Validation of RSID™ Reader System for Analysis and Documentation of RSID™ Tests

Introduction

The Rapid Stain IDentification (RSID™) Reader System is a ruggedized strip test reader unit that allows analysis and documentation of RSID™ lateral flow strip tests. The unit features an advanced imaging system for capturing and analyzing images from RSID™ cassettes and a touch screen based on Palm Pilot technology (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 in the format shown in Figure 1B, and can be downloaded to the computer through USB cable.

A.

B.

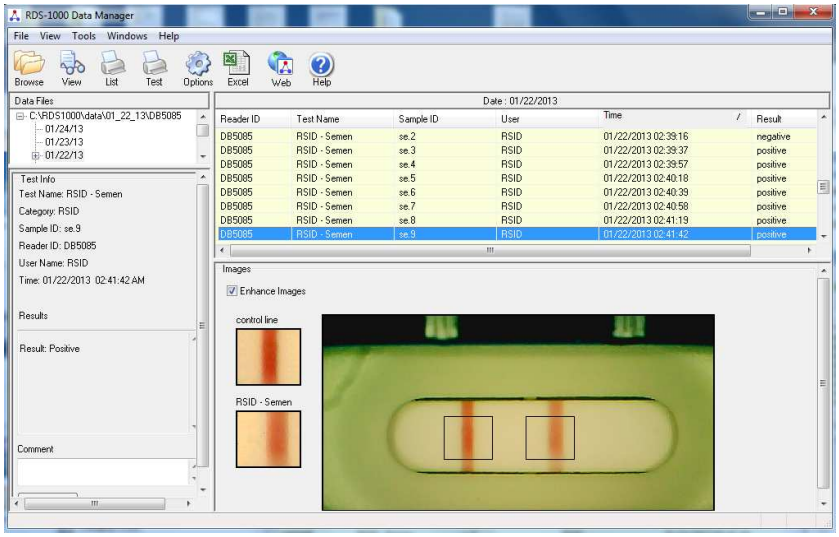

Figure 1. RSID™ Reader
(A) Ruggedized colorimetric unit with touch screen.
(B) Electronic documentation of sample name, time, date and results of individual tests.

In this study we validated the RSID™ Reader System for use with RSID™ Blood, RSID™ Saliva, RSID™ Semen and RSID™ Urine tests in the laboratory and field kit configurations of these tests.

RSID™ Reader Blood Experiments: Results and Conclusions

Sensitivity and specificity experiments for RSID™ Blood tests using RSID™ Reader device are described in Table 1. To prepare blood samples, 50 µL of whole blood was deposited on a sterile cotton swab and allowed 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 the corresponding RSID™ Blood extraction buffer. Assuming 100% extraction efficiency, each microliter of extract contained 50 nL of blood. In order to generate 50, 100 and 250 nL test volumes of blood 2, 4 and 10 µL of blood extract were adjusted to a total volume of 200 µL with RSID™ Blood running buffer and from each sample two aliquots of 100 µL each were applied to the sample window of two RSID™ cassettes (Table 1, cassettes 3, 4, 5, 6, 7, and 8). The test line signals were evaluated after 10 minutes.
Extraction negative controls were produced by extracting a sterile swab alongside the blood swabs and taking 15 µL of the extract for the analysis. Cross-reactivity control for RSID™ Blood tests consisted of 60 µL mixture of saliva, semen and urine extracts (20 µL each) and 40 µL of RSID™ Blood running buffer per cross-reactivity control replica.

Results:

Two replicas of each sample were analyzed by two RSID™ Readers. Extraction negative controls (Table 1, cassettes 1 and 2) and saliva-semen-urine cross-reactivity controls (Table 1, cassettes 9 and 10) were read as "negative" by both RSID™ Readers tested. Samples with estimated 50, 100 and 250 nL of blood were read as "positive" by both RSID™ Readers tested (Table 1).

Table 1. RSID™ Blood Experiments

Cassete #	Volume of Blood Analyzed *	RSID Reader # 1 Results	RSID Reader # 2 Results
1**	0	Negative	Negative
2**	0	Negative	Negative
3	50 nL	Positive	Positive
4	50 nL	Positive	Positive
5	100 nL	Positive	Positive
6	100 nL	Positive	Positive
7	250 nL	Positive	Positive
8	250 nL	Positive	Positive
9***	0	Negative	Negative
10***	0	Negative	Negative

*Volume of blood analyzed is an estimate based on 100% extraction efficiency.

**Extraction Negative Control.

***Saliva-Semen-Urine Cross-Reactivity Control.

Figure 2 shows results of the RSID™ Blood tests as they appear in a digital photograph of cassettes' test window taken by a digital camera (Nikon's Coolpix 5600) and in the digital images generated by RSID™ Reader.

