



Biological Screening Workshop

Saliva

Saliva

- **Colorless fluid secreted by three glands in the mouth:**
 - **Sublingual**
 - **Submandibular**
 - **Parotid**
- **Saliva from parotid glands contain amylases, enzymes, which aid in the digestion of carbohydrates**
- **Saliva is composed of electrolytes, enzymes, mucus**

Saliva

- **Screening for saliva is based on detection of high levels of amylase in the sample**
 - **It is not a confirmatory test; amylase is found in other body fluids:**
 - **Serum, urine, sweat, lip mucous, semen, feces, etc.**
 - **The concentration of amylase in saliva is variable among individual; if amylase is not detected in a sample it does not mean saliva is not present**

Saliva

- **Alternative Light Sources (ALS) can be used to aid in locating saliva stains**
 - **The intensity of the fluorescence can be affected by the substrate, concentration of the stain, and other body fluids**
 - **Saliva does not fluoresce as intensely as semen**

Saliva

- **α -amylase**
 - Found in humans elephants, rats, and pigs
 - Cleaves starch at its internal bonds (acts on α 1,4 glucosidic linkages) allowing for compound hydrolysis
 - The total breakdown of starch to maltose or glucose and dextrin

Amylase

- **One of the earliest tests for amylase was the starch-iodine test**
- **Iodine solutions cause starch to turn a deep blue color**
- **Amylase is a starch hydrolyzing enzyme**
- **The presence of amylase causes the disappearance of the blue color (due to hydrolysis of the starch) and can be used an indicator for the presence of amylase**

Amylase Testing – Limitations

- **Not confirmatory**
- **Not specific for human amylase**
- **Is specific for α -amylase**
 - **α -amylase can be found in other species**

Presumptive Amylase Tests

- **Starch Iodine Radial Diffusion Test**
- **Phadebas[®] Test**
- **SALigAE[®] Test**
- **Rapid Stain Identification (RSID[®]) of Saliva**

Starch Iodine Radial Diffusion Test - Overview

- **Gel test plate that contains Starch with sample and control wells**
- **Control s are commonly: a dilution of 1:100 and 1:500 of fresh saliva control and a negative sample**
- **The plate is incubated 6 hours to overnight and Iodine is added to stain the plate**
- **Starch with Iodine creates a blue color**
- **If saliva is present, it will break down the starch creating a clearing in the blue**
- **The clearing appears in a ring and are measured**

Starch Iodine Radial Diffusion Test – Results

- **A positive test is one in which the ring size is equal or greater in size than the positive control**
 - **Some laboratories assess the level if more than one positive control is run**
- **An inconclusive result is one in which the ring size is less than the positive control but greater than the negative control**
- **A negative result is an absence of any clear ring**

Amylase Diffusion Video

Phadebas[®] Test

- **Commercially available**
 - **Blue starch polymer**
 - **Blue dye covalently attached to the starch and upon hydrolysis a product is obtained which is colorimetrically evaluated**

Phadebas[®] Test – How to Perform

- 1. Place a small piece of the sample material in a 10 x 75 test tube.**
- 2. In a second tube, place an equal-sized piece of known saliva stain as a positive control.**
- 3. In a third tube add no sample (negative control).**
- 4. Add 1.0 ml distilled water and ¼ Phadebas[®] tablet to each tube using clean forceps.**
- 5. Vortex to mix thoroughly.**

