSID™) Reader System (Independent Forensics, Lombard, IL, USA) is a ruggedized, hand-held strip test reader instrument (Figure 1A). The unit features a touch screen based on Palm Pilot technology and charge-coupled device (CCD) imaging system for analyzing RSID™ cassettes. When determining test results, the reader performs optical interrogation of a cartridge, and the raw image data is collected, digitized, and individual regions of interest are analyzed for pixel density, *i.e.*, the Test Line and Control Line regions. The pixel density value of

these regions are then compared to pre-defined calibration data, thus yielding “positive”, or “negative” qualitative results. Calibration data were derived from more than 800 samples of RSID™ Blood, Saliva, Semen and Urine tests from a collection of retained lots of RSID™ tests.

2.4. Assessment of Inter- and Intra-Run Variability

Two RSID readers were tested in this study. In addition, two replicates for each tested extract volume were analyzed by both readers. Test strips near the experimental limit of detection of the corresponding body fluids were analyzed in triplicate by both readers.

2.5. RSID™ Blood and RSID™ Reader Experiments

Two, four and ten microlitres of blood extract, prepared as above, were adjusted to a total volume of two hundred microlitres with RSID™ running buffer (*i.e.*, a dilution of 1:100, 1:50 and 1:20 from the original extraction). From each diluted sample a 100 µL aliquot was applied to the sample window of an RSID™ Blood cassette. The test line signals were evaluated after 10 min. Cross-reactivity controls for RSID™ Blood consisted of a 60 µL mixture of saliva, semen and urine extracts (20 µL each) brought to 100 µL with RSID™ running buffer.

2.6. RSID™ Saliva and RSID™ Reader Experiments

0.4, 1, 2, and 10 µL of saliva extract were adjusted to a total volume of 200 µL with RSID™ running buffer (*i.e.*, a dilution of 1:500, 1:200, 1:100, and 1:20 from the original extraction). From each diluted sample a 100 µL aliquot was applied to the sample window of an RSID™ Saliva cassette. The test line signals were evaluated after 10 min. Cross-reactivity controls for RSID™ Saliva consisted of a 60 µL mixture of blood, semen and urine extracts (20 µL each) brought to 100 µL with RSID™ running buffer.

2.7. RSID™ Semen and RSID™ Reader Experiments

Using a standard semen extract, a first set of dilutions (1:20, 1:10, and 1:5) was made with RSID™ extraction buffer. These diluted extracts were then again diluted 1:100 into RSID™ running buffer. From each sample an aliquot of 100 µL was then applied to the sample window of RSID™ cassette. The test line signals were evaluated after 10 min. Cross-reactivity control for RSID™ Semen consisted of 60 µL mixture of blood, saliva and urine extracts (20 µL each), and 40 µL of RSID™ running buffer.

2.8. RSID™ Urine and RSID™ Reader Experiments

In order to generate 1 and 5 µL test extracts, 20 µL and 100 µL of standard urine extract was adjusted to a final volume of 200 µL with RSID™ Urine buffer. From each sample an aliquot of 100 µL was then applied to the sample window of RSID™ cassette. For the 10 µL test extract, 100 µL of urine extract was used directly. The test line signals were evaluated after 15 min. Cross-reactivity control for RSID™ Urine consisted of 60 µL mixture of blood, saliva and semen extracts (20 µL each), and 40 µL of RSID™ Urine buffer.

2.9. RSID™ Field Kits and RSID™ Reader Experiments

RSID™ Field Kits for Blood, Saliva and Semen are supplied with RSID™ Universal Buffer for both extraction and diluent/running buffer. These kits come with a calibrated transfer pipette and sample tubes pre-filled with Universal Buffer. For tests of RSID™ Field kits with the RSID™ Reader, the cotton batting of a collection swab, containing 50 µL of whole blood, saliva, or semen was added to the pre-loaded tube which contained 750 µL of RSID™ Universal Buffer. Using the supplied transfer pipette, four drops of extract were placed into the sample window of an RSID™ cassette. The test line signals were evaluated after 10 min. The Cross-Reactivity Controls were performed as described for RSID™ Reader Blood, Saliva and Semen Experiments, respectively.