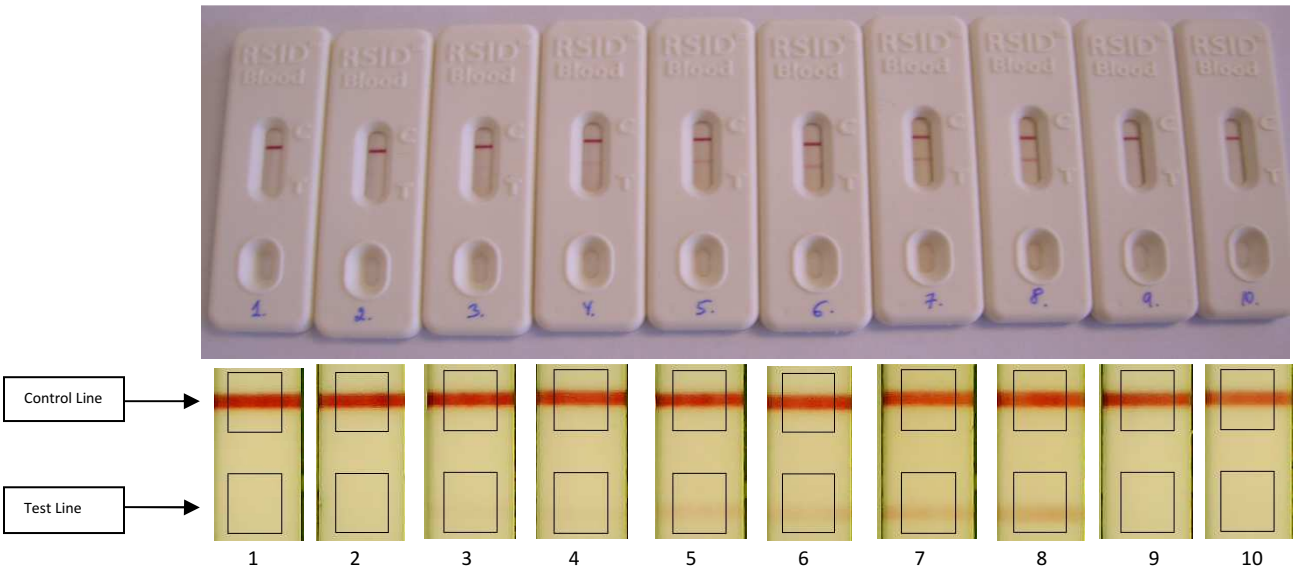

Figure 2. Results of RSID™ Blood Experiments (all experiments performed in duplicates, see Table 1)
Top - Digital photograph of the cassettes (C – Control; T – Test). Bottom - RSID™ Reader images.
Note: Photograph and RSID™ Reader images are slightly *less* sensitive than visual observation of the cassettes.

Cassettes 3 and 4, on which 50 nL of blood samples were tested had very light positive signal, which was expected since 50 nL of blood is at the detection limit of RSID™ Blood test (1). To measure reproducibility of RSID™ Reader, cassette 3 was analyzed three times on both RSID™ Readers (Table 2). Both RSID™ Readers detected signal in all three replicas (Table 2).

Table 2. RSID™ Blood Experiments: Triplicate RSID Reader Measurements

Cassete # (RSID Reader Measurement Replica)	Volume of Blood Analyzed *	RSID Reader # 1 Results	RSID Reader # 2 Results
3 (1)	50 nL	Positive	Positive
3 (2)	50 nL	Positive	Positive
3 (3)	50 nL	Positive	Positive

* Volume of blood analyzed is an estimate assuming 100% extraction efficiency

Conclusions:
RSID™ Reader accurately and reproducibly reported and recorded correct results from RSID™ Blood tests.

RSID™ Reader Blood Field Kit Experiments: Results and Conclusions

RSID™ Blood Field Kit uses RSID™ Universal buffer for extraction and running of samples. The extraction is performed in750 µL of the RSID™ Universal buffer. And from the extract, four drops are applied to the sample window of the RSID™ cassette using a dropper pipette supplied with RSID™ Blood Field Kit. Four drops of RSID™ Blood Field Kit dropper pipette are equivalent to 80 µL.

To prepare blood samples, 50 µL of whole blood was deposited on a sterile cotton swab and allowed to air-dry. The cotton batting was removed using laboratory clean technique, placed in a 1.5 mL microcentrifuge tube and extracted in 750 µL of the RSID™ Universal buffer. Assuming 100% extraction efficiency, each microliter of extract contained ~ 66.67 nL of blood. Blood extract volumes of 2, 4 and 10 µL were adjusted to a total volume of 200 µL with RSID™ Universal buffer and from each sample two aliquots of 80 µL each were applied to the sample window of two RSID™ cassettes (Table 3, cassettes 3 - 8). Additionally, two undiluted aliquots of 80 µL each were tested (Table 3, cassettes 11 and 12). The test line signals were evaluated after 10 minutes.
Extraction negative controls were produced by extracting a sterile swab alongside the blood swabs and taking 80 µL of the extract for the analysis. Cross-reactivity control for RSID™ Blood Field Kit tests consisted of 60 µL mixture of saliva, semen and urine extracts (20 µL each) and 40 µL of RSID™ Universal buffer.

Results:

Two replicas of each sample were analyzed by RSID™ Reader. Extraction negative controls (Table 3, cassettes 1 and 2) and saliva-semen-urine cross-reactivity controls (Table 3, cassettes 9 and 10) were read as “negative” , while samples with estimated 53, 107 and 267 nL, and 5.3 µL of blood were read as “positive” (Table 3 cassettes 3 – 8, 11, and 12).

Table 3. RSID™ Blood Field Kit Experiments

Cassete #	Volume of Blood Analyzed *	RSID Reader Results
1**	0	Negative
2**	0	Negative
3	53 nL	Positive
4	53 nL	Positive
5	107 nL	Positive
6	107 nL	Positive
7	267 nL	Positive
8	267 nL	Positive
9***	0	Negative
10***	0	Negative
11	5.3 µL	Positive
12	5.3 µL	Positive

*Volume of blood analyzed is an estimate based on 100% extraction efficiency.

**Extraction Negative Control. ***Saliva-Semen-Urine Cross-Reactivity Control.

Figure 3 shows results of the RSID™ Blood Field Kit tests as they appear in a digital photograph of cassettes' test window taken by a digital camera (Nikon's Coolpix 5600) and in the digital images generated by RSID™ Reader.

