

**Purpose:** To determine if shorter extraction times can be used with RSID™-Semen without loss of sensitivity.

**Experiment #1 - Testing shorter extraction times with RSID™-Semen**

**Protocol**

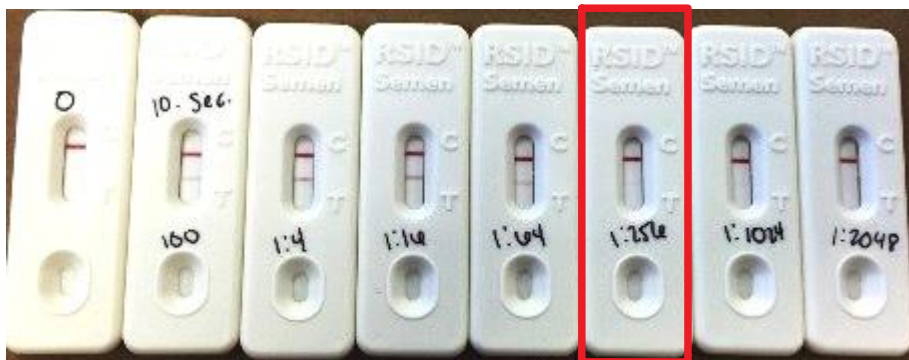
Positive control swabs were produced by depositing 50 µL of semen onto cotton swabs and allowing these to air-dry. The swabs were extracted in 1 mL of Universal buffer for: 10 sec, 30 sec, 1 min, 5 min, 20 min, 30 min, or 1 hour. The extractions were shaken either continuously for the 10 sec, 30 sec, or 1 min time points, or occasionally for the longer time points. After the indicated time points, 500 µl was removed from each extraction and placed into a separate tube. Serial dilutions of 1:4, 1:16, 1:64, 1:256, 1:1024, and 1:2048 were made from each extraction as indicated in the table below. For each time point, 100 µL of the extraction and of each dilution was added to an RSID™-cassette and results were recorded after 10 minutes.

			Vol buffer
1:4	A	100 µL extract	300 µL
1:16	B	100 µL sol A	300 µL
1:64	C	100 µL sol B	300 µL
1:256	D	100 µL sol C	300 µL
1:1024	E	100 µL sol D	300 µL
1:2048	F	100 µL sol F	200 µL

**Results**

Similar results were obtained with a positive control swab extracted for 10 seconds (with shaking) as compared to a sample extracted for 1 hour at all semen extract amounts tested (see below).

Regardless of extraction time, positive results were detected with RSID™-Semen up to 1:256 dilution of the semen extract from a positive control swab (please see red boxes below).



