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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**

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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**

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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**

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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

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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

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Amylase Testing – Limitations

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

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Presumptive Amylase Tests

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

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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**

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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

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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**

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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.**

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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.**

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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**

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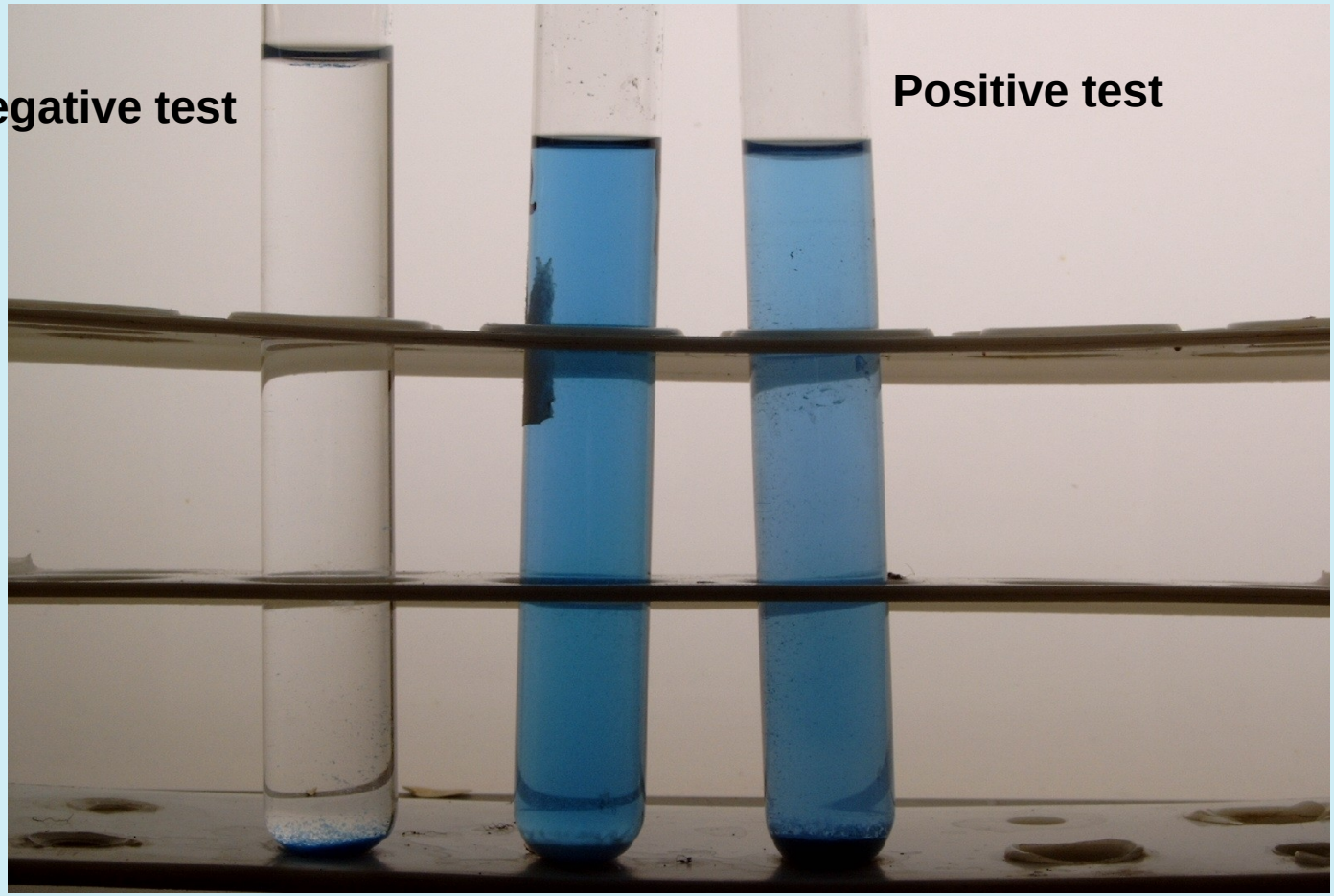
Phadebas® Test



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

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Phadebas Video

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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

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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.**

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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.**

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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**

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SALIgAE® Test

http://www.dnalabsinternational.com/SalivaValidation.pdf

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SALIGaE Video

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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.**

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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)**

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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**

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Questions?

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