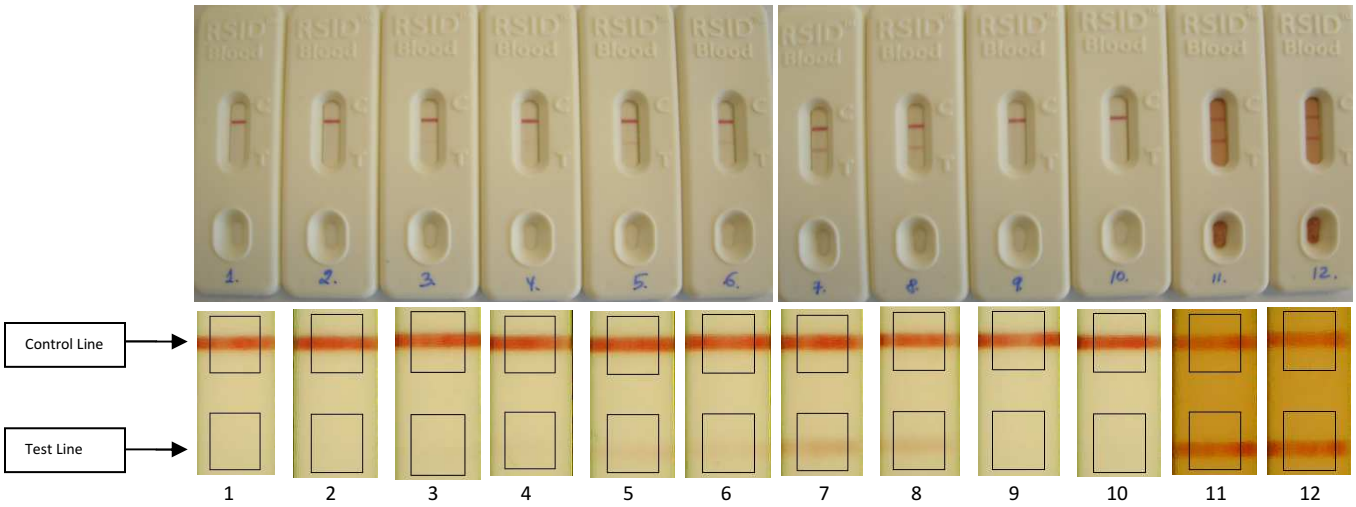

Figure 3. Results of RSID™ Blood Field Kit Experiments (all experiments performed in duplicates, see Table 3)
Top - Digital photograph of the cassettes (C – Control; T – Test). Bottom - RSID™ Reader images.
Note: Photograph and RSID™ Reader images are slightly *less* sensitive than visual observation of the cassettes.

Cassettes 3 and 4, on which 53 nL of blood samples were tested had very light positive signal, which was expected since 53 nL of blood is at the detection limit of RSID™ Blood test (1). To measure reproducibility of RSID™ Reader, cassette 3 was analyzed three times on RSID™ Reader (Table 4). RSID™ Reader detected signal in all three replicas (Table 4).

Table 4. RSID™ Blood Field Kit Experiments: Triplicate RSID Reader Measurements

Cassete # (RSID Reader Measurement Replica)	Volume of Blood Analyzed *	RSID Reader Results
3 (1)	53 nL	Positive
3 (2)	53 nL	Positive
3 (3)	53 nL	Positive

* Volume of blood analyzed is an estimate assuming 100% extraction efficiency

Conclusions:
RSID™ Reader accurately and reproducibly reported and recorded correct results from RSID™ Blood Field Kit tests.

RSID™ Saliva Experiments: Results and Conclusions

Sensitivity and specificity experiments for RSID™ Saliva tests using a RSID™ Reader device are described in Table 5. To prepare saliva samples, 50 µL of saliva was deposited on a sterile cotton swab and allowed 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 the corresponding RSID™ Saliva extraction buffer. Assuming 100% extraction efficiency, each microliter of extract contained 50 nL of saliva. In order to generate 10, 25, 50 and 250 nL test volumes of saliva in duplicates, 0.4, 1, 2 and 10 µL of saliva extract were adjusted to a total volume of 200 µL with RSID™ Saliva running buffer and from each sample two aliquots of 100 µL each were applied to the sample window of two RSID™ cassettes (Table 5, cassettes 3, 4, 5, 6, 7, 8, 9 and 10). The test line signals were evaluated after 10 minutes. Extraction negative controls were produced by extracting a sterile swab alongside the saliva swabs and taking 15 µL of the extract for the analysis. Cross-reactivity control for RSID™ Saliva tests consisted of 60 µL mixture of blood, semen and urine extracts (20 µL each) and 40 µL of RSID™ Saliva running buffer per cross-reactivity control replica.

Results:

Two replicas of each sample were analyzed by two RSID™ Readers. Extraction negative controls (Table 5, cassettes 1 and 2) and blood-semen-urine cross-reactivity controls (Table 5, cassettes 11 and 12) were read as "negative" by both RSID™ Readers tested. Samples with estimated 10, 25, 50 and 250 nL of saliva were read as "positive" by both RSID™ Readers tested (Table 5).

Table 5: RSID™ Saliva Experiments

Cassete #	Volume of Saliva Analyzed *	RSID Reader # 1 Results	RSID Reader # 2 Results
1**	0	Negative	Negative
2**	0	Negative	Negative
3	10 nL	Positive	Positive
4	10 nL	Positive	Positive
5	25 nL	Positive	Positive
6	25 nL	Positive	Positive
7	50 nL	Positive	Positive
8	50 nL	Positive	Positive
9	250 nL	Positive	Positive
10	250 nL	Positive	Positive
11***	0	Negative	Negative
12***	0	Negative	Negative

*Volume of saliva analyzed is an estimate assuming 100% extraction efficiency.

**Extraction Negative Control.

*** Blood-Semen-Urine Cross-Reactivity Control.

Figure 4 shows results of the RSID™ Saliva tests as they appear in a digital photograph of cassettes' test window taken by a digital camera (Nikon's Coolpix 5600) and in the digital images generated by RSID™ Reader.

