

PHADEBAS® PRESS TEST SHEETS FOR THE DETECTION OF α -AMYLASE; A COMMERCIAL REPLACEMENT OF THE SPOTTY PAPER TEST

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1. Introduction

The forensic detection of saliva stains on case items generally relies on the detection of α -amylase, found in high levels in saliva. One established method used for locating saliva stains on fabrics has been the Spotty Paper Test¹, which has been routinely used at FSSA.

In the past, laboratory staff prepared spotty paper test papers by crushing a Phadebas® test tablet to distilled water and spraying the suspension onto a filter paper. The paper was then dried and stored awaiting use.

A new commercially manufactured version of the Spotty Paper Test, the Phadebas® Press Test Sheet is now available. It has a long expiry date, and the commercial production results in an even, consistent application of the Phadebas® test reagent to the paper substrate.

This poster presents results from specificity and sensitivity studies of the Phadebas® Press Test Sheet. The results for the detection of aged saliva stains on different fabric types and mock case items such as underwear and nappies are also presented.

2. Method

Screening Procedure

When screening an item for saliva stains, the test paper (Spotty Paper or Phadebas® Press Test sheet) is wetted with water and applied to the suspected saliva stain. The test paper is held down with weights to ensure good contact between the test paper and the stain, and then incubated at 37°C for 45 minutes. Any α -amylase in the suspected stain will hydrolyse bonds between the insoluble starch dye complex in the test sheet, solubilising and releasing the dye. A blue stain on the test paper indicates a positive result.

Sensitivity

A comparison of the Phadebas® Press Test Sheet and the Spotty Paper Test was performed. 50 μ l saliva samples of neat, 1/20 and 1/100 dilutions were deposited on three fabric types (acrylic, polyester and cotton). Tests were carried out in triplicate.

Specificity

Substances tested included: biological fluids, food stuffs – potentially containing β -amylase, chemical substances that might hydrolyse bonds to cause a false positive and saliva from common domestic pets (cats and dogs). Only Phadebas® Press Test sheets were tested, and all substances were stains prepared on cotton material.

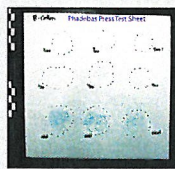
Case and Age Studies

- Aged stains greater than 2 years old were tested using a small range of fabrics with different thicknesses.
- Mock case items, which included children's underwear and nappies were supplied by staff with children. These items were not saliva stained, and represented items that are difficult to assess, often seen in casework.

3. Results

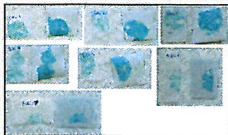
Sensitivity

- No significant difference was observed between Spotty Paper and Phadebas® Press Test sheets.
- Press Test Sheets produced slightly stronger reactions for the neat and 1/20 dilutions of saliva.
- The Spotty Paper produced slightly stronger reactions for the 1/100 saliva dilution.

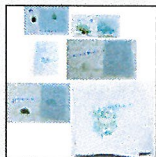


Specificity

- Saliva, faeces and animal saliva were the only substances that produced a positive reaction in this study (see Table 1).



Saliva Test Results (all strong positive)



Faeces Test Results (1 strong positive, 4 positive and 1 negative)

Table 1: Specificity Summary

Test Stain	No Positive	No Tested	Comments
Saliva	7	7	All strong positives
Faeces	5	6	4 positive and 1 strong positive
Blood	0	8	
Sweat	0	5	
Urine	0	5	
Semen	0	3	
Vaginal Secretions	0	2	
Food Samples	0	4	Wheat, sweet potato, soybean, barley
Non-biological	0	6	Spray n' wipe, bleach, Pine O clean, Ajax, coke and vinegar
Cat Saliva	3	5	Variable reactions
Dog Saliva	7	8	Variable reactions

Aged Stains

- All aged stains (greater than 2 years) produced positive reactions.
- Variations in reactions were observed between in fabric types and fabric thicknesses (see Table 2).

