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

Introduction

The Rapid Stain IDentification (RSID™) Reader System is a 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 down-loaded to the computer through USB cable.

In this study we validated the RSID™ Reader System for use with RSID™ Blood, RSID™ Saliva, RSID™ Semen and RSID™ Urine tests.

A.

B.

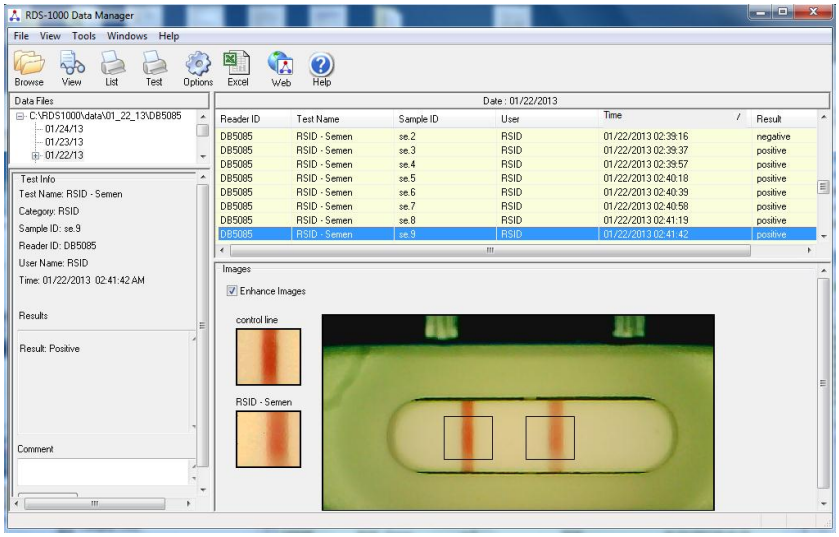

Figure 2. RSID™ Reader

(A) Ruggedized colorimetric unit with touch screen.

(B) Electronic documentation of sample name, time, date and results of individual 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

Similar results were obtained with RSID™ Universal buffer, data not shown.

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.

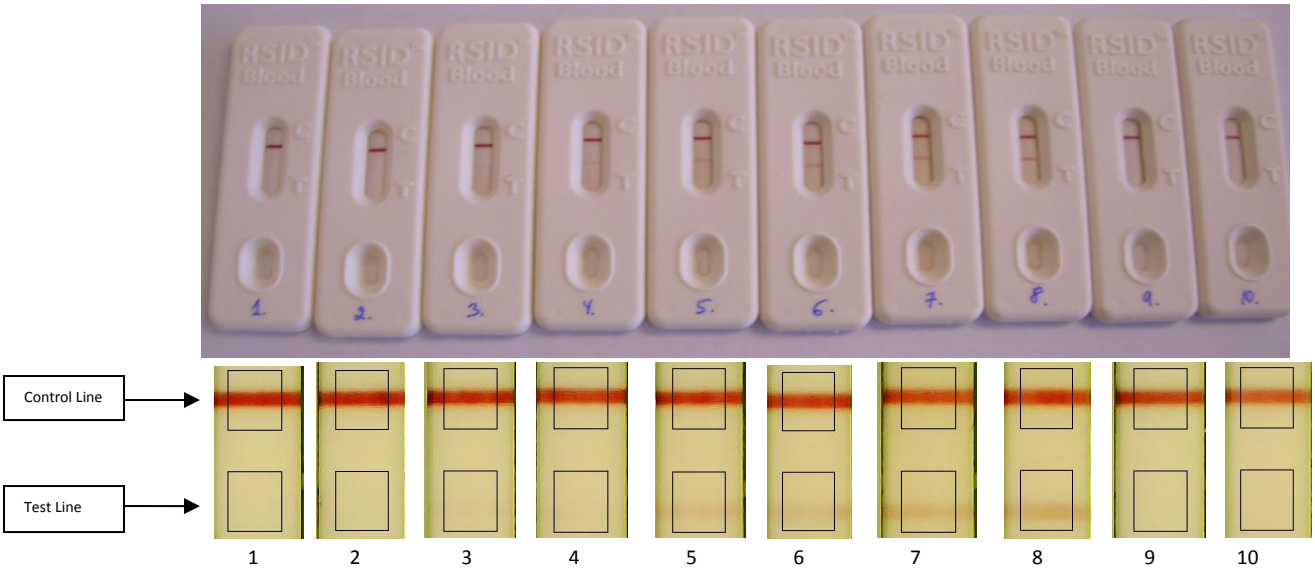

Figure 2. Results of RSID™ Blood Experiments (all experiments performed in duplicates, see Table 1)
Top - Digital photograph of the cassettes (C – Control; T – Test). Bottom - RSID™ Reader images.
Note: Photograph and RSID™ Reader images are slightly *less* sensitive than visual observation of the cassettes.

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 in a row 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™ Saliva Experiments: Results and Conclusions

Sensitivity and specificity experiments for RSID™ Saliva tests using a RSID™ Reader device are described in Table 3. 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 3, 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 3, cassettes 1 and 2) and blood-semen-urine cross-reactivity controls (Table 3, 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 3).