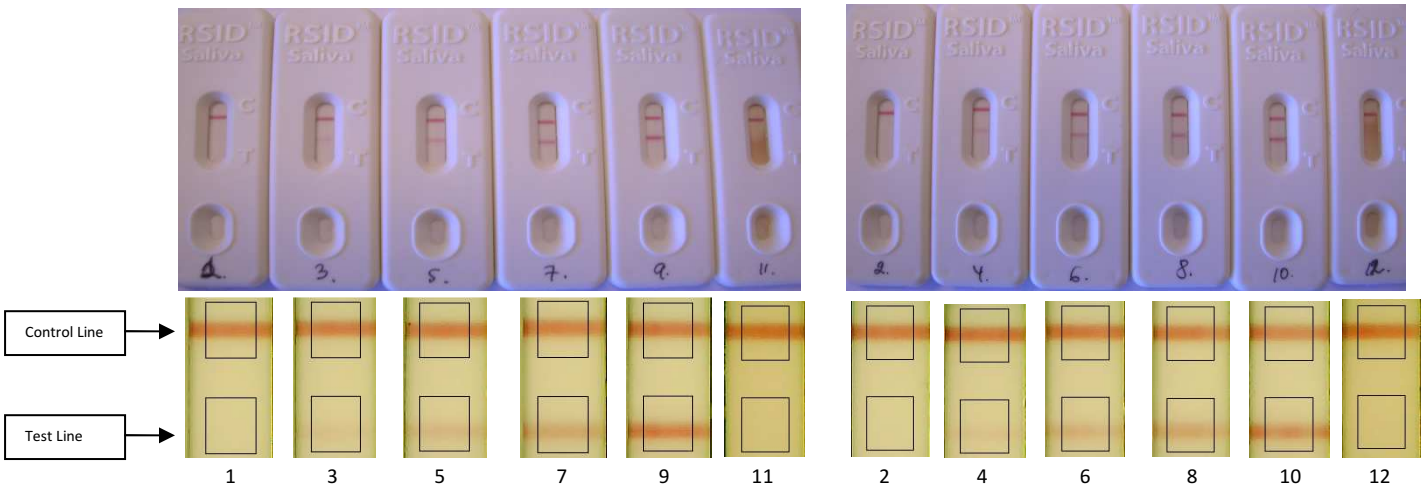

Figure 4. Results of RSID™ Saliva Experiments (all experiments performed in duplicates, see Table 5)
Top - Digital picture of the cassettes (C – Control; T – Test). Bottom - RSID Reader images.
Note: Photograph and RSID™ Reader images are slightly *less* sensitive than visual observation of the cassettes.

Cassettes 3 and 4, on which 10 nL of saliva samples were tested had very light positive signal, which was expected since 10 nL of saliva is at the detection limit of RSID™ Saliva test (2). To measure reproducibility of RSID™ Reader, cassette 3 was analyzed three times on both RSID™ Readers (Table 6). Both RSID™ Readers detected signal in all three replicates (Table 6).

Table 6: RSID™ Saliva Experiments: Triplicate RSID Reader Measurements

Cassete # (RSID Reader Measurement Replica)	Volume of Saliva Analyzed *	RSID Reader # 1 Results	RSID Reader # 2 Results
3 (1)	10 nL	Positive	Positive
3 (2)	10 nL	Positive	Positive
3 (3)	10 nL	Positive	Positive

* Volume of saliva analyzed is based on 100% extraction efficiency

Conclusions:
RSID™ Reader accurately and reproducibly reported and recorded correct results from RSID™ Saliva tests.

RSID™ Reader Saliva Field Kit Experiments: Results and Conclusions

RSID™ Saliva Field Kit uses RSID™ Universal buffer for extraction and running of samples. The extraction is performed in750 µL of the RSID™ Universal buffer. And from the extract, four drops are applied to the sample window of the RSID™ cassette using the dropper pipette supplied with RSID™ Saliva Field Kit. Four drops of RSID™ Saliva Field Kit dropper pipette are equivalent to 80 µL.

To prepare saliva samples, 50 µL of saliva was deposited on a sterile cotton swab and allowed to air-dry. The cotton batting was removed using laboratory clean technique, placed in a 1.5 mL microcentrifuge tube and extracted in 750 µL of the RSID™ Universal buffer. Assuming 100% extraction efficiency, each microliter of extract contained ~66.67 nL of saliva. Saliva extract volumes of 0.4, 1, 2 and 10 µL were adjusted to a total volume of 200 µL with RSID™ Universal buffer and from each sample two aliquots of 80 µL each were applied to the sample window of two RSID™ cassettes (Table 7, cassettes 3 - 10). Additionally, two undiluted aliquots of 80 µL each were tested (Table 7, cassettes 13 and 14). The test line signals were evaluated after 10 minutes. Extraction negative controls were produced by extracting a sterile swab alongside the saliva swabs and taking 80 µL of the extract for the analysis. Cross-reactivity control for RSID™ Saliva tests consisted of 60 µL mixture of blood, semen and urine extracts (20 µL each) and 40 µL of RSID™ Universal buffer.

Results:

Two replicates of each sample were analyzed by RSID™ Reader. Extraction negative controls and blood-semen-urine cross-reactivity controls, - Table 7, cassettes 1, 2 and 11, 12, respectively - were read as “negative”, while samples with estimated 11, 27, 53 and 267 nL, and 5.3 µL of saliva were read as “positive” (Table 7).

Table 7: RSID™ Saliva Field Kit Experiments

Cassete #	Volume of Saliva Analyzed *	RSID Reader Results
1**	0	Negative
2**	0	Negative
3	11 nL	Positive
4	11 nL	Positive
5	27 nL	Positive
6	27 nL	Positive
7	53 nL	Positive
8	53 nL	Positive
9	267 nL	Positive
10	267 nL	Positive
11***	0	Negative
12***	0	Negative
13	5.3 µL	Positive
14	5.3 µL	Positive

*Volume of saliva analyzed is an estimate assuming 100% extraction efficiency.

