Testing Shorter Incubation Times with

RSID™-Saliva

Experiment #1: Testing Shorter Extraction Times with **RSID™-Saliva** Using Positive Control Swabs

- **Protocol**

  Positive control swabs were produced by depositing 50 µL of saliva 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 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™ -Saliva cassette and results were recorded after 10 minutes. Cassettes from 10 sec, 5 min, 30 min, and 1 hour are shown below.

- Dilution Table -

			Volume 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 saliva extract amounts tested (see below).

Regardless of extraction time, positive results were detected with **RSID™-Saliva** up to the 1:256 dilution of the saliva extract from a positive control swab (as indicated by the red boxes, below).

10 Second Extraction Results

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5 Minute Extraction Results

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30 Minute Extraction Results

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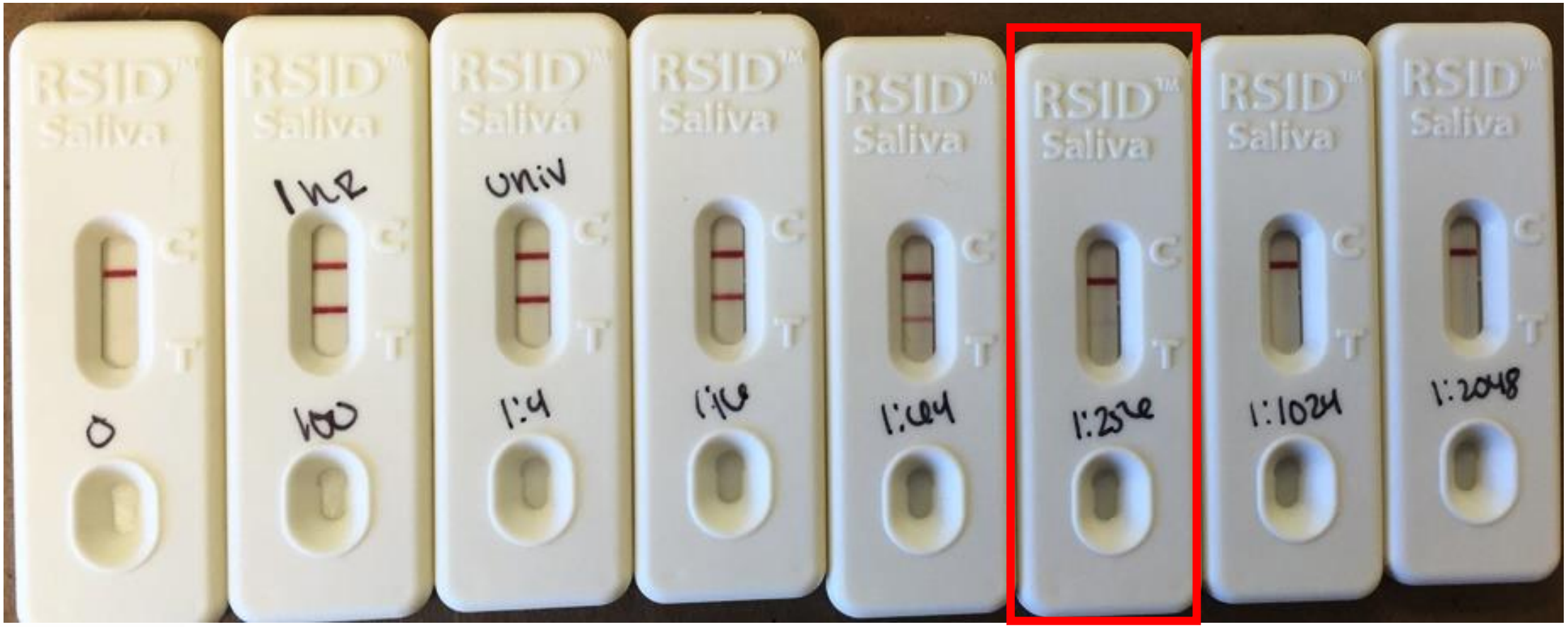
1 Hour Extraction Results