Table 3: 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

Similar results were obtained with RSID™ Universal buffer, data not shown.

Figure 3 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.

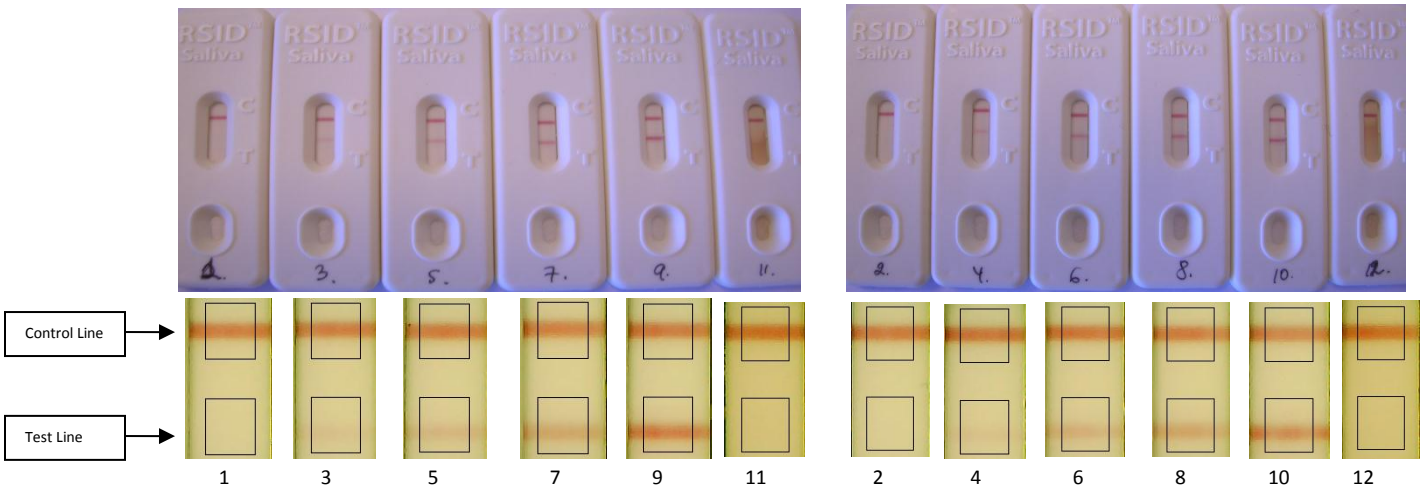

Figure 3. Results of RSID™ Saliva Experiments (all experiments performed in duplicates, see Table 3)
Top - Digital picture of the cassettes (C – Control; T – Test). Bottom - RSID Reader images.
Note: Photograph and RSID™ Reader images are slightly *less* sensitive than visual observation of the cassettes.

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 in on both RSID™ Readers (Table 4). Both RSID™ Readers detected signal in all three replicates (Table 4).

Table 4: 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™ Semen Experiments: Results and Conclusions

Sensitivity and specificity experiments for RSID™ Semen tests using RSID™ Reader device are described in Table 5. 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 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 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 5, cassettes 1 and 2) and blood-saliva-urine cross-reactivity controls (Table 5, 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 5).

Table 5: 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

Identical results were obtained with RSID™ Universal buffer, data not shown.

Figure 4 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.