**Extraction Negative Control. ***Blood-Semen-Urine Cross-Reactivity Control.

Figure 5 shows results of the RSID™ Saliva Field Kit tests as they appear in a digital photograph of cassettes' test window taken by a digital camera (Nikon's Coolpix 5600) and in the digital images generated by RSID™ Reader.

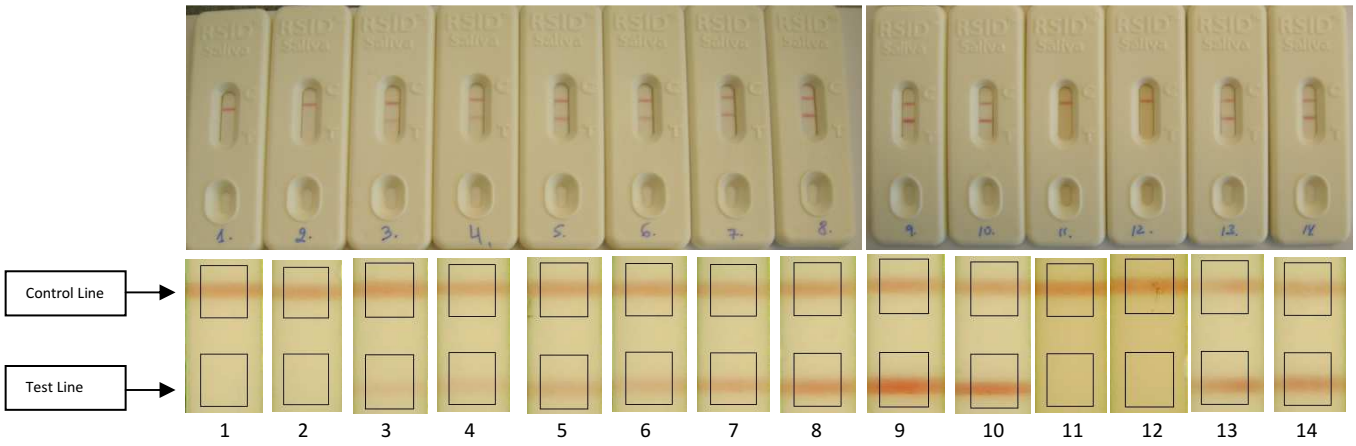

Figure 5. Results of RSID™ Saliva Field Kit Experiments (all experiments performed in duplicates, see Table 7)
Top - Digital picture of the cassettes (C – Control; T – Test). Bottom - RSID Reader images.

Note: Photograph and RSID™ Reader images are slightly *less* sensitive than visual observation of the cassettes.

Cassettes 3 and 4, on which 11 nL of saliva samples were tested had very light positive signal, which was expected since 11 nL of saliva is at the detection limit of RSID™ Saliva test (2). To measure reproducibility of RSID™ Reader, cassette 3 was analyzed three times on RSID™ Reader (Table 8). RSID™ Reader detected signal in all three replicates (Table 8).

Table 8: RSID™ Saliva Field Kit Experiments: Triplicate RSID Reader Measurements

Cassete # (RSID Reader Measurement Replica)	Volume of Saliva Analyzed *	RSID Reader Results
3 (1)	11 nL	Positive
3 (2)	11 nL	Positive
3 (3)	11 nL	Positive

* Volume of saliva analyzed is based on 100% extraction efficiency

Conclusions:

RSID™ Reader accurately and reproducibly reported and recorded correct results from RSID™ Saliva Field Kit tests.

RSID™ Semen Experiments: Results and Conclusions

Sensitivity and specificity experiments for RSID™ Semen tests using RSID™ Reader device are described in Table 9. To prepare semen samples, 50 µL of semen was deposited on a sterile cotton swab and allowed 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 the corresponding RSID™ Semen extraction buffer. Assuming 100% extraction efficiency, each microliter of extract contained 50 nL of semen. Using RSID™ Semen 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 in duplicates, 2 µL of semen dilutions (1:20, 1:10 and 1:5) were adjusted to a total volume of 200 µL with RSID™ Semen running buffer. In order to generate 50 nL test volumes of semen in duplicates, 2 µL of undiluted semen extract were adjusted to a total volume of 200 µL with RSID™ Semen running buffer. From each sample two aliquots of 100 µL each were applied to the sample window of two RSID™ cassette (Table 9, cassettes 3 - 10). The test line signals were evaluated after 10 minutes.

Extraction negative controls were produced by extracting a sterile swab alongside the semen swabs and taking 15 µL of the extract for the analysis. Cross-reactivity control for RSID™ Semen tests consisted of 60 µL mixture of blood, saliva and urine extracts (20 µL each) and 40 µL of RSID™ Semen running buffer per replicate.

Results:

Two replicates of each sample were analyzed by two RSID™ Readers. Extraction negative controls (Table 9, cassettes 1 and 2) and blood-saliva-urine cross-reactivity controls (Table 9, cassettes 11 and 12) were read as "negative" by both RSID™ Readers tested. Samples with estimated 2.5, 5, 10 and 50 nL of semen were read as "positive" by both RSID™ Readers tested (Table 9).

Table 9: RSID™ Semen Experiments

Cassete #	Volume of Semen Analyzed *	RSID Reader # 1 Results	RSID Reader # 2 Results
1**	0	Negative	Negative
2**	0	Negative	Negative
3	2.5 nL	Positive	Positive
4	2.5 nL	Positive	Positive
5	5 nL	Positive	Positive
6	5 nL	Positive	Positive
7	10 nL	Positive	Positive
8	10 nL	Positive	Positive
9	50 nL	Positive	Positive
10	50 nL	Positive	Positive
11***	0	Negative	Negative
12***	0	Negative	Negative

* Volume of semen analyzed is based on 100% extraction efficiency.