RSID™ Field Kit for Urine is supplied with sample tubes pre-filled with 1 mL of RSID™ Urine buffer for both extraction and running. Transfer pipettes, supplied with the kit were used to place four drops of extract into the sample window of an RSID™ cassette. The test line signals were evaluated after 15 min. The Cross-Reactivity Controls were performed as described for RSID™ Reader Urine Experiment.

3. Results and Discussion

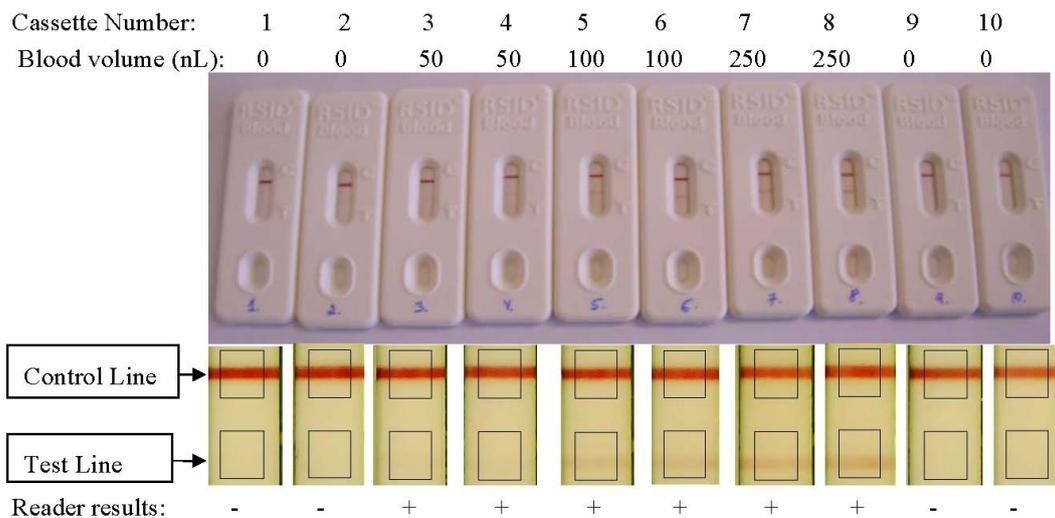
3.1. RSID™ Blood and RSID™ Reader Experiments

Assuming 100% extraction efficiency, 50 µL of whole blood deposited on a sterile cotton swab and extracted in 1 mL of RSID™ extraction buffer will produce an extract that contains 50 nL of blood per microliter of extract. When 2, 4 and 10 µL of blood extract is adjusted to a total volume of 200 µL with RSID™ running buffer, 100 µL of this extract will contain 50, 100 and 250 nL of blood, respectively. Two different Readers were evaluated in this study, each with two sets of RSID™ cassette replicates. All results were concordant.

Figure 2 shows results of RSID™ Blood as they appear in a digital photograph (Nikon's Coolpix 5600) and in the digital images generated by the RSID™ Reader. Negative controls (Figure 2, cassettes 1 and 2) and saliva-semen-urine cross-reactivity controls (Figure 2, cassettes 9 and 10) were read as "negative" by both RSID™ Readers tested. Samples with calculated amounts (50, 100 and 250 nL) of blood were read as "positive" by both RSID™ Readers tested (Figure 2, cassettes 3–8).

Cassettes 3 and 4, on which 50 nL of blood were tested had very light positive signals, which was expected since 50 nL of blood is at the detection limit of RSID™ Blood Test [1]. To measure the reproducibility of the RSID™ Reader, cassette 3 was analyzed three times in a row on both RSID™ Readers. Both RSID™ Readers detected signal and registered positive for blood in all three replicates.