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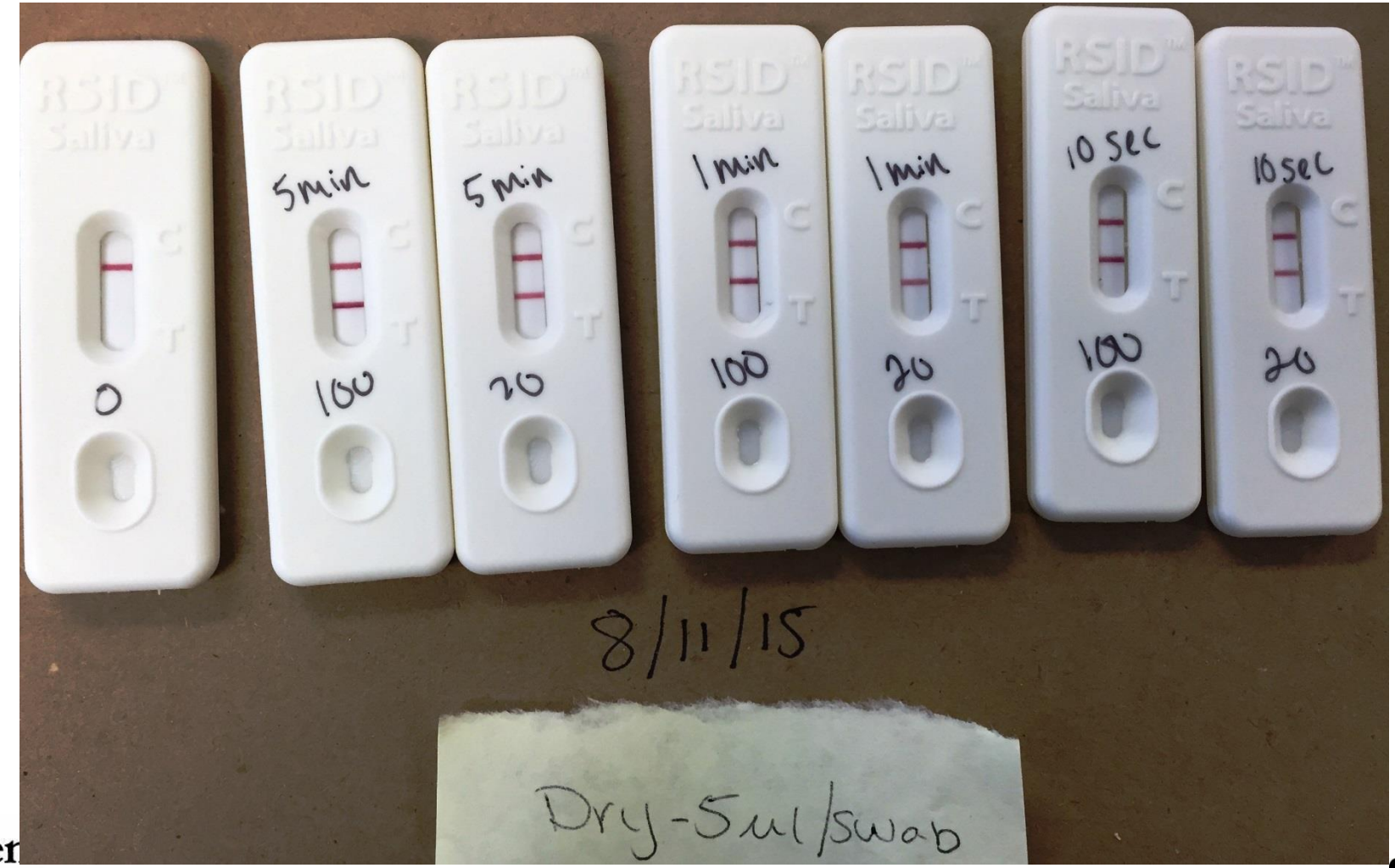
Experiment #2 - Shorter incubation times - test of sensitivity with lower volumes of saliva on control swab

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

Results:
Similar results for all dilutions were observed – no difference in sensitivity due to extraction times was seen for the 1 µL and 5 µL swabs or for 1 µL of 1:2 dilution or for the 1 µL of 1:10 saliva dilutions. Results are shown below; again, no difference in sensitivity at any extraction time was seen.