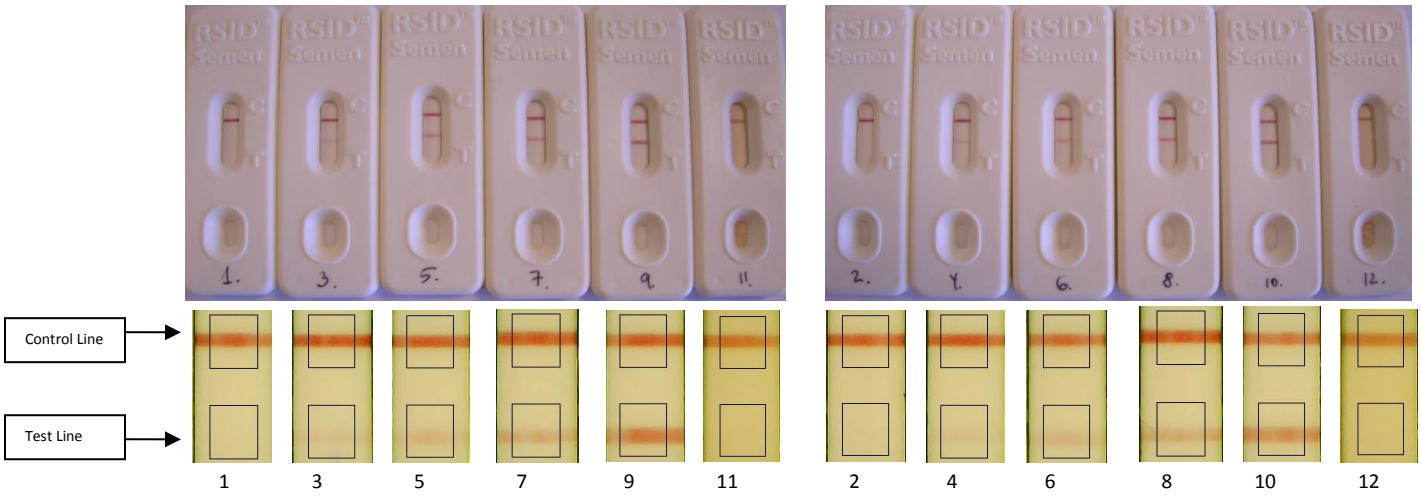

Figure 4. Results of RSID™ Semen Experiments (all experiments performed in duplicates, see Table 5)
Top - Digital picture of the cassettes (C – Control; T – Test). Bottom - RSID Reader images.
Note: Photograph and RSID™ Reader images are slightly *less* sensitive than visual observation of the cassettes.

Cassettes 3 and 4, on which 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 6). Both RSID™ Readers detected signal in all three replicates (Table 6).

Table 6. 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™ Urine Experiments: Results and Conclusions

Sensitivity and specificity experiments for RSID™ Urine tests using RSID™ Reader device are described in Table 7. 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 7, cassettes 3, 4, 5, 6, 7, and 8). 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™ Blood tests consisted of 60 µL mixture of blood, saliva and semen extracts (20 µL each) and 40 µL of RSID™ Urine running buffer per cross-reactivity control replicate.

Results:

Two replicates of each sample were analyzed by two RSID™ Readers. Extraction negative controls (Table 7, cassettes 1 and 2) and blood-saliva-semen cross-reactivity controls (Table 7, cassettes 9 and 10) were read as "negative" by both RSID™ Readers tested. Samples with estimated 1, 5 and 10 µL of urine were read as "positive" by both RSID™ Readers tested (Table 7).

Table 7. 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

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

** Extraction Negative Control

** Blood-Saliva-Semen Cross-Reactivity Control

Figure 5 shows results of the RSID™ Urine 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.

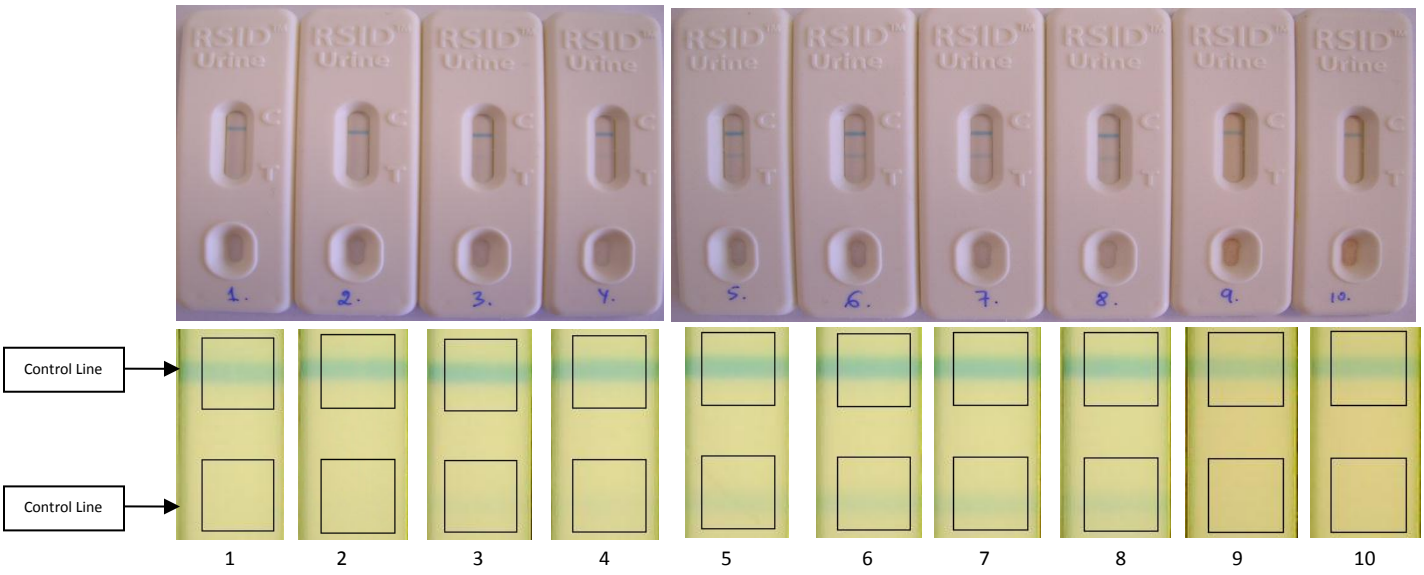

Figure 5. Results of RSID™ Urine Experiments (all experiments performed in duplicates as explained in Table 7)
Top - Digital picture of the cassettes (C – Control; T – Test). Bottom - RSID Reader images.
Note: Photograph and RSID™ Reader images are slightly *less* sensitive than visual observation of the cassettes.

Cassettes 3 and 4, on which 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 8). Both RSID™ Readers detected signal in all three replicates (Table 8).

Table 8: 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 test.

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)