**10 second extraction**



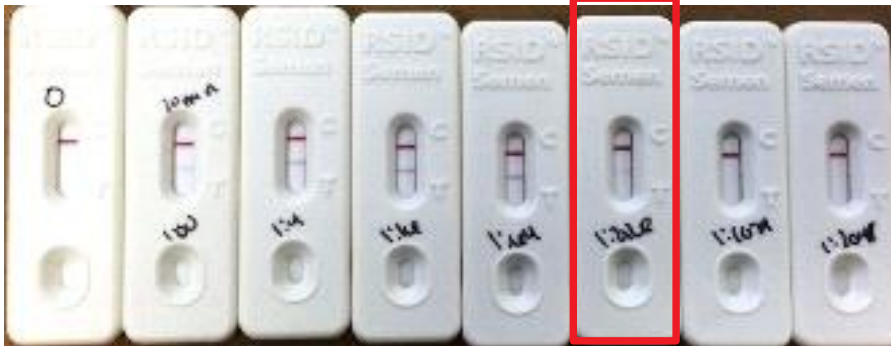
30 second extraction



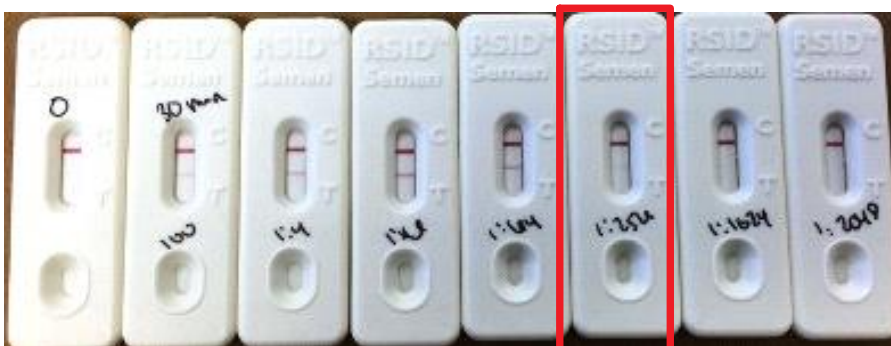
1 minute extraction



5 minute extraction



20 minute extraction



30 minute extraction



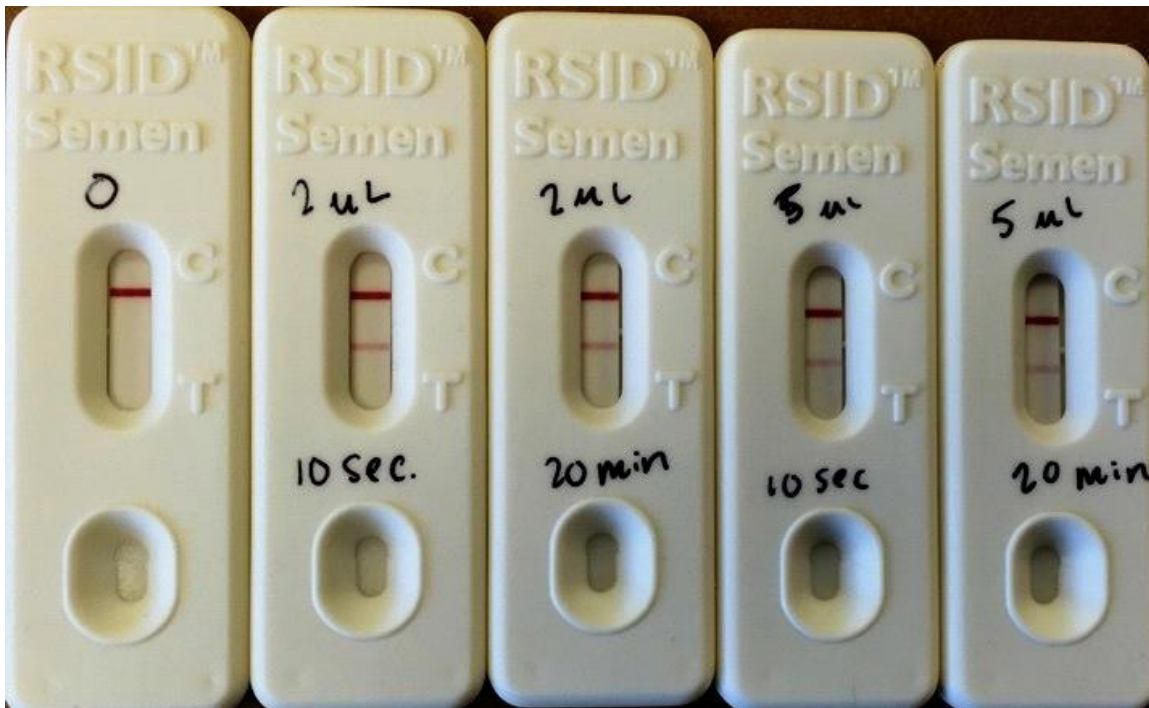
1 hour extraction

## Experiment #2 - Shorter incubation times - test of sensitivity.

**Protocol:** To determine if shorter extraction times can be used for samples with limiting amounts of semen, positive control swabs were produced on cotton swabs with 2 and 5  $\mu\text{L}$  of semen and allowed to air-dry. The swab heads were extracted in 300  $\mu\text{L}$  extraction buffer for either 10 seconds (with shaking) or 20 minutes (with occasional shaking), and 100  $\mu\text{L}$  was loaded onto an RSID™-Semen cassette. Results were recorded after ten minutes.