Figure 2. Results of RSID™ Blood and RSID™ Reader Experiments. Results of two replicates for each analyzed blood volume from one Reader are shown. Cassette number and calculated volume of blood added to each cassette are shown (top). Digital camera capture of cassettes and RSID™ Reader images are shown below, respectively. **Top**—Digital photograph of the cassettes (C—Control; T—Test lines) with blood volumes analyzed indicated above the images of the corresponding RSID™ cassettes. **Bottom**—Images taken from RSID™ Reader. RSID™ Reader results (positive [+] or negative [-]) are indicated under each RSID™ Reader image. Note: Camera capture or RSID™ Reader images are slightly *less* sensitive than visual observation of the cassettes.



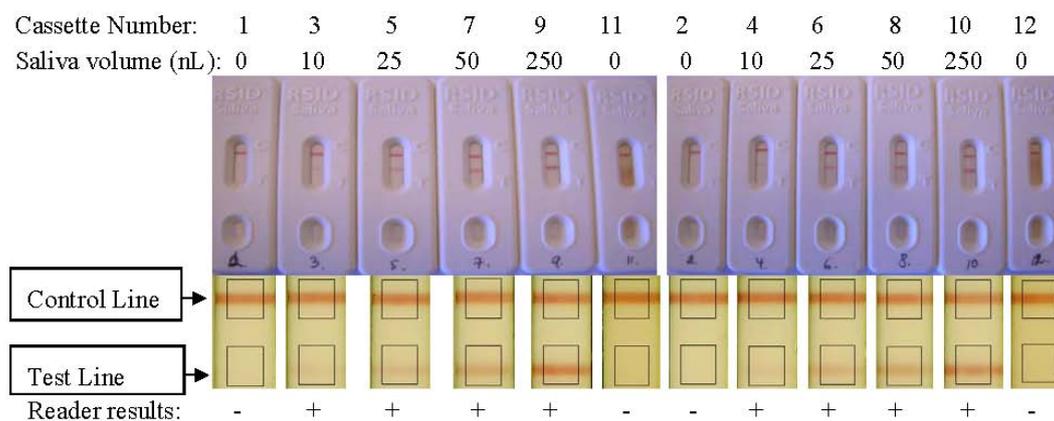
3.2. RSID™ Saliva and RSID™ Reader Experiments

Assuming 100% extraction efficiency, 50 µL of saliva deposited on a sterile cotton swab and extracted in 1 mL of the RSID™ extraction buffer will produce an extract that contains 50 nL of saliva per microliter of extract. When 0.4, 1, 2, and 10 µL of saliva extracts are adjusted to a total volume of 200 µL with RSID™ running buffer, 100 µL of these extracts will contain 10, 25, 50, and 250 nL of saliva, respectively. Two different Readers were evaluated in this study, each with two sets of RSID™ cassette replicates. All results were concordant.

Figure 3 shows results of RSID™ Saliva as they appear in a digital photograph (Nikon’s Coolpix 5600) and in the digital images generated by the RSID™ Reader. Extraction negative controls (Figure 3, cassettes 1 and 2) and blood-semen-urine cross-reactivity controls (Figure 3, cassettes 11 and 12) were scored as “negative” by both RSID™ Readers tested. Samples with calculated amounts (10, 25, 50, and 250 nL) of saliva were scored as “positive” by both RSID™ Readers tested (Figure 3, cassettes 3–10).

Cassettes 3 and 4, on which 10 nL of saliva were tested had very light positive signals, which was expected as 10 nL of saliva is at the detection limit of RSID™ Saliva test [2]. To measure reproducibility of the RSID™ Reader, cassette 3 was analyzed three times on both RSID™ Readers. Both RSID™ Readers detected signal and registered positive in all three replicates.

Figure 3. Results of RSID™ Saliva and RSID™ Reader Experiments. Results of two replicates for each analyzed saliva volume from one Reader are shown. Cassette number and calculated volume of saliva added to each cassette are shown (**top**). Digital camera capture of cassettes and RSID™ Reader images are shown below, respectively. **Top**—Digital photograph of the cassettes (C—Control; T—Test lines) with analyzed saliva volumes indicated above. **Bottom**—Images taken from RSID™ Reader. RSID™ Reader results (positive [+] or negative [-]) are indicated under each RSID™ Reader image. Note: Camera capture or RSID™ Reader images are slightly *less* sensitive than visual observation of the cassettes.