Extraction Negative Control. * Blood-Saliva-Urine Cross-Reactivity Control.

Figure 6 shows results of the RSID™ Semen tests as they appear in a digital photograph of cassettes' test window taken by a digital camera (Nikon's Coolpix 5600) and in the digital images generated by RSID™ Reader.

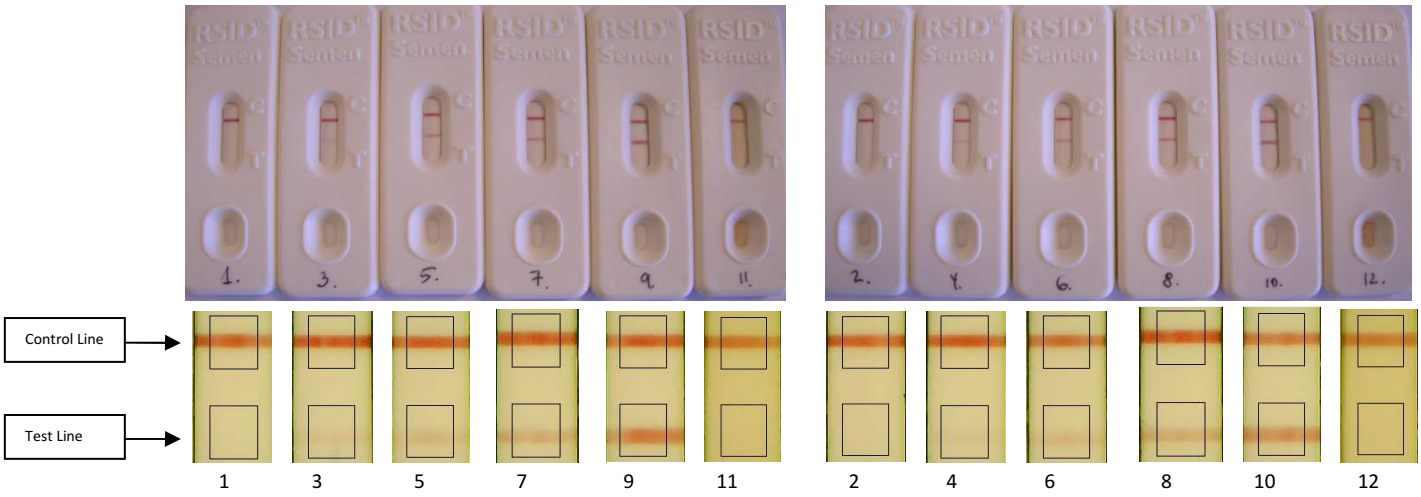

Figure 6. Results of RSID™ Semen Experiments (all experiments performed in duplicates, see Table 9)
Top - Digital picture of the cassettes (C – Control; T – Test). Bottom - RSID Reader images.
Note: Photograph and RSID™ Reader images are slightly *less* sensitive than visual observation of the cassettes.

Cassettes 3 and 4, on which an estimated 2.5 nL semen samples 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 (3). To measure reproducibility of RSID™ Reader, cassette 3 was analyzed three times on both RSID™ Readers (Table 10). Both RSID™ Readers detected signal in all three replicates (Table 10).

Table 10. RSID™ Semen Experiments: Triplicate RSID Reader Measurements

Cassete # (RSID Reader Measurement Replica)	Volume of Semen Analyzed *	RSID Reader # 1 Results	RSID Reader # 2 Results
3 (1)	2.5 nL	Positive	Positive
3 (2)	2.5 nL	Positive	Positive
3 (3)	2.5 nL	Positive	Positive

* Volume of semen analyzed is an estimate assuming 100% extraction efficiency

Conclusions:
RSID™ Reader accurately and reproducibly reported and recorded correct results from RSID™ Semen tests.

RSID™ Reader Semen Field Kit Experiments: Results and Conclusions

RSID™ Semen Field Kit uses RSID™ Universal buffer for extraction and running of the samples. The extraction is performed in750 µL of the RSID™ Universal buffer. And from the extract, four drops are applied to the sample window of the RSID™ cassette using the dropper pipette supplied with RSID™ Semen Field Kit. Four drops of RSID™ Semen Field Kit dropper pipette are equivalent to 80 µL.

To prepare semen samples, 50 µL of semen was deposited on a sterile cotton swab and allowed to air-dry. The cotton batting was removed using laboratory clean technique, placed in a 1.5 mL microcentrifuge tube and extracted in 750 µL of the RSID™ Universal buffer. Assuming 100% extraction efficiency, each microliter of extract contained 66.67 nL of semen. Using RSID™ Universal buffer, three dilutions of the extract were made, - 1:20, 1:10 and 1:5 - from which 2 µL were added to 198 µL of RSID™ Universal buffer. Also, 2 µL of undiluted semen extract were adjusted to a total volume of 200 µL with RSID™ Universal buffer. From each sample two aliquots of 80 µL each were applied to the sample window of two RSID™ cassettes (Table 11, cassettes 3 - 10). Additionally, two undiluted aliquots of 80 µL each were tested (Table 11, cassettes 13 and 14). The test line signals were evaluated after 10 minutes. Extraction negative controls were produced by extracting a sterile swab alongside the semen swabs and taking 80 µL of the extract for the analysis. Cross-reactivity control for RSID™ Semen Field Kit tests consisted of 60 µL mixture of blood, saliva and urine extracts (20 µL each) and 40 µL of RSID™ Universal buffer.

