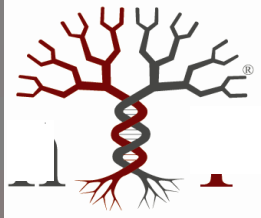


# Sensitivity, Specificity, & Suitability of RSID®-Saliva Confirmatory Test for Forensic Casework

Meaghan Roche, BS & Timothy Kupferschmid, MFS

Sorenson Forensics, Salt Lake City, UT



## Background

Screening methods for detecting saliva in forensic casework have traditionally taken the form of colorimetric biochemical assays to determine the activity of the enzyme amylase. Unfortunately, these methods are generally quite labor- and time-intensive, and they only function as presumptive tests. Independent Forensics (Hillside, IL) recently developed an ELISA-based immunochromatographic strip test that is specific for human salivary  $\alpha$ -amylase. However, the validation for this test process has not been published in a peer-reviewed journal, and thus we felt it was necessary to conduct an in-house validation.

At Sorenson Forensics, we conducted an extensive validation of this method in order to determine its suitability for use as a confirmatory saliva test in a forensic capacity. Included in this validation were liquid saliva and dried saliva stains, serial dilutions to assess sensitivity, body fluid testing to assess specificity, mixtures of saliva with other body fluids commonly encountered in forensic casework, species testing, and mock casework samples. Additionally, we tested different buffers to determine their effects on the test.

The RSID®-Saliva kit is an immunochromatographic membrane test which employs two mouse monoclonal antibodies that are specific for human salivary  $\alpha$ -amylase. One of these antibodies is deposited on the test pad in the sample well, and the other is affixed to the membrane at the test line indicated on the plastic cartridge. If human salivary  $\alpha$ -amylase is present in a sample, it will bind to the antibody when it is deposited in the sample well, creating an antibody-antigen complex. The sample travels by bulk fluid flow to the membrane phase of the test strip, where any antibody-antigen complexes that are present bind to the antibody on the membrane, causing a red line to appear at the test line indicated on the plastic cartridge. Finally, the bulk fluid flow moves to the area of the membrane labeled as the control line, where anti-mouse antibodies are affixed. The antibodies from the sample well bind here to create a red line, which serves as an internal control to ensure that the test strip is functioning properly. Thus, the presence of a red line in the control area and in the test area indicates a positive result, while the presence of a red line only in the control area indicates a negative result. Furthermore, if no line appears in the control area, the test is considered invalid and must be repeated. See Figure 1 below for photo of expected positive and negative control results.

Figure 1: RSID®-Saliva Kit Negative & Positive Controls



## Results

Specificity tests indicate that the RSID®-Saliva kit does not interact with a variety of body fluids that are commonly encountered in a forensic casework setting (see Table 1 below). The one notable exception to this specificity is breast milk, which may not be a true cross-reaction because breast milk is known to contain digestive enzymes.

Species specificity experiments indicate that higher primates, such as gorillas, produce a low-level cross-reaction with the RSID®-Saliva kit. Other species that were tested produced no detectable cross-reaction with the test (see Table 2 below).

The mock casework samples that were tested all produced positive results with both buffers (see Table 3 below).

Mixture studies indicate that saliva can be detected using the RSID®-Saliva kit at a ratio of 10:1 with other body fluids, which is significant for forensic casework purposes due to the likelihood of receiving evidence samples that contain mixtures (see Table 4 below).

Sensitivity studies show that liquid saliva is detectable with both TE<sup>-4</sup> and TBS<sup>-</sup> buffers at dilutions nearing 1:10,000 (see Table 5 below and Figure 2 to right), while dried saliva stains are detectable at dilutions of up to 1:1,000 with both buffers (see Table 6 below).

Figure 2: RSID®-Saliva Kit Results on Liquid Saliva Dilutions

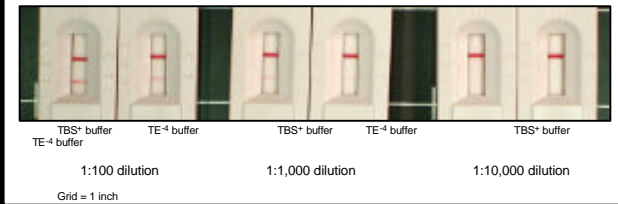


Table 1: Body Fluid Specificity of RSID®-Saliva

	Blood	Semen	Saliva	Vaginal swab	Urine sample	Breast milk	Sweat	Tears
<b>TBS<sup>-</sup> buffer</b>	(-)	(-)	+	(-)	(-)	+(wk)	(-)	(-)
<b>TE<sup>-4</sup> buffer</b>	(-)	(-)	+	(-)	(-)	+(wk)	(-)	(-)

Table 4: Mixture Studies of RSID®-Saliva Kit

	Blood : saliva	Semen : saliva	Vaginal swab : saliva	Urine : saliva
<b>TBS<sup>-</sup> buffer</b>				
1:1 ratio	+	+	+	+
5:1 ratio	+	+	+(wk)	+
10:1 ratio	+(wk)	+(wk)	+(wk)	+
<b>TE<sup>-4</sup> buffer</b>				
1:1 ratio	+	+	+	+
5:1 ratio	+	+	+(wk)	+
10:1 ratio	+	+(wk)	+(wk)	+(wk)