Extraction of 5.0 µL saliva from swab



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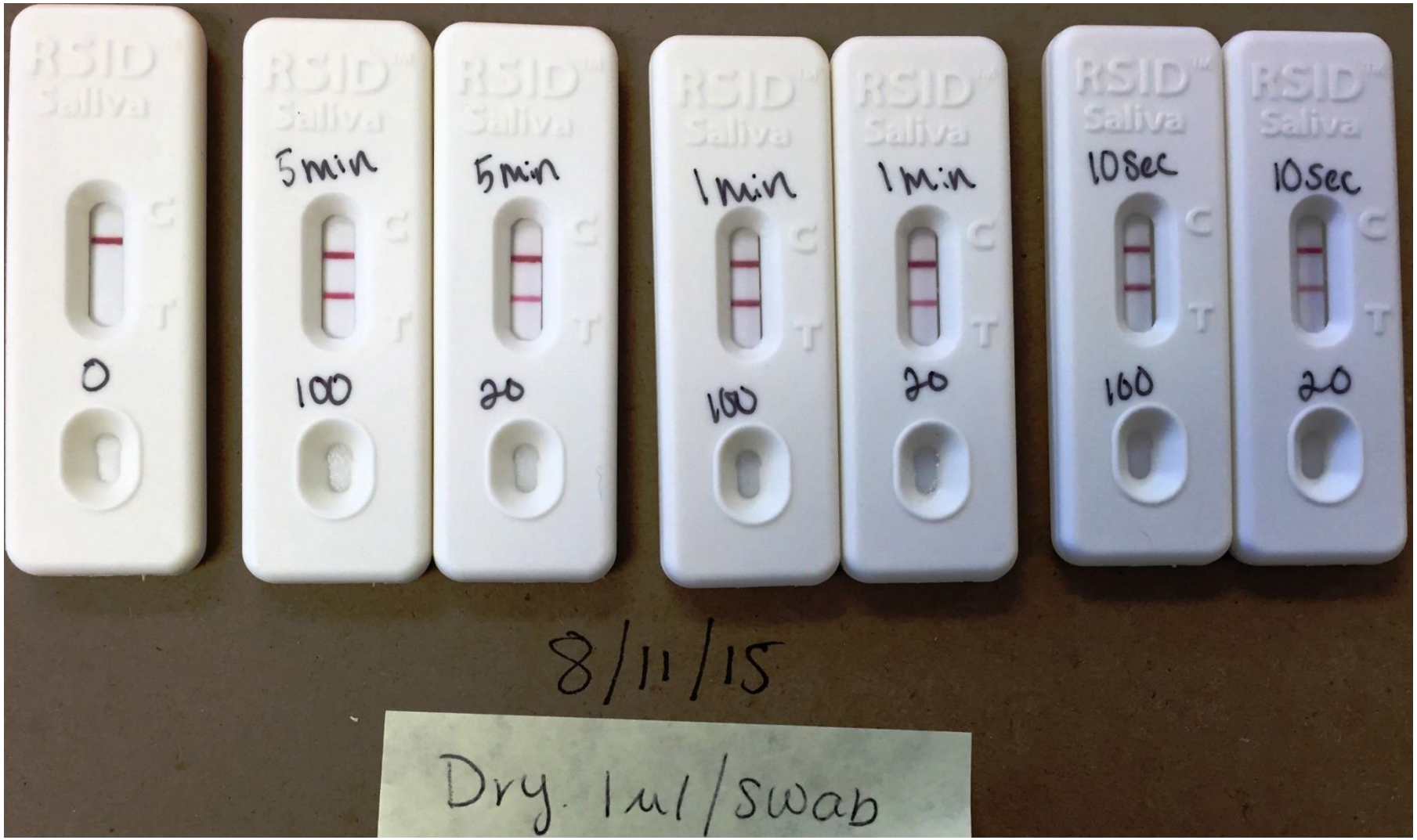
Extraction of 1.0 µL saliva from swab