Results:

Two replicates of each sample were analyzed by RSID™ Reader. Extraction negative controls (Table 11, cassettes 1 and 2) and blood-saliva-urine cross-reactivity controls (Table 11, cassettes 11 and 12) were read as “negative” by RSID™ Reader. Samples with estimated 2.7, 5.3, 11 and 53 nL and 5.3 µL of semen were read as “positive” by both RSID™ Readers tested (Table 11, cassettes 3 – 10, 13 and 14).

Table 11: RSID™ Semen Field Kit Experiments

Cassete #	Volume of Semen Analyzed *	RSID Reader Results
1**	0	Negative
2**	0	Negative
3	2.7 nL	Positive
4	2.7 nL	Positive
5	5.3 nL	Positive
6	5.3 nL	Positive
7	11 nL	Positive
8	11 nL	Positive
9	53 nL	Positive
10	53 nL	Positive
11***	0	Negative
12***	0	Negative
13	5.3 µL	Positive
14	5.3 µL	Positive

* Volume of semen analyzed is based on 100% extraction efficiency. **Extraction Negative Control. ***Blood-Saliva-Urine Cross-Reactivity Control.

Figure 7 shows results of the RSID™ Semen Field Kit tests as they appear in a digital photograph of cassettes' test window taken by a digital camera (Nikon's Coolpix 5600) and in the digital images generated by RSID™ Reader.

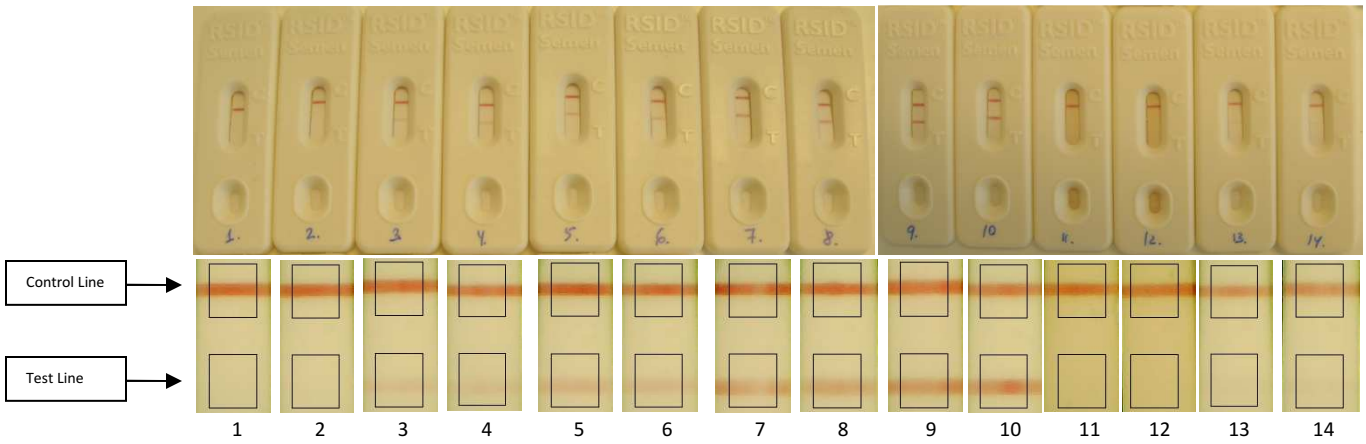

Figure 7. Results of RSID™ Semen Field Kit Experiments (all experiments performed in duplicates, see Table 11)
Top - Digital picture of the cassettes (C – Control; T – Test). Bottom - RSID Reader images.
Note a weak signal for samples with estimated 5.3 μL of semen (cassettes 13 and 14) due to the High Dose Hook Effect.
Note: Photograph and RSID™ Reader images are slightly *less* sensitive than visual observation of the cassettes.

Cassettes 3 and 4, on which an estimated 2.7 nL semen samples were tested had light positive signal, which was expected since 2.7 nL of semen is at the detection limit of RSID™ Semen test (3). To measure reproducibility of RSID™ Reader, cassette 3 was analyzed three times on RSID™ Reader (Table 12). RSID™ Reader detected signal in all three replicates (Table 12).

Table 12. RSID™ Semen Field Kit Experiments: Triplicate RSID Reader Measurements

Cassete # (RSID Reader Measurement Replica)	Volume of Semen Analyzed *	RSID Reader Results
3 (1)	2.7 nL	Positive
3 (2)	2.7 nL	Positive
3 (3)	2.7 nL	Positive

* Volume of semen analyzed is an estimate assuming 100% extraction efficiency

Conclusions:
RSID™ Reader accurately and reproducibly reported and recorded correct results from RSID™ Semen Field Kit tests.

RSID™ Reader Urine and RSID™ Reader Urine Field Kit Experiments: Results and Conclusions

RSID™ Urine and RSID™ Urine Field Kit use the same RSID™ Urine buffer.

Sensitivity and specificity experiments for RSID™ Urine tests using RSID™ Reader device are described in Table 13. To prepare urine samples, 100 µL of urine was deposited on a sterile cotton swab and allowed 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 the corresponding RSID™ Urine buffer. Assuming 100% extraction efficiency, each microliter of extract contained 100 nL of urine. In order to generate 1, 5 and 10 µL test volumes of urine in duplicates, 20, 100 and 200 µL of urine extract were adjusted to a total volume of 200 µL with RSID™ Urine buffer and from each sample two aliquots of 100 µL each were applied to the sample window of RSID™ cassette (Table 13, cassettes 3, 4, 5, 6, 7, and 8). Additionally, two undiluted aliquots of 80 µL each (8 µL of urine) were tested (Table 13, cassettes 11 and 12) to simulate the RSID™ Urine Field Kit configuration (4 drops from Field Kit dropper pipette = 80 µL). The test line signals were evaluated after 15 minutes. Extraction negative controls were produced by extracting a sterile swab alongside the urine swabs and taking 100 µL of the extract for the analysis. Cross-reactivity control for RSID™ Urine tests consisted of 60 µL mixture of blood, saliva and semen extracts (20 µL each) and 40 µL of RSID™ Urine buffer per cross-reactivity control replicate.

