



EXAMINATION OF ITEMS FOR THE PRESENCE OF SALIVA

DESCRIBED TEST

Phadebas[®] Forensic tube test

Background

The tube test is more sensitive than the press test and used semi-quantitatively for the presumptive testing of saliva deposits.

If it is suspected that the stain to be tested is a weak saliva stain, or if testing the supernatant from an extracted stain or swab, Phadebas tube test offers a better method than using Phadebas paper.

A tube test may also be carried out if *amylase* is detected (using the Phadebas[®] Forensic Press test) in an area that exhibits other staining such as semen, blood or heavy vaginal deposits;

The enzyme *α-amylase* is found in very high levels in saliva. Its activity in stains is used as an indicator for the presence of saliva. The test used to identify *amylase* uses Phadebas[®], consisting of starch microspheres with a blue dye cross-linked to the starch. In the presence of *amylase* the starch is digested, releasing the water soluble dye into solution. The resulting blue colour is read semi-quantitatively using a spectrophotometer.

Although the Phadebas[®] tests are specific for *amylase*, they're not specific for saliva. *Amylase* is found in other body fluids, although normally at much lower levels than in saliva. Generally, *amylase* found in other body fluids will not be present in sufficient quantity for detection by the Phadebas[®] methods. As a reference on the differences in *amylase* activity between saliva and other fluids, the below list was compiled from the results published in reference 1¹:

- **Saliva:** 263000 to 376000 IU/L
- **Urine:** 263 to 940 IU/L
- **Blood:** 110 IU/L
- **Semen:** 35 IU/L
- **Nasal secretion:** Undetectable levels
- **Sweat:** Undetectable levels

When positive results are obtained on an item, it is recommended that a substrate control sample also is submitted for DNA analysis at the same time, with the exception of swabs.

¹ P.H. Whitehead, Ann E. Kipps; *J. Forens. Sci. Soc.* (1975), **15**, 39-42



Phadebas® Forensic tube test protocol

If it is suspected that the stain to be tested is a weak saliva stain, or if testing the supernatant from an extracted stain or swab, Phadebas reagent used as a tube test is more sensitive than Phadebas paper. When testing swab(s) for the presence of saliva they may need to be extracted according to a separate protocol. The supernatant from the extraction procedure can then be tested using the Phadebas tube test protocol below.

1. Prepare suitable positive (saliva) and negative (distilled water) control samples, in tubes (for example Eppendorf tubes).
2. Extraction of samples:
 - i. STAIN: Cut out a small portion of the stain (approx. 3x3 mm) and transfer to a sterile tube. Add 0,5-1 ml of sterile distilled water and leave to soak for 1 minute. Agitate vigorously using a mechanical shaker for 30 seconds.
 - ii. SWAB: Follow internal protocol for extracting swabs.
3. Pipette 0,5-1 ml of supernatant from the stain or swab extraction to another tube.
4. Add 1 (one) Phadebas tablet to each of the test and control tubes.
5. Top up the sample and control tubes to 1 ml with sterile distilled water and agitate using a mechanical shaker.
6. Incubate the tubes at 37° C for 30 minutes. Remove and agitate each tube.
7. Centrifuge the tubes at 10,000 g for 1 minute.
8. A positive amylase reaction will produce a blue coloured supernatant solution, the depth of colour depending upon the amylase concentration. A negative reaction will result in a clear supernatant.



Phadebas tube test, with absorbance spectrophotometry

If it is necessary to quantify the saliva detected, absorbance spectrophotometry can be used in conjunction with the following procedure:

1. Stains: remove a piece of material approximately 3mm x 3mm and place into a centrifuge tube.
2. Extracts: remove 250µl of supernatant to tube as above.
3. Place suitable positive and negative controls into tubes. Add 4ml of sterile distilled water to another tube as a blank control.
4. Add 4ml of sterile distilled water to all tubes except the blank control.
5. Using forceps, add 1 (one) Phadebas tablet (from a single batch) to each tube and mix well. Cover the tubes with sealing film.
6. Incubate at 37° C for 30 minutes.
7. Stop the reaction by adding 1ml of 0.5M NaOH. Mix and then centrifuge at 10,000g for 1 minute.
8. Measure the absorbance of the supernatant at a wavelength of 620nm.
9. Note if the controls have given expected results and record findings.
10. Convert the absorbance results to α -amylase activity in International Units per litre, using the standard curve supplied with the Phadebas tablets. Ensure that the lot number on the tablets corresponds with that printed on the standard curve. Record findings.