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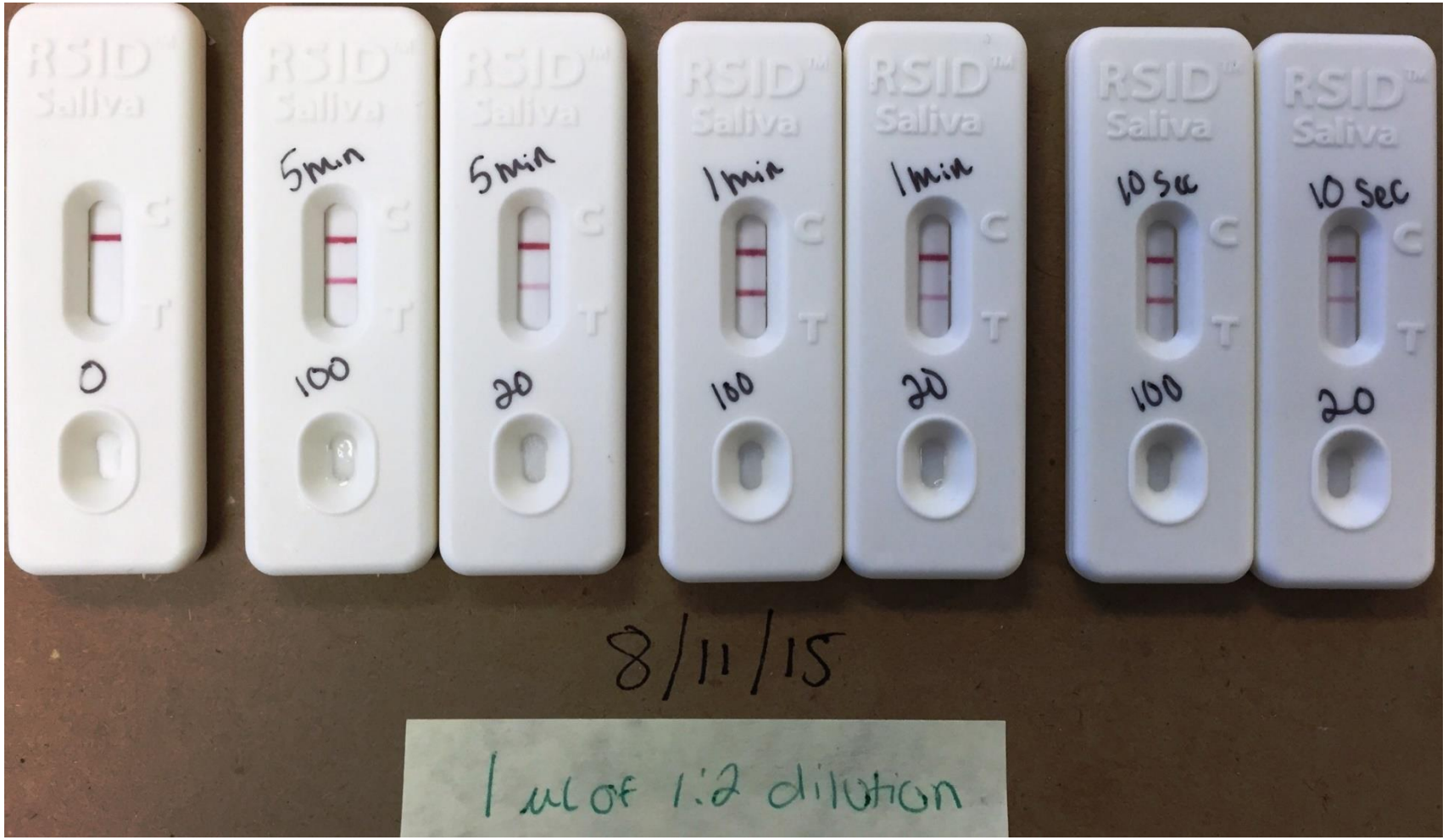
Extraction of 0.5 µL saliva (1 µL of 1:2 dilution) from swab



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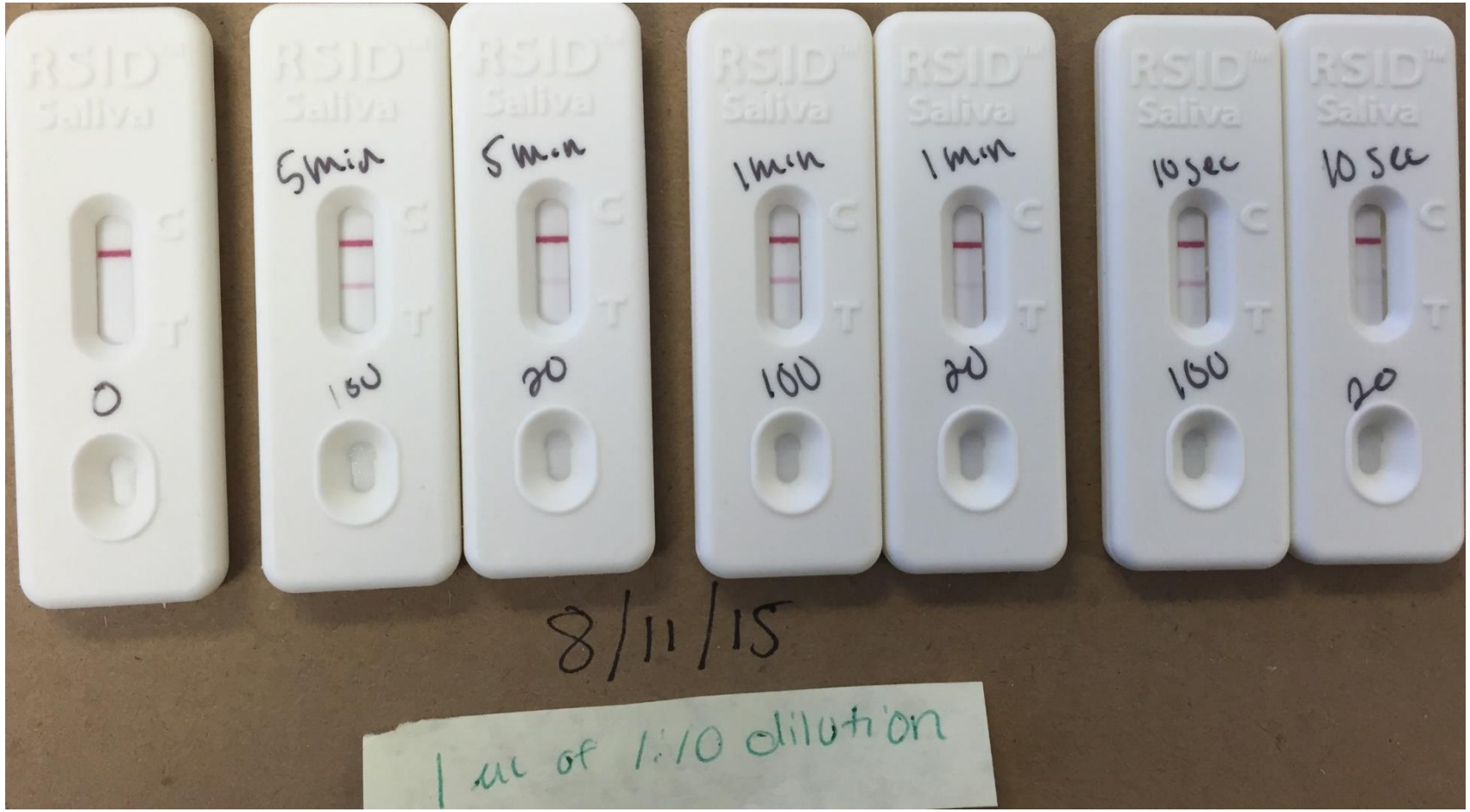
Extraction of 0.1 µL saliva (1 µL of 1:10 dilution) from swab