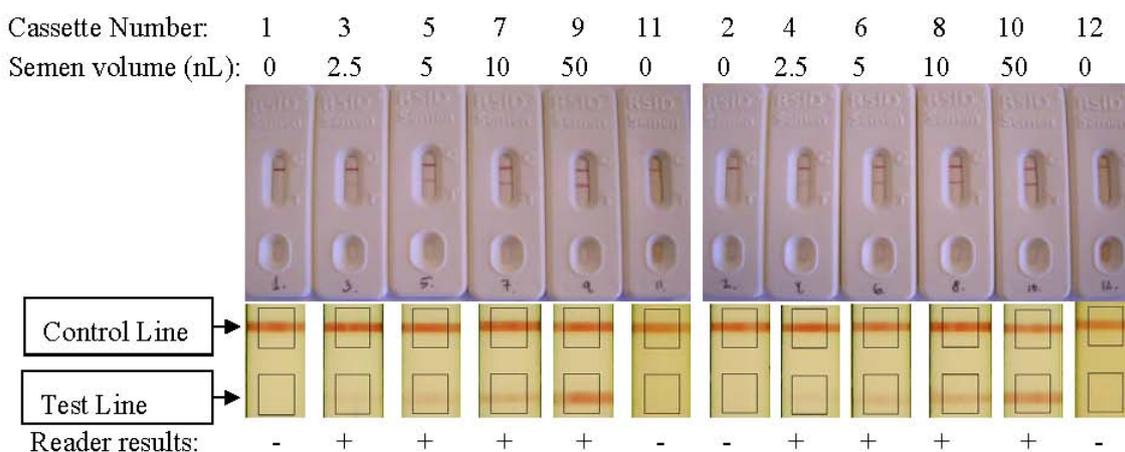
3.3. RSID™ Semen and RSID™ Reader Experiments

Assuming 100% extraction efficiency, 50 μ L of semen deposited on a sterile cotton swab extracted in 1 mL of RSID™ extraction buffer will produce an extract that contains 50 nL of semen per microliter of extract. Using RSID™ extraction buffer, three dilutions of the extract were made: 1:20, 1:10, and 1:5. In order to generate 2.5, 5, and 10 nL test volumes of semen, 2 μ L of each of the semen dilutions (1:20, 1:10, and 1:5) were adjusted to a total volume of 200 μ L with RSID™ running buffer. In order to generate 50 nL test volumes of semen, 2 μ L of undiluted semen extract were adjusted to a total volume of 200 μ L with RSID™ running buffer. Final tested (calculated) volumes of semen were 2.5, 5, 10 and 50 nL. Two different Readers were evaluated in this study, each with two sets of RSID™ cassette replicates. All results were concordant.

Figure 4 shows results of RSID™ Semen as they appear in a digital photograph (Nikon’s Coolpix 5600) and in the digital images generated by the RSID™ Reader. Extraction negative controls (Figure 4, cassettes 1 and 2) and blood-saliva-urine cross-reactivity controls (Figure 4, cassettes 11 and 12) were read as “negative” by both RSID™ Readers tested. Samples with calculated amounts of 2.5, 5, 10 and 50 nL of semen were read as “positive” by both RSID™ Readers tested (Figure 4, cassettes 3–10).

Cassettes 3 and 4, on which an estimated 2.5 nL of semen were tested had very light positive signal, which was expected since 2.5 nL of semen is at the detection limit of RSID™ Semen test [4]. To measure reproducibility of RSID™ Reader, cassette 3 was analyzed three times on both RSID™ Readers. Both RSID™ Readers detected signal in all three replicas.

Figure 4. Results of RSID™ Semen and RSID™ Reader Experiments. Results of two replicates for each analyzed semen volume from one Reader are shown. Cassette number and calculated volume of semen added to each cassette are shown (**top**). Digital camera capture of cassettes (C—Control; T—Test lines) with analyzed semen volumes indicated above. **Top**—Digital photograph of the cassettes (C—Control; T—Test lines) with analyzed semen volumes indicated above. **Bottom**—Images taken from RSID™ Reader. RSID™ Reader results (positive [+] or negative [-]) are indicated under each RSID™ Reader image. Note: Camera capture or RSID™ Reader images are slightly less sensitive than visual observation of the cassettes.