Phadebas[®] Test – How to Perform

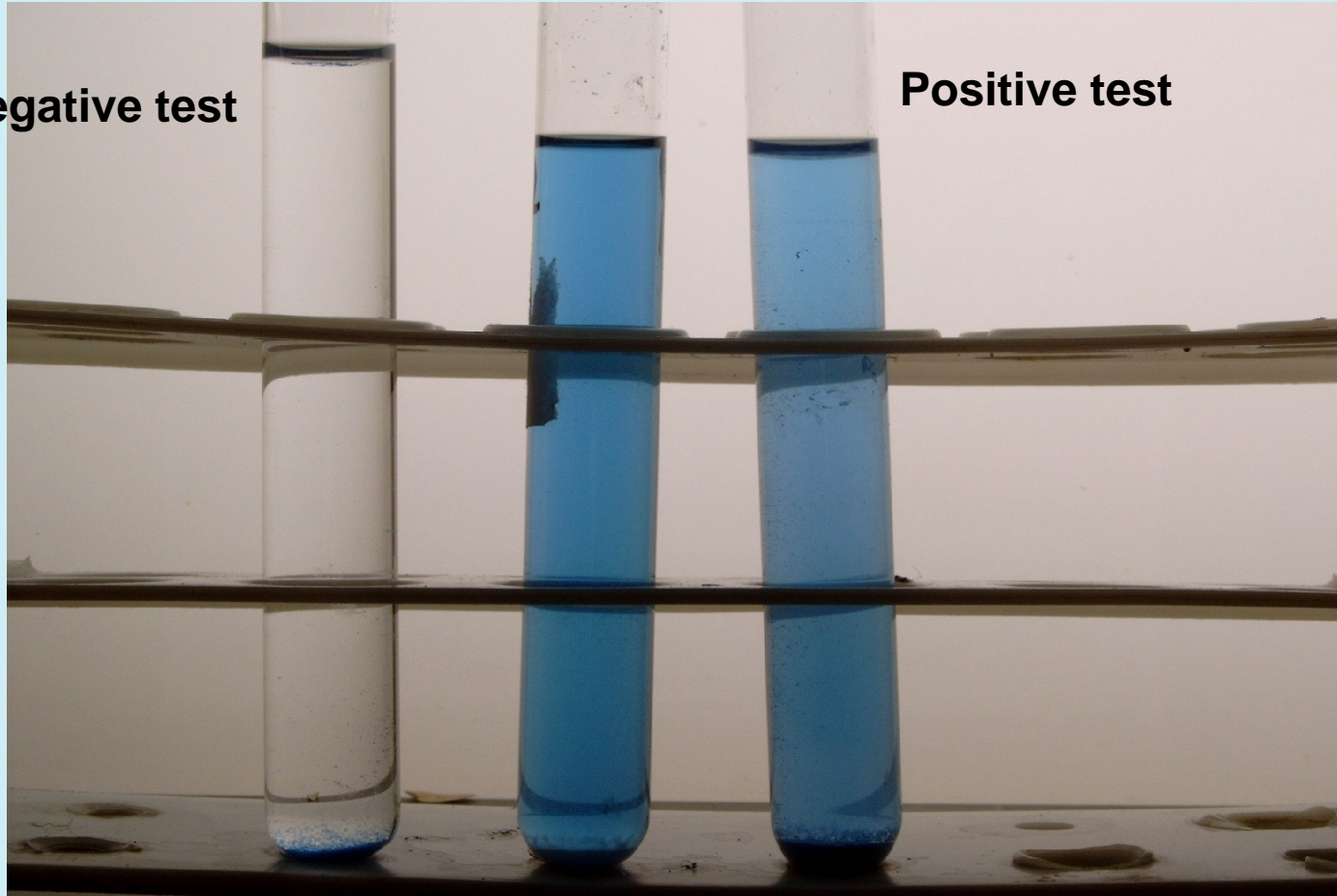
- 6. Incubate at 37°C for 30 minutes.**
- 7. Add 0.25 ml of 0.5 M sodium hydroxide to each tube to stop the reaction.**
- 8. Centrifuge for five minutes.**

Phadebas[®] Test – Interpretation

- **A transparent dark blue supernatant of equal or greater intensity than the positive control is regarded as a positive test for amylase activity**
- **A blue color that is less intense than the positive control but darker than the negative control is considered inconclusive for presence of amylase**
- **No blue color is considered negative for presence of amylase**

Phadebas[®] Test

Negative test



Positive test

<http://www.uni-wuerzburg.de/ddch/liquits/Amylasedirekt.jpg>

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Phadebas Video

SALIgAE[®] Test

- **Test available from Abacus Diagnostics[®]**
- **Sensitive, simple, and reported to be more accurate than other tests**
- **Solution in a tube**
 - **Changes color with addition of an extract containing saliva**
 - **Exact mechanism – proprietary**
 - **Sensitivity – about 1:1000**

SALigAE[®] Test – How to Perform

- 1. Place approximately 5 mm² cutting or ½ of a swab into a sterile 1.5 ml microcentrifuge tube.**
- 2. Place an equal-sized piece of known saliva stain as a positive control in a separate sterile 1.5 ml microcentrifuge tube.**
- 3. In a third tube add no sample (negative control).**
- 4. Pipette 30 µl to 50 µl of sterile deionized water into the tube.**

SALIgAE[®] Test – How to Perform

- 5. Incubate for 30 minutes at room temperature.**
- 6. Allow the test vials to warm to room temperature.**
- 7. Remove bubbles from the test vials by gently tapping the vials.**
- 8. Add 8 μ l of sample to the test vial.**
- 9. Mix gently.**

SALIgAE[®] Test – How to Perform

10. Read the result after 10 minutes.

- A yellow color change indicates a positive result**
- No color change indicates a negative result**
 - A negative result indicates that there is no saliva present or is below the limit of detection of the test**

SALigAE[®] Test



<http://www.dnabsinternational.com/SalivaValidation.pdf>

SALIGaE Video

Rapid Stain Identification (RSID[®]) of Saliva – How to Perform

- 1. Place a small cutting of the stain into a 1.5 ml microcentrifuge tube.**
- 2. Add 200 to 300 μ l RSID Extraction Buffer.**
- 3. Incubate for 1 to 2 hours at room temperature.**
- 4. Remove 20 μ l of extracted sample and add it to 80 μ l of RSID TBS Running Buffer in a new tube.**
- 5. Add the total 100 μ l to the sample well of the RSID[®] card.**
- 6. Read result after 10 minutes.**

Rapid Stain Identification (RSID®) of Saliva – How to Perform

- **Positive result**
 - Test line and control line are both present
- **Negative result**
 - Only control line is present
- **Invalid result**
 - No line at all or only test line is present (control line absent)

Rapid Stain Identification (RSID®) of Saliva – Limitations

- **False positives with breast milk, fecal material, and vaginal fluid**
- **False negative – high dose hook effect**
 - **Sample containing up to 50 µl saliva did not result in high dose hook effect**
 - **If high dose hook effect is possible, dilute using 1:100 dilution of sample**

Questions?