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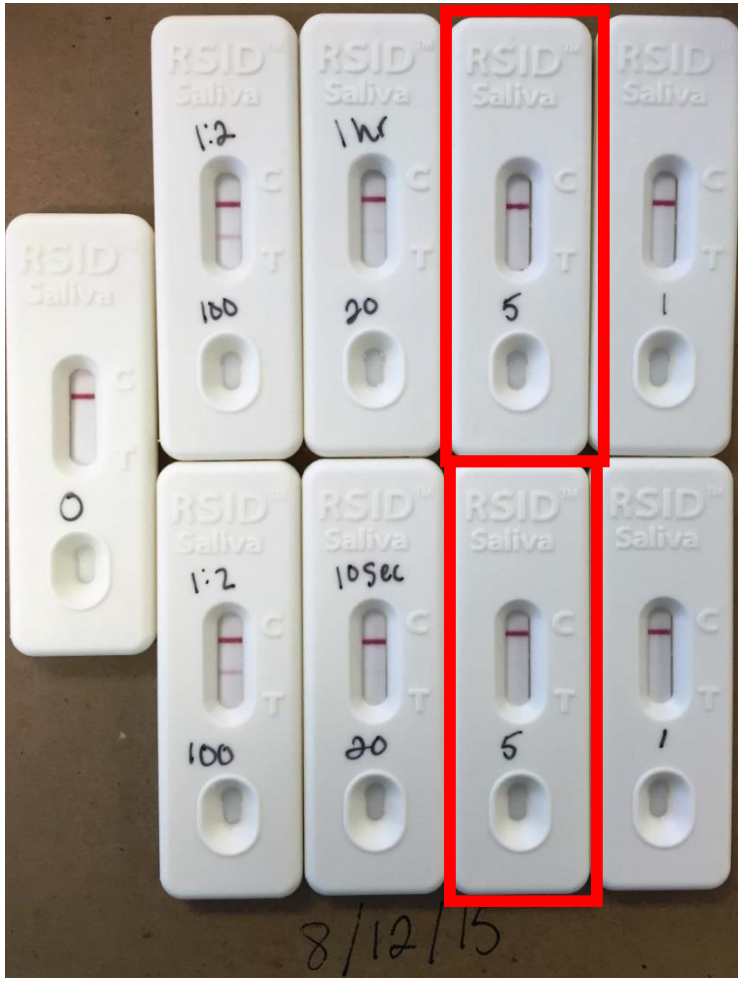
Experiment #2.1 - Shorter incubation times – further test of sensitivity

Protocol: Saliva swabs were prepared by depositing 1 µL of 1:2 saliva dilution (equivalent to 0.5 µL saliva) onto cotton swabs and allowing to air-dry. The swab heads were extracted in 300 µL extraction buffer for either 10 seconds (with shaking) or 1 hour (with occasional shaking), and 1, 5, 20 and 100 µL was loaded onto an RSID™-Saliva cassette. Results were recorded after ten minutes.



Results:
The same results were seen when comparing swabs extracted for 10 seconds as compared to 1 hour extraction. Weak positives were seen with 5 µL of extract volume, and the same test line intensity was seen for 20 and 100 µL extract volumes for both incubation times. For both incubation times, 1 µL extract was negative.

Experiment #3 - Shorter incubation times – extraction of older swab from 2009

Protocol: A swab from 2009 with 50 µL saliva was extracted in 300 µL extraction buffer for either 10 seconds (with shaking) or 1 hour (with occasional shaking) and 1, 5, 20 and 100 µL was loaded onto an **RSID™-Saliva** cassette. Results were recorded after ten minutes.