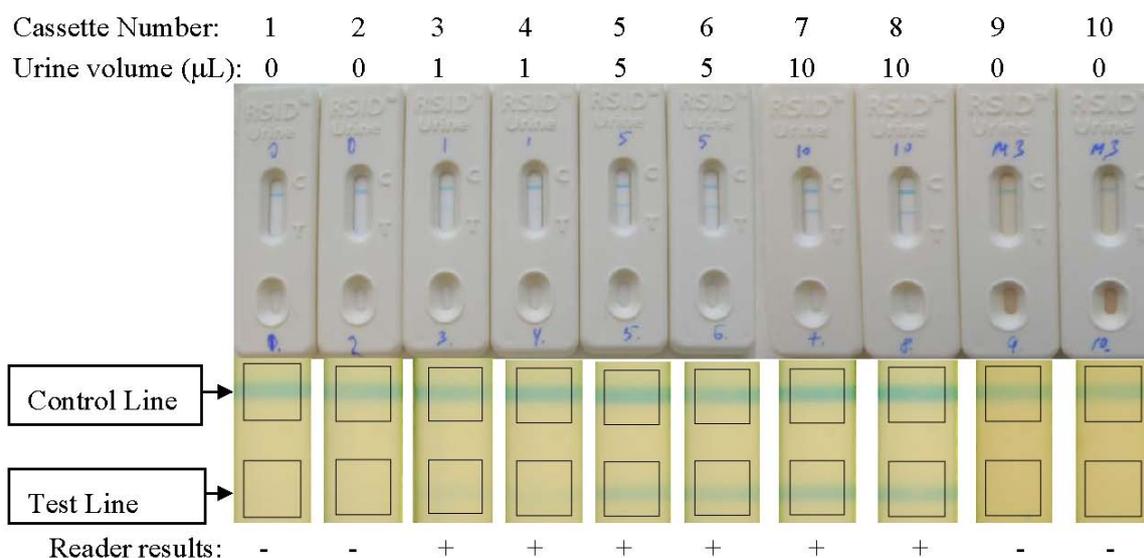
3.4. RSID™ Urine and RSID™ Reader Experiments

Assuming 100% extraction efficiency, 100 µL of urine on a sterile cotton swab extracted in 1 mL of the RSID™ Urine buffer will produce an extract that contains 100 nL of urine per microliter of extract. In order to generate 1, 5 and 10 µL test volumes of urine, 20, and 100 µL of urine extract were adjusted to a total volume of 200 µL with RSID™ Urine buffer (for the 10 µL urine sample, 200 µL of extract was used directly). Two different Readers were evaluated in this study, each with two sets of RSID™ cassette replicates. All results were concordant.

Figure 5 shows results of RSID™ Urine as they appear in a digital photograph (Nikon’s Coolpix 5600) and in the digital images generated by the RSID™ Reader. Extraction negative controls (Figure 5, cassettes 1 and 2) and blood-saliva-semen cross-reactivity controls (Figure 5, cassettes 9 and 10) were scored as “negative” by both RSID™ Readers tested. Samples with calculated 1, 5 and 10 µL of urine were read as “positive” by both RSID™ Readers tested (Figure 5, cassettes 3–8).

Cassettes 3 and 4, on which a calculated 1 µL of urine were tested had very light positive signal, which was expected since 1 µL of urine is at the detection limit of RSID™ Urine test [5]. To measure reproducibility of RSID™ Reader, cassette 3 was analyzed three times on both RSID™ Readers. Both RSID™ Readers detected signal in all three replicates.

