

Procedure for Saliva Analysis

1.0 Purpose - This procedure specifies the method of performing saliva analysis in forensic casework.

2.0 Scope - This procedure applies to those Forensic Scientists who have been released to do saliva analysis in forensic casework.

3.0 Definitions

- **α -Amylase**- an enzyme found in high concentrations in saliva which is responsible for the breakdown of starch into simple sugars.

4.0 Equipment, Materials and Reagents

- Sterile disposable scissors or sterile scalpel blade
- Known saliva sample
- RSID kits which contain the test cards and universal buffer
- 1.5 mL centrifuge tube

5.0 Procedure

5.1 Requirements for testing:

5.1.1 Saliva analysis shall be performed on vaginal swabs (including, but not limited to, external vaginal swabs and others similarly identified), penile swabs, and the underwear from the victim in cases where no semen or sperm was detected during initial screening of that item under the following circumstances.

5.1.1.1 When the victim has stated that cunnilingus or fellatio has occurred.

5.1.1.2 When it is unknown whether cunnilingus or fellatio has occurred because the victim was unconscious or deceased, or because the information provided is incomplete.

5.1.1.3 When the suspect is alleged to have licked his/her fingers before digitally penetrating the victim.

5.1.1.4 To validate or refute a statement made by a victim, alleged suspect, witness, or other individual involved in the case.

5.1.1.5 Saliva analysis will not be performed on areas that could be contaminated with feces.

5.1.2 Before a sample that tested positive with the RSID-Saliva according to **5.1.1** is sent for DNA analysis, confirmatory semen/sperm testing shall be done.

5.1.3 Saliva analysis shall be performed on any item to validate a statement made by the victim if no other evidence is available.

5.2 RSID-Saliva

5.2.1 Procedure

- 5.2.1.1 Cut a small sample, approximately 0.5 cm² (depending on the concentration of the stain), from the evidence sample using sterile disposable scissors or a sterile scalpel blade and place the cutting into a 1.5 mL centrifuge tube.
- 5.2.1.2 Add a minimum of 300 µL, up to 1 mL, of RSID universal buffer to each sample and mix well. (The amount of buffer added will depend on the sample size; buffer should cover the sample completely.)
- 5.2.1.3 Allow the sample to extract for 2 hours. For weaker or older samples, Forensic Scientists should use a larger quantity of material and/or an extended extraction time to include overnight (not to exceed 24 hours).
- 5.2.1.4 After completing the extraction process, pipette 20 µL of the extracted sample into 80 µl of universal buffer and add this entire 100 µl into the sample well on the RSID card.

5.2.2 Results

- 5.2.2.1 A positive reaction will have two lines appear in the test window. One line will appear in the area marked "C" for control and one line will appear in the area marked "T" for test. A positive result may be recorded as soon as both of these lines appear, but after no longer than 10 minutes. The lines must be reddish in color.
- 5.2.2.2 If a line does not appear in the "T" area within 10 minutes, the test is considered negative. A line must appear at the area marked "C" to ensure that the test is working properly.
- 5.2.2.3 If no line appears at the area marked "C," the test shall be repeated. If no line is seen in the "C" window in the repeated test, the test is considered inconclusive. If this occurs, the Body Fluid Technical Leader shall be notified as soon as possible. Refer to Forensic Biology Section Administrative Policy and Procedure.
- 5.2.2.4 The RSID reader shall be used to read the test cards immediately following the 10 minutes.
 - 5.2.2.4.1 Turn on the RSID Reader using the power button. Note: The stylus must be used to make selection choices on the reader screen.
 - 5.2.2.4.2 Select RSID icon on the Main Menu screen.
 - 5.2.2.4.3 Select RSID-Saliva on the Select Test Screen. Then select Run Tests.
 - 5.2.2.4.4 Using the alpha or numeric keys, enter the name of the sample. Select Done. Review the sample name. If name is correct, select Proceed. If name is incorrect, select Back and enter the correct name.
 - 5.2.2.4.5 Insert test card into the reader until the green status light is engaged. The Test Area of the cassette must enter the reader's port first and face out to ensure proper positioning. The message, "Analyzing Cassette. Please

Wait,” will appear on the screen. Both the positive and negative controls shall be read first, followed by the unknown card(s).

Note: Take care to insert the card completely and do not remove it prior to results being obtained by the reader.

5.2.2.4.6 Read the results from the reader. These results are the results that shall be reported.

Note: The reader notes inconclusive as invalid. If the reader reports an invalid result, the test shall be repeated. If the repeated test is inconclusive (invalid), the Body Fluid Technical Leader shall be notified as soon as possible.