Results:

Two replicates of each sample were analyzed by two RSID™ Readers. Extraction negative controls (Table 13, cassettes 1 and 2) and blood-saliva-semen cross-reactivity controls (Table 13, cassettes 9 and 10) were read as "negative" by both RSID™ Readers tested. Samples with estimated 1, 5, 8 and 10 µL of urine were read as "positive" by both RSID™ Readers tested (Table 13).

Table 13. RSID™ Urine Experiments

Cassete #	Volume of Urine Analyzed *	RSID Reader # 1 Results	RSID Reader # 2 Results
1**	0	Negative	Negative
2**	0	Negative	Negative
3	1 µL	Positive	Positive
4	1 µL	Positive	Positive
5	5 µL	Positive	Positive
6	5 µL	Positive	Positive
7	10 µL	Positive	Positive
8	10 µL	Positive	Positive
9***	0	Negative	Negative
10***	0	Negative	Negative
11 (Field Kit)	8 µL	Positive	Positive
12 (Field Kit)	8 µL	Positive	Positive

* Volume of urine analyzed is an estimate assuming 100% extraction efficiency

** Extraction Negative Control

** Blood-Saliva-Semen Cross-Reactivity Control

Figure 8 shows results of the RSID™ Urine and RSID™ Urine Field Kit tests as they appear in a digital photograph of cassettes' test window taken by a digital camera (Nikon's Coolpix 5600) and in the digital images generated by RSID™ Reader.

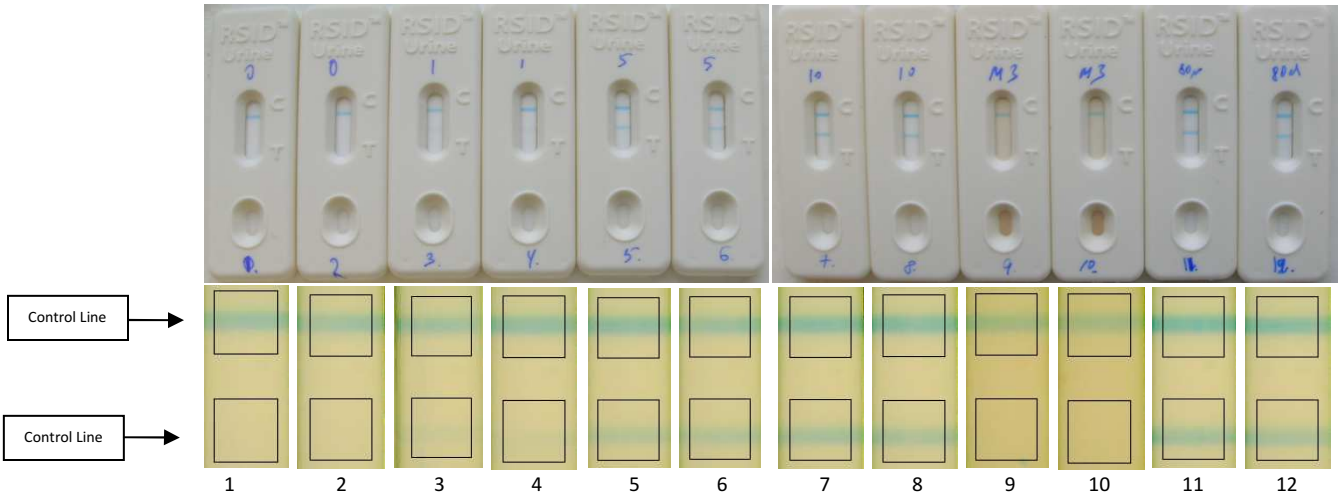

Figure 8. Results of RSID™ Urine Experiments (all experiments performed in duplicates as explained in Table 13)
Top - Digital picture of the cassettes (C – Control; T – Test). Bottom - RSID Reader images.
Cassettes 11 and 12 show RSID™ Urine Tests performed in a Field Kit configuration: 80 µL (equivalent of 4 drops from Field Kit dropper pipette) of urine extract applied to the cassette's window.

Note: Photograph and RSID™ Reader images are slightly *less* sensitive than visual observation of the cassettes.

Cassettes 3 and 4, on which an estimated 1 µL urine samples were tested had very light positive signal, which was expected since 1 µL of urine is at the detection limit of RSID™ Urine test (4). To measure reproducibility of RSID™ Reader, cassette 3 was analyzed three times on both RSID™ Readers (Table 14). Both RSID™ Readers detected signal in all three replicates (Table 14).

Table 14: RSID™ Urine Experiments: Triplicate RSID Reader Measurements

Cassete # (RSID Reader Measurement Replica)	Volume of Urine Analyzed *	RSID Reader # 1 Results	RSID Reader # 2 Results
3 (1)	1 µl	Positive	Positive
3 (2)	1 µl	Positive	Positive
3 (3)	1 µl	Positive	Positive

* Volume of urine analyzed is an estimate assuming 100% extraction efficiency

Conclusions:

RSID™ Reader accurately and reproducibly reported and recorded correct results from RSID™ Urine and RSID™ Urine Field Kit tests.

References:

1. Developmental Validation of RSID™ Blood Test (Independent Forensics of Illinois, 2010)
2. Developmental Validation of RSID™ Saliva Test (Independent Forensics of Illinois, 2010)
3. Developmental Validation of RSID™ Semen Test (Independent Forensics of Illinois, 2010)
4. Developmental Validation of RSID™ Urine Test (Independent Forensics of Illinois, 2011)