Table 2: Species Specificity of RSID®-Saliva Kit

	Charlie (terrier mix)	Smokoy (Russian blue cat mix)	Bug (leopard gecko)	Papa (black & white ruffed lemur)	Aquila (black & white ruffed lemur)	Tino (Western Lowland gorilla)
<b>TBS<sup>-</sup> buffer</b>	(-)	(-)	(-)	(-)	(-)	+(wk)
<b>TE<sup>-4</sup> buffer</b>	(-)	(-)	(-)	(-)	(-)	+(wk)

Table 5: Sensitivity of RSID®-Saliva Kit with Liquid Saliva

	Neat saliva	1:100 dilution	1:1,000 dilution	1:9,000 dilution	1:10,000 dilution
<b>TBS<sup>-</sup> buffer</b>	+	+	+	+(wk)	(-)
<b>TE<sup>-4</sup> buffer</b>	+	+	+	+(wk)	(-)

Table 3: Mock Casework Samples

	Plastic coffee cup lid	Plastic water bottle lip	Plastic straw	Aluminum can lip
<b>TBS<sup>-</sup> buffer</b>	+	+	+	+
<b>TE<sup>-4</sup> buffer</b>	+	+	+	+(wk)

Table 6: Sensitivity of RSID®-Saliva Kit with Dried Saliva Swabs

	Neat sample	1:100 dilution	1:1,000 dilution	1:2,000 dilution
<b>TBS<sup>-</sup> buffer</b>	+	+	+(wk)	(-)
<b>TE<sup>-4</sup> buffer</b>	+	+	+(wk)	(-)

## Methods

All experiments were conducted in accordance with the manufacturer's specifications. The product insert enclosed in each case of RSID®-Saliva kits recommends extracting known samples in 1ml of PBS buffer and evidence samples in 200-300µl of PBS buffer for 1-2 hours at room temperature. However, it is noted that other buffers may be used for extraction. Because PBS buffer is not included in the RSID® saliva kit, and because the buffer used routinely by Sorenson Forensics is an in-house preparation of TE<sup>-4</sup> buffer (pH = 8.0), this validation was performed using both in-house TE<sup>-4</sup> buffer and the provided TBS<sup>-</sup> buffer to extract samples. The product insert further instructs the user to combine 20µl of extract with 80µl of the provided TBS<sup>-</sup> buffer and to load this 100µl volume into the test cartridge's sample well, recording results after ten minutes.

All of the samples tested in this validation, regardless of the buffer they were extracted in, were added to the sample well in the following manner: 20µl of extract was deposited in the sample well, followed by 80µl of the provided TBS<sup>-</sup> buffer. Samples were incubated at room temperature for varying amounts of time, ranging from 15 minutes to two hours. Depending on the sample size (snippet, ½ swab, or whole swab head), the amount of extraction buffer (TE<sup>-4</sup> or TBS<sup>-</sup>) also varied, ranging from 200µl to 1ml.

The specificity experiment samples were prepared as follows: 50µl of the appropriate body fluid was deposited on a sterile cotton swab head and allowed to air-dry. After drying, the entire head was removed from the swab using sterile technique and transferred to a 1.5ml microcentrifuge tube.

Extracts from the specificity experiment were combined at ratios of 1:1, 5:1, and 10:1 for the mixture studies. For the species specificity study, oral swabs were collected from three domestic animals and three primates: a mixed-breed (terrier mix) dog, a mixed-breed (Russian blue mix) cat, a leopard gecko, two black and white ruffed lemurs, and a Western Lowland gorilla. A snippet from each swab was removed using sterile technique and placed in a 1.5ml microcentrifuge tube.

To test sensitivity, a dilution series was prepared using both liquid and dried saliva. The "neat" liquid saliva sample consisted of 20µl of liquid saliva and 80µl of TBS<sup>-</sup> buffer, while the "neat" dried saliva sample consisted of 20µl of extract from ½ of a dried saliva swab (placed into a microcentrifuge tube and incubated at room temperature in 300µl of extraction buffer) and 80µl of TBS<sup>-</sup> buffer.

For the mock casework samples, items that closely mirror forensic casework samples were collected from laboratory personnel, including a plastic straw, aluminum soda can, plastic coffee cup lid, and plastic water bottle. A sterile cotton swab was used to collect a sample from the area of each substrate that was most likely to have been in direct contact with human mouths. This cotton swab was cut in half using sterile technique, and each half was placed into a microcentrifuge tube.

## Discussion

High levels of sensitivity and body fluid specificity, as well as positive results from mixtures and mock casework samples, indicate that this test is robust and reproducible. Weak positive results were obtained when oral swabs from a Western Lowland gorilla were tested with the RSID®-Saliva kit, which indicates that saliva from higher primates may cross-react with the test. Further studies could address this issue by testing oral swabs from other higher primates such as chimpanzees. Overall, this validation demonstrates the suitability of the RSID®-Saliva kit as a confirmatory saliva test for use in forensic casework.

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## Contact Information

Meaghan Roche can be reached at [mroche@sorensonforensics.com](mailto:mroche@sorensonforensics.com) and Timothy Kupferschmid can be reached at [kupfers@sorensonforensics.com](mailto:kupfers@sorensonforensics.com). You can also visit the Sorenson Forensics website at [www.sorensonforensics.com](http://www.sorensonforensics.com) for further information regarding our casework and validation services.