TEST FOR AMYLASE

16.1 PURPOSE

16.1.1: To determine the presence of amylase in Forensic samples utilizing the Phadebas® Test.

A. Theory

1. Amylase is a digestive enzyme that catalyzes the breakdown of starch into simple sugars and is generally found in very high levels in saliva. The Phadebas® Test may be used to to detect the presence of amylase in stains.

2. The Phadebas® reagent consists of starch polymers which are bonded to blue dye molecules. In water, these starch polymer/dye complexes are insoluble. However, in the presence of amylase, these starch polymer/dye complexes are digested, liberating the blue dye and changing the solution from clear to blue.

3. The Phadebas® Test is specific for detecting amylase activity.

B. Limitations

1. This test is not human specific.

2. Amylase is generally found in much higher concentrations in saliva and fecal material, and can be found in other body fluids as well.

3. This test does not differentiate between salivary amylase and pancreatic amylase.

16.1.2: To quality control new Phadebas® reagent.

16.2 RESPONSIBILITY

16.2.1: Test Procedure – Personnel qualified to perform Forensic Biology duties.

16.2.2: Preparation/QC Procedure – Personnel qualified to perform Forensic Biology duties. Ordering information is maintained in a log book and/or electronically in the Forensic Biology Unit. New reagents and kits are purchased according to GL-6 (Purchasing). For additional information, refer to the Biological Inventory located in Appendix 3.

16.3 SAFETY

Use appropriate measures for the proper handling of biohazardous material according to GL-2 (Safety Manual) and the Safety Data Sheets.

16.4 DEFINITION/ABBREVIATIONS

A. RSID™: Rapid Stain Identification
B. QRW(s): Quality Record Worksheet(s) (Appendix 1)

16.5 TEST PROCEDURE

A. This test will be performed at the discretion of the examiner, with input from the Unit Lead(s) when applicable, based on the submitting agency requests, case information and the condition of the evidence.

B. A sample is considered limited when it appears to be of low quantity and compromised when it appears to be in poor condition. The conditions the evidence may have been exposed to prior to submission shall be considered when assessing the sample tested and/or collected.

16.5.1: Materials

A. Phadebas® powder
B. dH_2O
C. RSID™-Universal Buffer
D. Controls: positive (known saliva stain) and negative (blank substrate),
additional controls as needed: positive (mixture of known saliva stain and known blood stain) and negative (known blood stain), substrate control from the evidence
E. Samples previously extracted in RSID™-Universal Buffer
F. Disposable pipets
G. Test tubes
H. Filter paper
I. Forceps
J. Scissors/scalpel(s)

16.5.2: Procedure

A. Record the extraction solution(s) used on the appropriate QRW(s) and the lot numbers on the General Reagent Sheet (FBQR-09).

B. Test a positive and negative control with the following procedure (steps D - K).
1. The controls may be run concurrently with the questioned samples as an intermediate check. Reagent QC is always conducted prior to use on case samples. The controls must be made with the same extraction solution used for the questioned samples.

A. If the sample(s) being tested for amylase contain(s) blood, run the following additional controls for interpretation purposes: positive (mixture of known saliva stain and known blood stain) and negative (known blood stain).

B. Run a substrate control from the evidence if you suspect interference.

2. If the questioned sample is limited, run the controls prior to testing the questioned sample. If the controls yield the appropriate results then immediately test the questioned sample.

3. If the controls do not yield the appropriate results, review the procedure and retest the controls prior to the questioned sample. If the controls still do not yield the appropriate results, then inform the Unit Lead to try to determine the root cause.

C. For a questioned liquid sample, prepare a stain on filter paper or a swab, air dry and proceed with questioned stain testing.

D. For questioned stains:

1. In a labeled glass test tube, combine a portion of the questioned stain, Phadebas® powder (approximately 0.025g) and a sufficient amount of dH_2O to allow easy observation of the supernatant color.

2. Agitate/vortex each test tube to mix the contents.

3. Incubate at 37°C for a minimum of 15-20 minutes.

4. Agitate/vortex each tube to re-suspend the contents and centrifuge for approximately one (1) minute.

5. Observe the color of the supernatant of each sample.

E. For questioned extracts:

1. Remove the supernatant of the questioned extract (i.e. RSID-Universal Buffer or dH_2O) from its original tube leaving the pellet intact, if present.

