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

• $\alpha$-amylase
  – Found in humans, elephants, rats, and pigs
  – Cleaves starch at its internal bonds (acts on $\alpha$ 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
- Controls 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
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 mm\(^2\) 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 µl 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.dnalabsinternational.com/SalivaValidation.pdf
SALIGaE Video
Biological Screening Workshop: Saliva

**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 µl RSID Extraction Buffer.
3. Incubate for 1 to 2 hours at room temperature.
4. Remove 20 µl of extracted sample and add it to 80 µl of RSID TBS Running Buffer in a new tube.
5. Add the total 100 µl 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?