

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

- Phadebas tablets
- Deionized water
- 13 x 100 mm disposable culture tubes
- Vortex
- Sero-fuge
- Incubator set at 37 °C
- 0.5 N NaOH (see QC procedure)
- 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 Phadebas

5.1.1 Requirements for testing: Saliva analysis should be performed in all cases when requested and additionally, without a request, under the following circumstances:

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

5.1.1.2 Where the alleged suspect has licked, or possibly licked, his/her fingers before penetrating the victim.

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

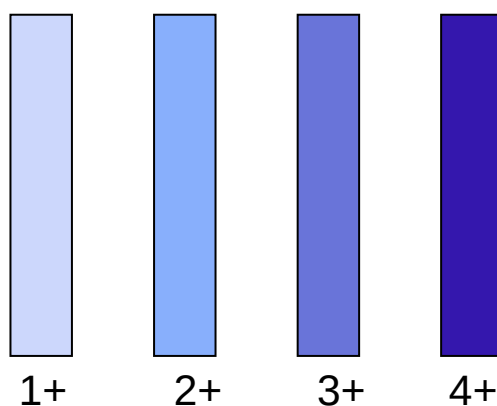
5.1.1.4 Saliva analysis will not be performed on rectal swabs or other areas that could be contaminated with feces.

5.1.1.5 If limited sample is available only the RSID-Saliva test shall be run.

5.1.2 Procedure

- 5.1.2.1 Using sterile disposable scissors or scalpel blade, make approximately ¼ cm squared cutting of the suspected stain or the tip of each swab and place the cutting in a 13 x 100 mm labeled culture tube.
- 5.1.2.2 Add one Phadebas tablet to the tube.
- 5.1.2.3 Add 4 mL deionized water to the culture tube and vortex the mixture for a few seconds or until the material is mixed well.
- 5.1.2.4 Incubate at 37 °C for thirty minutes.
- 5.1.2.5 If the test is not going to be observed immediately following the 30 minute incubation, add 1 mL of 0.5 N NaOH to stop the reaction.
- 5.1.2.6 Vortex and spin in a sero-fuge for five minutes.
- 5.1.2.7 Evaluate the supernatant for the presence of a blue color. Note the chart below for the appropriate grading of the color. Color will be noted as negative if it is absent or below the 1+ value.

Phadebas Table



5.2 RSID-Saliva

- 5.2.1 **Requirements for testing:** If the Phadebas is 1+ or greater, a RSID-Saliva test shall be run. If multiple areas tested from one item give a positive Phadebas result, the RSID-Saliva test need be run only from the area that gives the strongest color change unless there is a reason to believe more than one saliva donor may be present on an item.

5.2.2 Procedure

- 5.2.2.1 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.

5.2.2.2 Add a minimum of 150 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.)

5.2.2.3 Allow the sample to extract for a minimum of 15 min. For weaker or older samples, Forensic Scientists should use a larger quantity of material and/or an extended extraction time to include overnight.

5.2.2.4 After completing the extraction process, pipette 100 µL of the extracted sample into the sample well on the RSID card.

5.2.3 Results

5.2.3.1 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.

5.2.3.2 If a line does not appear in the "T" area within ten minutes, the test is considered negative. A line must appear at the area marked "C" to ensure that the test is working properly.

5.2.3.3 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.

5.3 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 if the Phadebas Test is negative:

Examination of a sample(s) taken from _____ (Item(s) ____), using the Phadebas Test, failed to reveal elevated levels of amylase, which is an indicator of, but not specific for, saliva.

5.3.2 This phrase shall be used when the presumptive test (Phadebas) is positive:

Examination of a sample(s) taken from _____ (Item(s) ____), using the Phadebas test, revealed elevated levels of amylase, which is an indicator of, but not specific for, saliva.

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

Further examination of sample(s) taken from ____ (Item(s)____), using the RSID Saliva test, failed to give reactions consistent with the presence of human saliva.

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

Further examination of sample(s) taken from ____ (Item(s)____), using the RSID Saliva test, gave reactions consistent with the presence of human saliva.

5.3.5 This phrase shall be used when no confirmatory saliva testing is done.

No confirmatory saliva testing was performed.

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

5.4.1 Phadebas: A positive control which is a known saliva sample. A negative control which contains only the reagents. A substrate control, if available. A substrate control is a cutting taken from an apparently “unstained” area of the same material that the suspected stain is cut from.

5.4.2 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: The Phadebas test is used for the detection of the enzyme α -amylase. This enzyme is also found elsewhere in the body, including blood and semen, though the levels are much greater in saliva.

RSID-Saliva- Because the RSID saliva test can cross react with breast milk, the statement used in the report is “gave reactions consistent with 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