



## FORENSIC EXAMINATION OF ITEMS FOR THE PRESENCE OF SALIVA

### DESCRIBED TEST

Phadebas® Forensic Press test

### Background

The enzyme  $\alpha$ -*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. The blue starch microspheres are immobilised on filter paper sheets. In the presence of *amylase* the starch is digested, releasing the water soluble dye, which diffuses through the pores of the filter paper. The resulting blue colour is visually observed on the non-reagent side of the Phadebas® paper.

The Press test is performed when it is necessary to localise an *amylase* positive area on an item. If a very strong reaction is obtained with the Press test and there is no other obvious contaminating material, this is interpreted as an indication of saliva.

Although the Phadebas® test is specific for *amylase*, it is 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 Press test method. 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 1\*:

- **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.

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



### **Phadebas® Forensic Press test protocol**

1. As a positive control, and before testing the item, tear a corner from the Phadebas® paper and envelop a fresh (damp) saliva-containing swab within it. Record the time that a positive reaction is first observed. *Note:* If no colour develops within 5 minutes take another corner and repeat using a different saliva donor. If again, no colour change is observed within 5 minutes, discard the Phadebas® paper and repeat using a fresh piece and a third saliva donor.
2. Place the item to be tested on a flat surface. The item can for example be stretched out over a piece of glass board, covered in a plastic wrap.
3. Using a spray bottle, dampen the item with distilled water.
4. Place a piece of Phadebas® paper over the area to be tested, with the blue reagent side in contact with the item. On the non-reagent side, write the case and item number, date and initial.
5. Spray distilled water liberally onto the Phadebas® paper. The paper must not dry out during the test period but it also should not be too wet.
6. Trace a rough outline of the area being tested on the Phadebas® paper so that it can be accurately placed back on the item at a later time if necessary.
7. Lay another (plastic-wrapped), clean glass board on top of the paper and place a weight on top of the glass. Use a 4 kg or heavier weight, to ensure good contact between the item and the paper and always use the same weight for all Press tests.
8. At this point start a timer.
9. The test is to be observed for a time period of maximum 40 minutes. Record in the examination notes the time that (a) positive reaction(s) is/are first observed. As well, record the progression of the test frequently for the first ten minutes, and then at 5-minute intervals until 30 minutes has elapsed, and finally at 40 minutes. *Note:* If a strong positive reaction has occurred then the test may be stopped before 40 minutes, depending on the circumstances (consult reporting scientist).
10. Record in the examination notes the time that any positive reaction(s) is/are first observed. Also note the dimensions and intensities (e.g., weak, moderate or strong) of any *amylase* positive areas observed during this time. *Note:* A positive is identified as a distinct area of diffuse blue colour on the non-reagent side of the paper.
11. Remove the glass board leaving the Phadebas® paper in contact with the item.
12. Mark the boundaries and any garment markings, such as seams, directly on the Phadebas® paper using an appropriate pen.
13. Using a wooden applicator stick, outline any positive areas by piercing small holes around the area and, using a suitable pen, dabbing ink onto the fabric.
14. The Phadebas® paper can now be removed and hung to dry. Negative press papers can be discarded but positive press papers must be retained with item. If a positive area is to be submitted for DNA analysis, cut an approximately 1cm x 1cm portion of the material and the corresponding piece of the Phadebas® paper into a labelled tube. An unstained area from the item and the corresponding piece of the Phadebas® paper may also be submitted as controls.
15. In cases where the DNA analysis yields a low-level poor profile or if the profile is a complex mixture where you cannot derive a major contributor, you may try to submit another piece



*from the positive area of the Phadebas paper only* for DNA analysis. This has been known to sometimes yield an interpretable DNA profile.

**Important:** If the same area of the item must be checked for *acid phosphatase* activity, it can be done once testing for *amylase* is complete. Apply the *acid phosphatase reagent* directly to the Phadebas® paper. The paper can be replaced on the item and the potential semen stain delineated (as above) and excised as necessary.

### **False Positives**

In-house testing at several independent forensic laboratories has determined that no other forensically relevant body fluid (sweat, semen and vaginal secretion) will react within 10 minutes using the current protocol, even after repeated deposition. The exception is faecal stains that may contain levels of *amylase* as high as those found in saliva. For this reason positive observations within areas obviously contaminated with faeces should not be interpreted for the presence of saliva. The presence of potential faecal material on an item should be recorded in the examination notes.

### **False Negatives**

*Amylase* activity within and among individuals can vary and it may be possible that a saliva containing stain may not react, even after 40 minutes.