SER: Saliva – Amylase Filter Paper Overlay

Principle

Amylase is an enzyme present in saliva at elevated levels and in other body fluids (such as semen, vaginal fluid, urine) at lower levels. Amylase hydrolyzes starch.

The amylase overlay procedure is used to locate possible saliva stains.

Equipment and supplies

This procedure uses the following laboratory equipment and supplies:

- moisture chamber
- oven
- filter paper
- spray bottle

Reagents

This procedure uses the following reagents:

- α-amylase standard (1 U/µL)
  - Reconstitute lyophilized amylase in enough deionized water to yield a one unit per µl solution (e.g. reconstitute a 500U bottle with 500 µL DI water). Aliquot the reconstituted standard and store at -20°C ± 5°C.
- Stock iodine solution
  - 1.65 g potassium iodide
  - 2.5 g iodine
    - Dissolve in 30 mL warm deionized water and filter.
- Working iodine solution
  - Mix 1 milliliter of the *Stock iodine solution* with 99 mL of deionized water.
- Starch Solution
  - Using heat, dissolve 1 gram of starch in 250 mL of deionized water.

Controls

The following controls must be run with each test:

- Positive control- neat saliva or α-amylase standard placed on a substrate (filter paper, cotton, etc.)
- Negative control- saline placed on a substrate (filter paper, cotton, etc.)

Continued on next page

SER: Saliva – Amylase Filter Paper Overlay, Continued

Filter paper preparation

Prepare the filter paper (Whatman #3 or greater, or filter paper of similar thickness) by saturating it with the *Starch solution* and allowing it to dry.

Store treated filter paper in refrigerator at 5°C ± 3°C.

Procedure

Use the following procedure to perform amylase overlay.

Step	Action
1	Place the substrates with the positive and negative control on a glass or plastic plate.
2	Spread out the evidence item to be analyzed.
3	Saturate filter papers for evidence item and controls with deionized water.
4	Place the treated filter paper over item and a separate treated filter paper on the controls.
5	Allow the filter papers to maintain good contact with the samples for approximately 20 minutes. Mark seams or other reference points for orientation.
6	Remove the filter papers.
7	Place filter papers in a moisture chamber and incubate in an oven at 40°C ± 5°C for about 1 hour.
8	Remove filter papers from moisture chamber and allow to air dry.
9	Spray the filter papers with the *Working Iodine Solution* until a blue color develops.

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SER: Saliva – Amylase Filter Paper Overlay, Continued

Interpretation

Starch will react with the iodine solution and produce a blue product. Where the starch has been hydrolyzed by amylase, the filter paper will not be stained blue.

A white area on the filter paper is a positive (+) result for the presence of amylase. To determine the approximate amount of amylase present, perform an amylase radial diffusion test (see *SER: Saliva- Radial Diffusion for Amylase*).

The absence of white areas is a negative (-) result for the presence of amylase.

References

The following references were used in the development of this procedure.

Houde, J., "Saliva Mapping," *CACNews*, Fall 1993, pg. 4.

Forensic Examination of Sexual Assault Evidence (Section 25). California Criminalistics Institute. 1992.