Figure 5. Results of RSID™ Urine and RSID™ Reader Experiments. Results of two replicates for each analyzed urine volume from one Reader are shown. Cassette number and calculated volume of urine added to each cassette are shown (**top**). Digital camera capture of cassettes and RSID™ Reader images are shown below, respectively. **Top**—Digital photograph of the cassettes (C—Control; T—Test lines) with analyzed urine volumes indicated above. **Bottom**—Images taken from RSID™ Reader. RSID™ Reader results (positive [+] or negative [-]) are indicated under each RSID™ Reader image. Note: Camera capture or RSID™ Reader images are slightly *less* sensitive than visual observation of the cassettes.

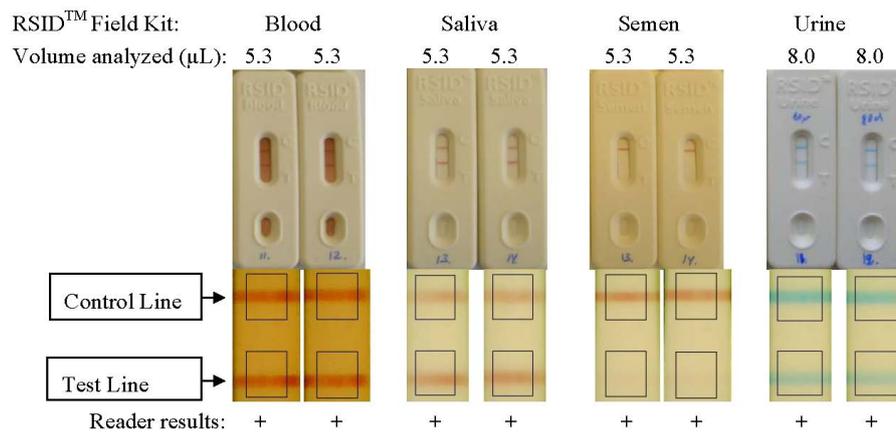


3.5. RSID™ Field Kits and RSID™ Reader Experiments

In these experiments the RSID™ Field kit protocol was followed. Briefly, an extract was prepared from the RSID™ Field kit O-ring tubes and four drops of this extract, using the supplied disposable transfer pipette, was applied to the sample well of RSID™ cassettes; in other words the extracts were used without further dilution. Using our standard body fluid swab (*i.e.*, 50 µL of the cognate body fluid) and assuming 100% extraction efficiency, the field kit protocol would measure ~5.3 µL of blood, saliva, or semen, respectively. The RSID™ Field Kit for Urine uses 1 mL of RSID™ Urine buffer for extraction and therefore using our standard urine body fluid swab (100 µL) this test would then measure ~8.0 µL of urine. Two different Readers were evaluated in this study, each with two sets of RSID™ cassette replicates. All results were concordant.

Extraction Blank and Cross-Reactivity controls were read as “negative”, while samples that contained body fluids were read as “positive” by the RSID™ Readers. Figure 6 shows results of the RSID™ Field Kit tests for undiluted extracts of blood, saliva, semen and urine as they appear in a digital image (Nikon’s Coolpix 5600) and from images taken from the RSID™ Reader. A weak Test Line signal for samples with an estimated 5.3 µL of semen is due to the High Dose Hook Effect [4], but was still scored positive by the RSID™ Reader.

Figure 6. Results of RSID™ Reader analysis using Field Kit Protocol. **Top**—Digital image of cassettes (C—Control; T—Test) with analyzed volumes indicated. **Bottom**—Images taken from RSID™ Reader. Results as scored by RSID™ Reader are shown (positive [+]) or negative [-]). The RSID™-Semen test with 5.3 µL of semen demonstrates a high dose hook effect, however a positive result was still scored by the RSID™ Reader. Note: Camera capture or RSID™ Reader images are slightly *less* sensitive than visual observation of the cassettes.



4. Conclusions

This work describes the evaluation of a Rapid Stain Identification Reader System. The Reader System is a ruggedized strip test reader unit that allows analysis and documentation of RSID™ lateral flow strip tests using pre-configured settings. The unit features a touch screen based on Palm Pilot technology and a charge-coupled device (CCD) imaging system for analyzing RSID™ cassettes. The test data, including reader ID, test name, sample ID, date and time of the test, results of the analysis and digital images of control and test lines of RSID™ strips are automatically recorded by and stored in the RSID™ Reader memory; these data can be downloaded through a USB cable to a computer and are then displayed in the format shown (Figure 1B).