2. Combine with Phadebas® powder (approximately 0.025g) in a labeled glass test tube. A sufficient amount of the same extraction solution may be added to allow easy observation of the supernatant color.

3. Follow steps 2-5 in paragraph D above.

F. The negative control may be used to aid in the interpretation of the results.

G. Record the results of the controls and samples on the appropriate QRW(s).

H. A second qualified examiner will observe/confirm the results and initial/date the appropriate QRW(s).

16.5.3: Results and Conclusions

A. Positive

1. The supernatant will be blue, indicating the presence of amylase activity.

2. Positive results may be designated and recorded as follows:
   ↓/vw(+) = very weak positive = very light blue
   w(+) = weak positive = light blue
   (+) = positive = blue
   ↑/s(+) = strong positive = dark blue

   The description of a positive result may vary between examiners. This variation is acceptable since all descriptions designate a positive result.

3. Suggested Report Wording:

   a.

Testing Performed	**Result**	**Conclusion**
Screening - Amylase	Positive	Amylase detected

Appendix:
Amylase is a component of saliva. This test does not determine the origin of the detected amylase.

   b. *[ ] gave a positive result(s) with a color screening test for the presence of amylase, a component of saliva.*

B. Negative

1. The supernatant will be clear, indicating the absence of amylase activity.

2. Suggested Report Wording:

   a.

Testing Performed	**Result**	**Conclusion**
Screening - Amylase	Negative	Amylase not detected

Appendix:
Amylase is a component of saliva. This test does not determine the origin of the detected amylase.

   b. *A color screening test for the presence of amylase, a component of saliva, was performed on [ ]. Amylase was not detected with this test.*

C. Inconclusive

1. If a blue color change of the supernatant could not be determined (i.e. when blood is present or there is interference from the substrate).

2. Suggested Report Wording:

a.

Testing Performed	**Result**	**Conclusion**
Screening - Amylase	Indeterminate	Inconclusive[1]
Comment: [1]Due to an indeterminate result and/or substrate interference, this test was determined to be inconclusive.		

Appendix:
Amylase is a component of saliva. This test does not determine the origin of the detected amylase.

b. *A color screening test for the presence of amylase, a component of saliva, was performed on [ ]. Due to indeterminate results and/or substrate interference, this/these test(s) was/were determined to be inconclusive.*

3. Record the reason a result is determined to be inconclusive on the appropriate QRW(s).

16.6 QC PROCEDURE

16.6.1: Materials

A. Phadebas® tablets
B. Mortar and pestle

16.6.2: Procedure

A. Crush tablets into a powder and return to original container.

B. Test each new lot before use with case samples according to the test procedure and the Phadebas® Reagent Log Sheet. Record the required information.

A second qualified examiner will observe/confirm the results and initial/date the Phadebas® Reagent Log Sheet.

C. If the appropriate results are not obtained, review the procedure and repeat the test. If the reagent still does not yield the appropriate results, then inform the Unit Lead to try to determine the root cause.

D. If the lot is acceptable for use, record the date received, date opened and examiner's initials on the bottle and store at room temperature.

The Phadebas® reagent is acceptable for use when a positive result is obtained with the saliva control and a negative result is obtained with the blank filter paper/negative control.

E. Discard/replace according to the manufacturer's expiration date or according to 21.4.3.E in FB SOP-21 (General Chemical and Reagent QC).

Manufacturer's expiration dates with only month and year indicated (i.e. 04/2014) expire the last day of the month noted.

F. Please refer to FB SOP-13 (Extraction of Samples for Semen) for the QC procedure for RSID™-Universal Buffer.

16.7 REFERENCES

A. Willott, G.M. 1974. "An improved test for the detection of salivary amylase in stains". J. Forensic Sci. Soc., 14: 341-344.
B. Li, Richard, Forensic Biology, Second Edition, CRC Press, FL, 2015: Chapter 15: "Identification of Saliva", pp. 277-281 and p. 282 (Figures 15.6 and 15.7).
C. GL-2 (Safety Manual)
D. GL-6 (Purchasing)
E. Safety Data Sheets