### Results

Positive results were obtained for both the 2  $\mu\text{L}$  and 5  $\mu\text{L}$  samples, regardless of extraction time. Similar test line intensities were observed for both the 10 sec or 20 minute extractions for both the 2  $\mu\text{L}$  and 5  $\mu\text{L}$  semen swabs (see image below).



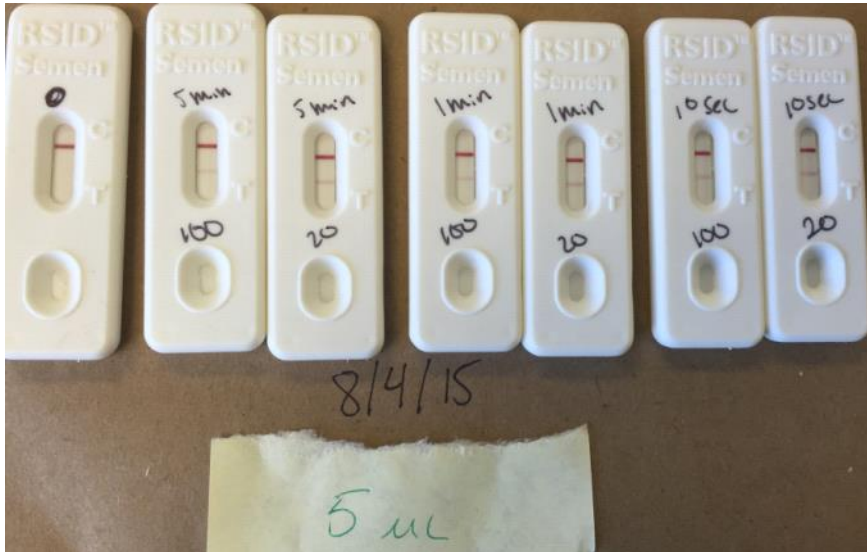
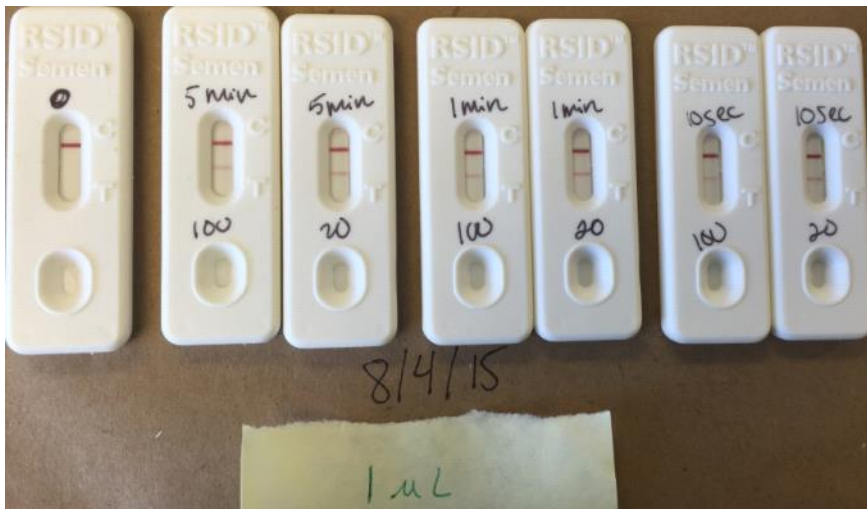
## Experiment #2.1 - Shorter incubation times - further test of sensitivity

**Protocol:** Positive control swabs were prepared by depositing 5  $\mu\text{L}$ , 1  $\mu\text{L}$ , 1  $\mu\text{L}$  of 1:2 semen dilution (equivalent to 0.5  $\mu\text{L}$  semen) and 1  $\mu\text{L}$  of 1:10 dilution (equivalent to 0.1  $\mu\text{L}$  of semen) onto cotton swabs and allowing to air-dry. The swab heads were extracted in 300  $\mu\text{L}$  extraction buffer for either 10 seconds (with shaking), 1 minute (with occasional shaking), or 5 minutes (with occasional shaking) and 20 and 100  $\mu\text{L}$  was loaded onto an RSID™-Semen cassette. Results were recorded after ten minutes.