Results:
When comparing each extract volume tested, similar results were seen for both 10 sec and 1 hour incubation times.

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Experiment #4 - Shorter incubation times – Test with Dual Buffer

Protocol: Positive control swabs prepared with 50 µL saliva were extracted in 1 ml saliva dual extraction buffer for 10 seconds (with shaking), 5 min, 30 min, or 1 hour (occasional shaking for time points longer than 10 seconds). 1 µL of 1:10 extract dilution (0.1 µL saliva), 1 µL 1:2 extract dilution (0.5 µL saliva), 5, 20 and 100 µL extract were loaded onto RSID™-Saliva cassettes. Results were recorded after ten minutes.

Results: Similar results were obtained with a positive control swab extracted for 10 seconds (with shaking) as compared to a swab extracted for 5 min, 30 min and 1 hour at all saliva extract amounts tested (see below).

10 Second Extraction Results

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5 Minute Extraction Results

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30 Minute Extraction Results

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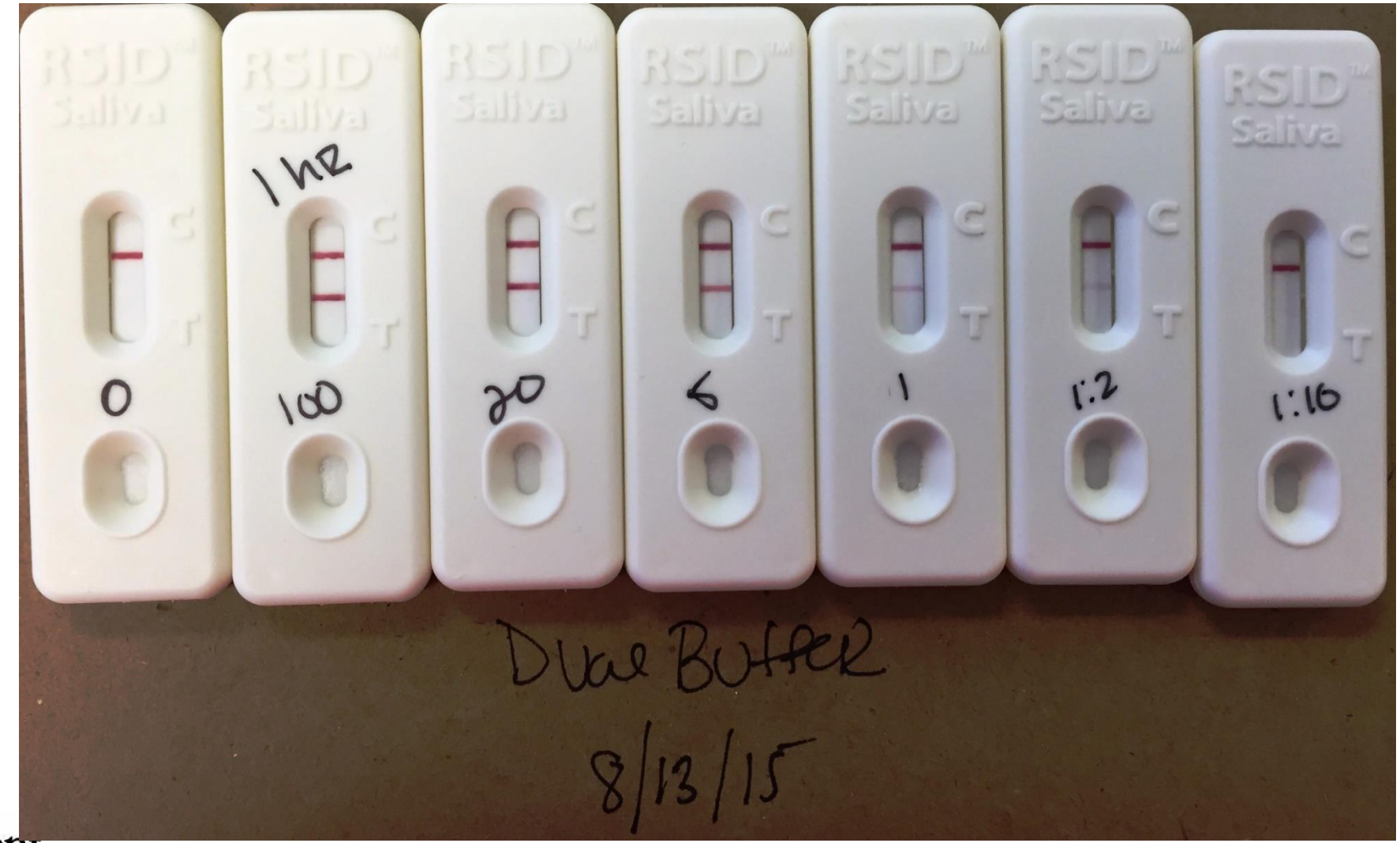
1 hour extraction