Table 2: Aged samples (2 years)

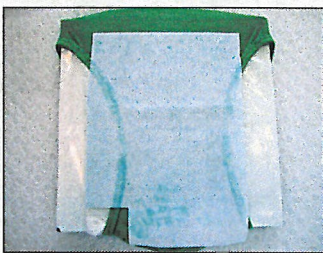
Type of fabric	Thickness	Result
Acrylic/nylon	Thick	Strong positive
Fleecy	Thick	Strong positive
Fleecy	Thick	Strong positive
Denim	Thick	Strong positive
Cotton	Medium	Positive
Cotton	Thin	Weak positive
Polyester/cotton	Medium	Strong positive
Polyester/cotton	Thin	Strong positive
Polyester/satin	Thin	Positive
Polyester/satin	Thin	Weak positive
Polyester	Thin	Weak positive

Mock Case Items

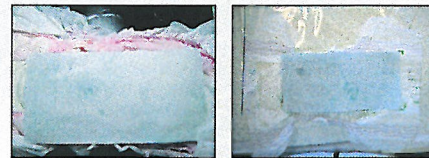
- These items produced pale, speckled positive patterns, making the interpretation difficult (see Discussion).



Underwear 1: Some pale and speckled reactions scattered over the test paper.



Underwear 2: A positive reaction was observed on the inner front and seams. A pale, speckled reaction was observed scattered over the remaining area of the test paper.



Nappies x3: Some weak reactions

4. Discussion

- Due to the low number of samples available for testing in this study, there was limited data available for interpretation.
- Saliva stains reacted strongly, however six faecal samples also gave positive reactions, but generally weaker than saliva stains.
- Some dog and cat saliva samples tested positive, indicating that the Phadebas® Test is not human-specific.
- Contact and moisture are critical to ensure reaction with the test paper. This is important to the interpretation of results, as thicker material generally gave stronger reactions. If the test paper dries out, false negatives may result.
- Results from aged samples indicate that there was no loss of activity up to the two year time period tested.
- Phadebas® Press Test sheets have the following advantages over Spotty Paper:
 - Even distribution of test reagent
 - Test reagent binds better to the substrate (issue when the paper is wet)
 - No preparation
 - Longer storage

Mock Case Work

The results from the underwear and nappies indicate that a positive result does not necessarily indicate that a child has been sexually assaulted. Innocent transfer of saliva could occur or faecal material could be present on the underwear. The positive reaction on the front of a boy's underwear (Underwear 2) was probably due to transfer. It was learned that he was known to chew his fingernails, which could have easily transferred saliva to his underpants.

The interesting speckled reaction observed is not easily explained. It could possibly be caused by transfer after contacting dried saliva on the skin. The children's underwear also showed strong positive results at the seams and this may be due to the better contact by the test paper to the fabric. This emphasises the effects of different fabric thickness, particularly where irregularities in the item surface cause uneven contact of the test paper.

Nappies produced very weak, inconsistent reactions. Due to the nature of nappies, moisture from the test paper was drawn into the nappy and some test papers required remoistening. This could explain the inconsistent results.

5. Conclusion

Sensitivity

There was no significant difference in the level of detection sensitivity between the Phadebas® Press Test Sheets and the inhouse Spotty Paper Test sheets.

Specificity

The Phadebas® Press Test sheet:

- Reacts strongly to saliva
- Has variable reactions to faeces
- Is not human specific
- Did not react with anything other than saliva and faeces in this study.

Interpretation

- Good contact and moist application is essential for a good test reaction. False negatives can occur if conditions are not correct.
- Care needs to be taken in the interpretation of results especially with underwear, as saliva is not the only source of amylase. Secondary transfer from hands was very likely the cause of the positive at the inner front area of one of the underwear (boy chewing fingernails). Not all staining is easily explained.
- Material effects should be considered; polyester material spreads out the stain. Thinner materials may give weaker reactions.

6. Reference

¹Willott GM, Griffith M. A new Method for Locating Saliva Stains – Spotty paper for Spotting Spit. Forensic Sci Int 1979 15: 79-83.