### Results

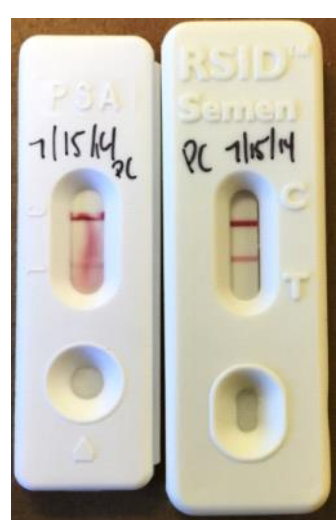
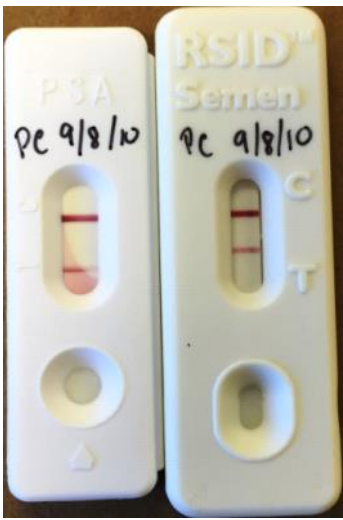
Similar results for the 1  $\mu\text{L}$  of 1:10 semen dilution were observed at all three extraction times tested (see top panel below). Results for 1  $\mu\text{L}$  of 1:2 dilution and the 1  $\mu\text{L}$  and 5  $\mu\text{L}$  swabs are shown below; again, no difference in sensitivity at any extraction time was seen.





**Experiment 3: Testing 10 second extractions of post coital swabs with RSID™-Semen and Bluestar**

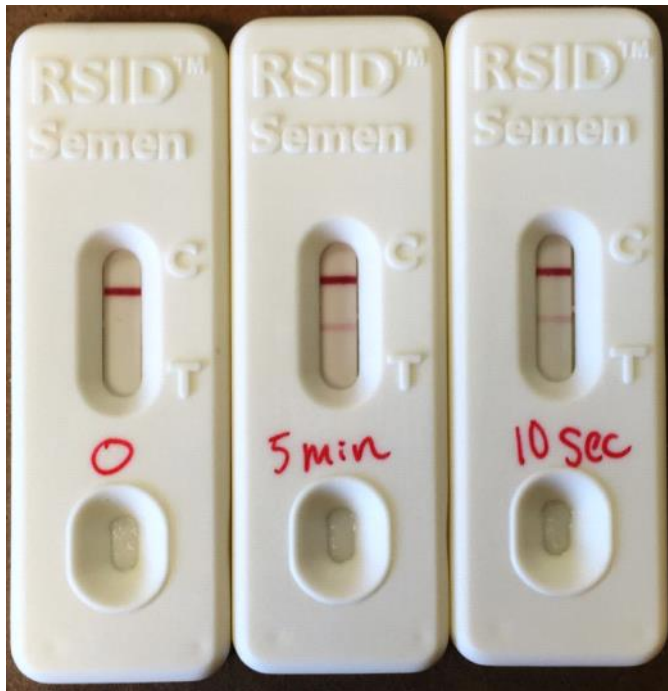
**Protocol:** Post coital swabs collected at 8 hrs (collected 2/12/15), 12 hrs (collected 7/15/14) and 14 hrs (collected 9/8/10) post intercourse were extracted and tested on RSID™-Semen and Bluestar PSA tests. 10 second extractions were used throughout. Note positive results for RSID-Semen and streaked, incomplete test lines on Bluestar.



### Experiment #3.1: Testing PC swabs with RSID™-Semen Field kit

**Protocol:** To determine if lower extraction times can be used when extracting PC swabs with the RSID™-Semen field kit, 2 post-coital swabs (8 hours after unprotected intercourse) were extracted in field kit extraction tubes containing 750 µL Universal buffer for either 10 seconds (with shaking) or 5 minutes (with occasional shaking). Using the disposable plastic transfer pipette included in the field kit, 5 drops of each extract was added to an RSID™-Semen cassette and results were recorded at ten minutes.