5.2.2.4.7 Select Finish. Continue with 5.2.2.4.3 to continue testing any additional cards. When all cards are read, select the home icon.

5.2.2.4.8 Turn the RSID Reader off using the power button.

5.3 Reporting guidelines – The results statements shall reflect only the work that is performed. Portions of the statements may be omitted to address testing actually performed. This interpretation may include or build upon one (1) or more of the following responses depending on the circumstances of the case and the nature of the examination.

5.3.1 This phrase shall be used when the RSID Saliva test is negative:

Examination of sample(s) taken from ____ (Item(s) ____), using the RSID Saliva Test and the RSID reader, failed to indicate the presence of human saliva. No confirmatory saliva testing was performed.

5.3.2 This phrase shall be used when the RSID Saliva test is positive.

Examination of sample(s) taken from ____ (Item(s) ____), using the RSID Saliva Test and the RSID reader, indicated but is not specific for the presence of human saliva. No confirmatory saliva testing was performed.

5.3.3 This phrase shall be used when the RSID Saliva test reads invalid:

Examination of sample(s) taken from ____ (Item(s) ____), using the RSID Saliva Test and the RSID reader, failed to give conclusive results for the indication of human saliva. No confirmatory saliva testing was performed.

5.3.4 This phrase shall be used when the RSID Saliva test is positive and samples are not being sent for STR DNA analysis:

This sample may be suitable for YSTR DNA testing. If you need further information regarding this sample(s), please do not hesitate to contact the Forensic Scientist or the Forensic Biology Forensic Scientist Manager at the North Carolina State Crime Laboratory.

5.4 Controls (to be set up on every case or every batch of cases):

RSID-Saliva: A positive control (applicable body fluid standard), and a negative control (100 µL of universal buffer) shall be run with every case or every batch of cases and the results will be recorded in the case notes as a positive or negative. If a reddish line is seen in the negative control “T” area, the test shall be rerun. If a reddish line appears again in the negative control “T” area, the test shall be considered inconclusive. If this occurs, the Technical Leader shall be notified immediately.

6.0 Limitations - Limitations include, but are not limited to, the following: Because the RSID saliva test can cross react with breast milk, the statement used in the report is “indicated but is not specific for the presence of human saliva” instead of “revealed the presence of human saliva.”

7.0 Safety - N/A

8.0 References

Forensic Biology Section Body Fluid training documents

Forensic Biology Section Procedure for Calibration and Maintenance

Forensic Biology Section Procedure for Aseptic Technique and Contamination Control

9.0 Records - N/A

10.0 Attachments - N/A

Revision History		
Effective Date	Version Number	Reason
10/26/2012	1	Original Document - Combined Procedure for Phadebas test and RSID-saliva portion of the RSID Procedure. Added reporting guidelines and allowed for changes to be made by the Forensic Scientist to address the testing actually performed. Removed +/- as a result choice for phadebas test
12/07/2012	2	5.2.1 - Added wording to clarify required analysis on an item that has multiple areas being tested.
02/15/2013	3	5.3.5 – changed “no further confirmatory” to “no confirmatory” 6.0 – clarified wording
09/13/2013	4	4.0 – removed phadebas equipment and reagents; 5.1 – removed phadebas requirements and reworded what sample types would be tested for saliva; 5.1.1.5 – removed limited sample statement; 5.1.2 – removed phadebas procedure; 5.2.1 – removed RSID-saliva requirements (now in 5.1); 5.3.1, 5.3.2, 5.3.5 – removed reporting guidelines for phadebas testing results; 5.4.1 – removed limitations for phadebas; 6.0- removed controls for phadebas

12/18/2013	5	Header – added issuing authority
08/29/2014	6	5.1 – removed underwear for saliva testing; 5.2.1.3 – adjusted extraction times; 5.2.2.3 – clarified wording; 5.2.2.4 – added RSID reader; 5.3.3 – added reporting statement for inconclusive result
02/27/2015	7	5.1 – updated testing requirements; 5.2.2.4 – required RSID reader to be used; 5.3.4 – added YSR statement for reports
12/28/2015	8	5.1.1 – updated wording, added clarification ; 5.2.1.2, 5.2.1., 5.2.1.4 – updated procedure as a result of validation; 5.3.1, 5.3.2, 5.3.3 – updated results statements; 6.0 – updated limitations statement wording