In this study we tested the RSID™ Reader with RSID™ Blood, Saliva, Semen and Urine Kits and RSID™ Field Kits for blood, saliva, semen and urine (Independent Forensics, Lombard, IL, USA). Although scoring these tests does not require electronic image analysis or an optical reader, there are administrative and legal pressures to create and maintain an electronic record of the identification of body fluids from forensic evidence. Additionally, an impartial scoring of the tests by a calibrated instrument may be useful in resolving potentially ambiguous results obtained from a sample with very small amount of biological material that will produce very faint bands at the Test line. This latter point is supported by the accurate and reproducible performance of the RSID™ Reader when testing volumes of blood, saliva, semen and urine that are at the respective detection thresholds. The RSID™ Reader accurately and reproducibly detected very weak bands generated by very small volumes of body fluids, —estimated 50 nL of blood (Figure 2, Cassettes 3 and 4), 10 nL of saliva (Figure 3, Cassettes 3 and 4), 2.5 nL of semen (Figure 4, Cassettes 3 and 4), and 1 µL of urine (Figure 5, Cassettes 3 and 4). It must be noted that faint bands produced by a high dose hook effect (arguably at

the other end of the detection threshold) were also scored accurately by the Reader (field kit protocol, 5.3 μ L of semen, Figure. 6).

The accuracy and reproducibility of the Reader system was achieved through extensive calibration testing using six RSID™ Readers and more than 800 samples of RSID™ Blood, Saliva, Semen and Urine tests from our extensive collection of retained lots of RSID™ tests. This background work was performed in our laboratory as part of the developmental validation of the RSID™ Reader System. Using this foundation, we have configured the Reader to be accurate, reproducible and an effective, if not required, tool for the identification of body fluids from forensic evidence.

Lateral flow strip tests are in general qualitative; only yes/no, positive/negative results can be assessed from the running of these tests. There is the formal possibility of using lateral flow tests for quantitative measurements; given the many unavoidable uncertainties involved in forensic crime analysis including trying to estimate the size of the original stain, the efficiency of extraction, the possibility of degradation over time, the effects of long-term storage, the stability of the bio-marker used by the test, converting these tests for quantitative analysis would offer little benefit to criminal investigators.

In the present configuration, the RSID™ Reader System provides only qualitative analysis of RSID™ tests, which are recorded as either positive or negative by the Reader. Further development of the unit's imaging system, as well as rigorous studies on the processing of biological evidence, will be needed in order to allow quantitative analysis of RSID™ tests.

Acknowledgments

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Conflict of Interest

The authors declare no conflict of interest.

References

1. Schweers, B.A.; Old, J.B.; Boonlayangoor, P.W.; Reich, K. Developmental validation of a novel lateral flow strip test for rapid identification of human blood (Rapid Stain Identification of Blood) *Forensic Sci. Int. Genet.* **2008**, *2*, 243–247.
2. Old, J.B.; Schweers, B.A.; Boonlayangoor, P.W.; Reich, K. Developmental validation of RSID-saliva: A lateral flow immunochromatographic strip test for the forensic detection of saliva. *J. Forensic Sci.* **2009**, *54*, 866–873.
3. Casey, D.G.; Price, J. The sensitivity and specificity of the RSID-saliva kit for the detection of human salivary amylase in the Forensic Science Laboratory, Dublin, Ireland. *Forensic Sci. Int.* **2010**, *194*, 67–71.

4. Old, J.B.; Schweers, B.A.; Boonlayangoor, P.W.; Fischer, B.; Miller, K.W.P.; Reich, K. Developmental validation of RSID™-Semen: A lateral flow immunochromatographic strip test for the forensic detection of human semen. *J. Forensic Sci.* **2012**, *57*, 489–499.
5. Developmental validation of RSID™-Urine. Independent Forensics, July 2010. Available online: <http://www.ifi-test.com/pdf/UrineValidation.pdf> (accessed on 14 June 2013).

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