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Experiment #5 Shorter incubation times – Test with RSID™-Saliva field kit format

Protocol: Saliva swabs were prepared by depositing 5 µL, 1 µL, 1 µL of 1:2 saliva dilution (equivalent to 0.5 µL saliva) and 1 µL of 1:10 dilution (equivalent to 0.1 µL of saliva) onto cotton swabs and allowing to air-dry. The swab heads were extracted in field kit extraction buffer tubes which contain 750 µL buffer for either 10 seconds (with shaking), 1 minute, 5 minutes, 20 minutes, or 1 hour. For time points longer than 10 seconds, the tubes were shaken occasionally. Using the disposable transfer pipette included in the field kit, 5 drops was loaded onto an **RSID™-Saliva** cassette. Results were recorded after ten minutes.

Results: Similar results for the 1 µL and 5 µL swabs, the 1 µL of 1:2 dilution and the 1 µL of 1:10 saliva dilution were observed at all extraction times tested (see below). No difference in sensitivity at any extraction time was seen.

RSID™-Saliva field kit; extraction of 0.1 µL saliva (1 µL of 1:10 dilution)

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RSID™-Saliva field kit; extraction of 0.5 µL saliva (1 µL of 1:2 dilution)

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RSID™-Saliva field kit; extraction of 1 µL saliva

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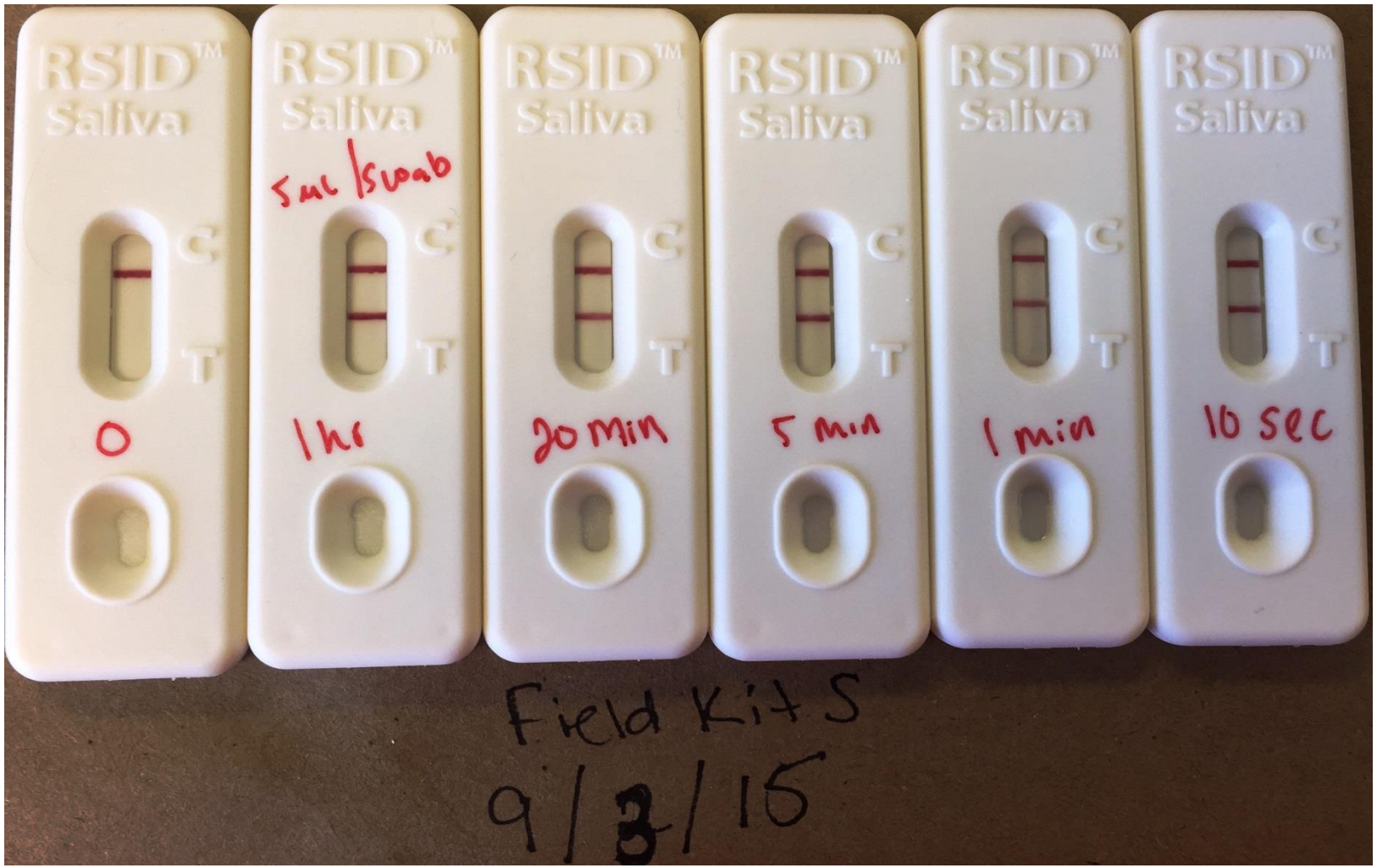
RSID™-Saliva field kit; extraction of 5 µL saliva