**Results:** Similar results were obtained when extracting the PC swabs at either 10 seconds or 5 minutes (see below).

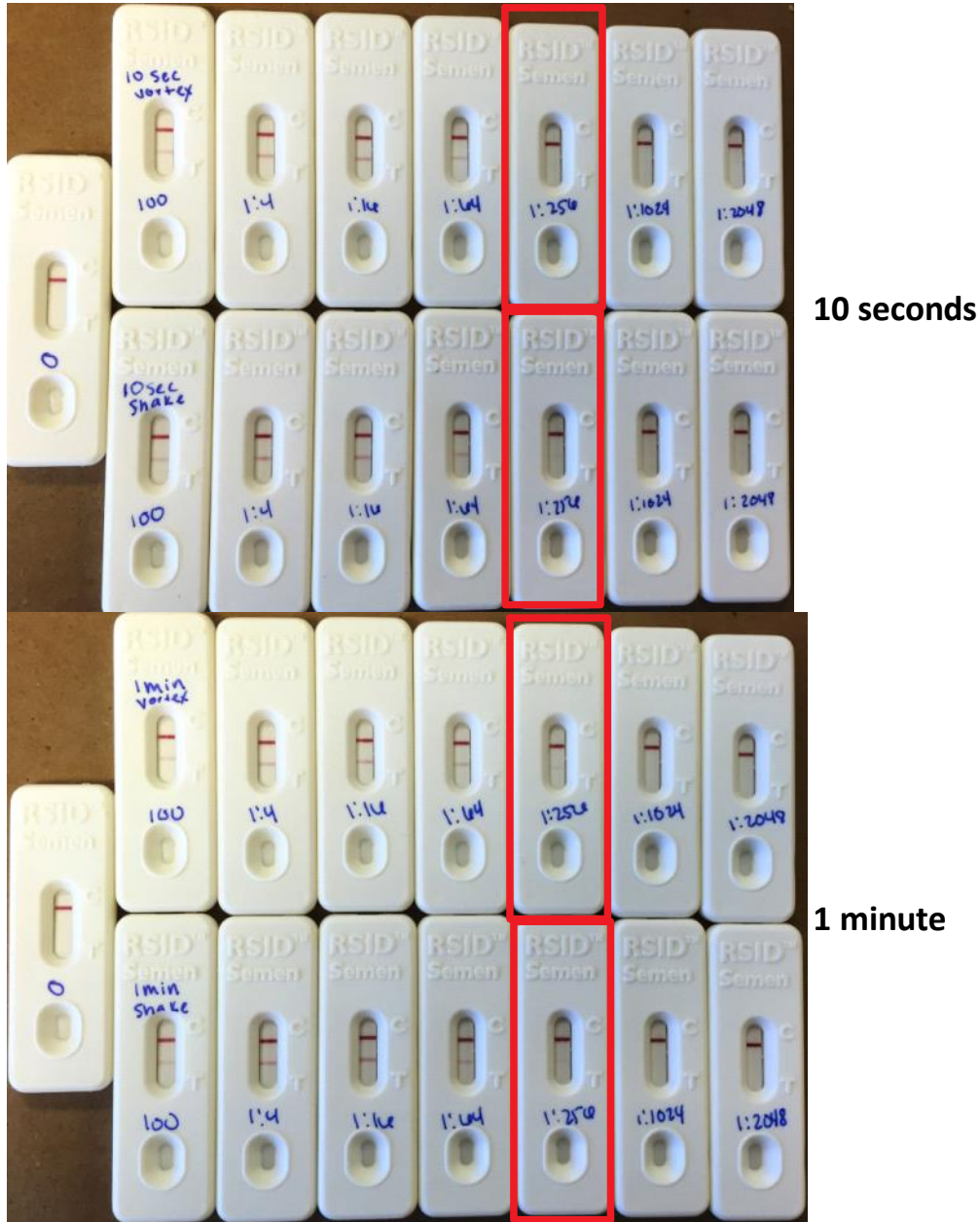


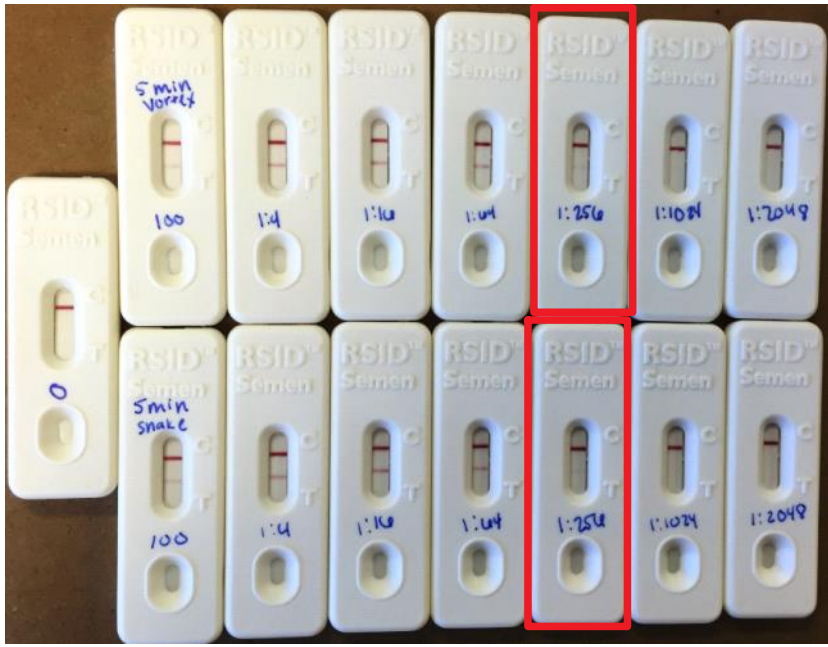
### Experiment #4: Comparing shaking vs vortexing for extraction of semen samples

**Protocol:** To determine if vortexing samples improved the signal when testing semen extracts, samples were either periodically shaken or vortexed for the following time points: 10 sec, 1 min, 5 min, 20 min, 30 min, and 1 hour. After the indicated time points, 500 µL was removed from each extraction and placed into a separate tube. Similar to experiment #1, serial dilutions of 1:4, 1:16, 1:64, 1:256, 1:1024, and 1:2048 were made from each extraction (please see table in experiment #1). For each time point and handling condition, 100 µL of the extraction and of each dilution was added to an RSID™-cassette and results were recorded after 10 minutes.

## Results

Similar results were obtained with a positive control swab extracted for 10 seconds with either vortexing or shaking (please see top figure below). Regardless of extraction time or handling condition, positive results were detected with RSID™-Semen up to 1:256 dilution of the semen extract from a positive control swab (please see red boxes below).





5 minutes



20 minutes





30 minutes



1 hour

### Summary and Overall Conclusions

Similar results were observed from extraction of semen from cotton swabs as short as 10 seconds up to one hour (please see experiment #1). In addition, extraction of lower volumes of semen (i.e. 2 and 5  $\mu\text{L}$ ) did not show a difference when extracting for 10 seconds or 20 minutes (please see experiment #2). There was no difference when extracting 5  $\mu\text{L}$ , 1  $\mu\text{L}$ , 0.5  $\mu\text{L}$ , and 0.1  $\mu\text{L}$  at 10 seconds, 1 minute, or 5 minutes (please see experiment #2.1). Regardless of extraction time, the results were similar for extractions of semen from various fabrics and aged samples (data not shown) as well as post-intercourse samples (please see experiment #3 and #3.1). In addition, vortexing extractions as compared to shaking did not increase the sensitivity since similar results were seen when both methods of handling the extractions were compared (please see experiment #4).

Based on these data, extraction of semen samples for as little as 10 seconds is sufficient for detection of semenogelin. Please note that longer incubation times are acceptable and the recommendations of 1-2 hours from the original protocol can remain in the protocol, if so desired.