TEST FOR AMYLASE

16.1 PURPOSE

16.1.1: To determine the presence of amylase in Forensic samples.
16.1.2: To quality control new Phadebas® reagent.

16.2 RESPONSIBILITY

16.2.1: Forensic Science Examiners from the Division of Scientific Services who have been trained in the discipline of testing for amylase according to FB SOP-26 (Training Manual and Checklist).
16.2.2: Forensic Science Examiners in the Forensic Biology Unit. Ordering information is maintained in a log book in the Forensic Biology Unit. New reagents and kits are purchased according to GL-6 (Purchasing). For additional information, refer to the Biological Inventory in Appendix 2.

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

RSID™: Rapid Stain Identification

16.5 TEST PROCEDURE

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

B. A sample is considered limited/compromised when it appears to be of low quantity and/or 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

16.5.2: Procedure

A. Record the extraction solution(s) used on the appropriate Quality Record Worksheet 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 and must be made with the same extraction solution used for the questioned samples.

   Note: 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).

   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, determine the root cause and correct.

C. For a liquid sample, prepare a stain on filter paper or swab, air dry and test that stain.

D. To each test tube add approximately 0.025g of Phadebas® powder.

E. Place a portion of the questioned sample or stain in a labeled test tube.

F. A sufficient amount of dH_2O should be added to each tube to easily cover the Phadebas® powder/sample and to observe the color of the supernatant.

G. For samples previously extracted in RSID™-Universal Buffer (as needed):

1. To each fresh tube add approximately 0.025g of Phadebas® powder.

2. Add the remainder of the extract (leave pellet intact) to each tube.

H. Gently shake each test tube to mix contents.

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

J. Gently shake each tube to re-suspend and centrifuge for approximately one (1) minute.

K. Observe the color of the supernatant of the samples.

L. It is important to compare results of the questioned samples against the positive and negative controls.

M. Record the results of the controls and samples on the appropriate Quality Record Worksheet.

N. A second examiner will observe and confirm the results and initial the appropriate Quality Record Worksheet.

16.5.3: Results and *Suggested Report Statements*

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

   Variation between examiners calling a positive result is acceptable since these are not critical designations and will not change downstream testing. The result will still be considered positive.

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

B. Negative

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

A color screening test for the presence of amylase, a component of saliva, was performed on [ ]. Saliva 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).

   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.

2. Record the reason a result is determined to be inconclusive on the appropriate Quality Record Worksheet.

16.6 QC PROCEDURE

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

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 according to the test procedure and the Phadebas® Reagent Log Sheet. Record the required information.

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, determine the root cause and correct.

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

E. Discard according to the manufacturer's expiration date.

F. Please refer to FB SOP-13 (Extraction of Samples) 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. Metropolitan Police Forensic Science Laboratory. Biology Methods Manual. 1978, pp. 3-10 to 3-11.
C. GL-2 (Safety Manual)
D. GL-6 (Purchasing)
E. Safety Data Sheets