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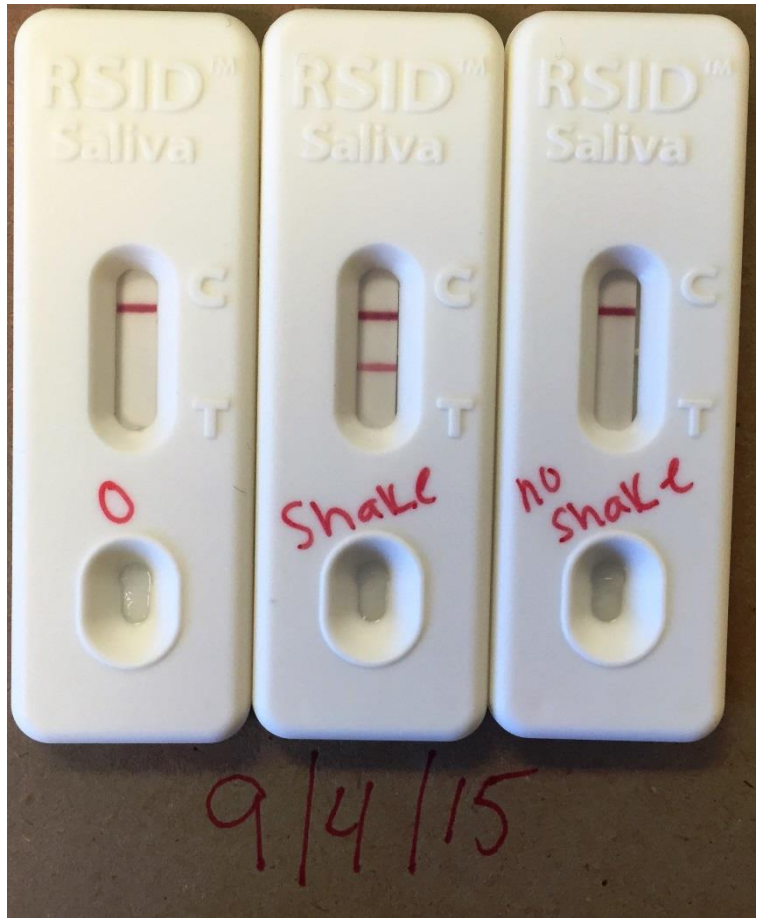
Expt. #6: Shaking vs. no shaking with **RSID™-Saliva** field kit

Protocol: To determine if shaking is required for optimal extraction efficiency, two saliva swabs prepared with 1 µl saliva each were extracted in field kit extraction tubes containing 750 µL extraction buffer for 10 seconds. During the 10 seconds, one tube was shaken continuously whereas the second tube was not shaken. Using the disposable transfer pipette included in the field kit, 5 drops was loaded onto an **RSID™-Saliva** cassette. Results were recorded after ten minutes.



Results: A strong positive was seen from the shaken tube whereas the tube that was not shaken was a very weak positive.

Summary and Overall Conclusions

Similar results were observed for extractions of our standard saliva positive control from cotton swabs using incubation times as short as 10 seconds to as long as one hour (see expt. #1). Extraction of lower volumes of saliva from cotton swabs also did not show a difference when extracting for 10 seconds, 1 minute, or 5 minutes (see expt. #2). Similar results were seen when extracting 0.5 µL saliva from swabs for either 10 seconds or 1 hour (see expt. #2.1). Regardless of extraction time, the results were similar for extractions of saliva from various fabrics (data not shown) and aged samples (see expt. 3). Similar results were seen when shorter incubation times were used with dual buffer (see expt. #4). There was no difference in sensitivity at any extraction time tested when using the **RSID™-Saliva** field kit format (see expt. #5). It is important to note that shaking during the 10 second incubation ***is required*** for optimal extraction efficiency (see expt. #6).

Based on these data, extraction of saliva samples for as little as 10 seconds is sufficient for detection of α-amylase without loss of sensitivity or specificity. Please note that longer incubation times are acceptable and the recommendations of 1-2 hours from the original protocol can remain, if so desired.