Forensic Preliminary Testing for Saliva (amylase)
Specialized Topics-Spring 2010

Supplies:
Phadebas tablets
0.5 M Sodium Hydroxide
test tubes
water
scalpel or razor blade
forceps
starch plates
eppendorf tubes (1.5 ml)
37°C incubator
iodine staining solution (diluted)

1. Two methods of saliva (amylase) testing will be performed. Each student will perform all described procedures using their own saliva. Saliva samples will be obtained by spitting into a small disposable beaker. Dilutions will be made using water, and all beakers and diluted material will be disposed of as biohazard waste.

2. Prepare dilutions of saliva as follows:
   neat saliva (undiluted)
   1/100 dilution
   1/500 dilution
   1/1000 dilution
   water (negative control)

3. Test for amylase
   A. Phadebas tablet method
      1. Place 50 ul of each described dilution from above into a test tube.
      2. Add 1.0 ml of water, and ¼ Phadebas tablet (cut with scalpel or razor blade CAREFULLY). Add the ¼ tablet using forceps, NOT fingers to handle/cut the tablet.
      3. Vortex to mix thoroughly.
      4. Incubate at 37°C for 30 minutes.
      5. Add .25 ml of 0.5 M Sodium Hydroxide to each tube to stop the reaction.
      6. Centrifuge for 5 minutes.
      7. A transparent dark blue supernatant will indicate a positive test for amylase/saliva.
**B. Starch Iodine test (Radial Gel Diffusion)**

1. Prepare reagents and plates as follows (students will not be performing this portion, the plates will already be prepared for them, but they should be aware of how to make them):

   a. **Phosphate Buffer, pH 6.9**
      
      NaH$_2$PO$_4$, anhydrous 2.7 grams (.01 moles)  
      Na$_2$HPO$_4$, anhydrous 3.9 grams (.01 moles)  
      NaCl 0.2 grams (.002 moles)  
      Distilled water to 500 ml

   b. **Pour plates**
      (this will make between 20-30 x 2 inch plates)
      
      Phosphate Buffer, pH 6.9 100 ml  
      Agarose 2 grams  
      Soluble starch 0.1 grams

      Heat to boiling in a microwave (not unlike preparing an agarose gel) and continue stirring constantly until all the agarose is dissolved. Divide gel solution by pouring into 2 inch disposable plastic petri dish (approximately 3 ml per plate). Allow to polymerize completely. Store gels inverted at 4°C.

   c. **Iodine Development Solution**
      
      KI 1.65 grams  
      I$_2$ 2.54 grams  
      distilled water 30 ml

      Dissolve by stirring for 5 minutes at 65°C in a fume hood. Store solution in a dark stoppered bottle.

2. Punch holes into plate with vacuum flask and pipette tip, (instructor will demonstrate). Each student should prepare one plate with five holes/wells.

3. For each well, 4 ul of each dilution/sample will be added.

4. Place dish at 37°C for 6 hours or overnight.

   *Will have to incubate and then resume protocol at next class meeting for results.*

5. Stain the plate by pouring a 1/50 dilution of the Iodine Development Solution onto the surface of the plate. Rinse with water.
6. Clear circles around the wells indicate areas of amylase activity. The diameter of the wells is proportional to the square root of the concentration of amylase. Record the diameter of each dilution clearing in the results portion of your write up.

7. A positive test is one in which the cleared area is equal to or greater than the positive control (which is usually 1/500 dilution of a known saliva sample).

8. An inconclusive result is one in which the ring size is less than the positive control but greater than the negative control.

9. A negative control is an absence of any clear ring.

Questions (please answer each of these as part of your conclusions in your write up for this experiment):

Based on your results, which test did you think was more sensitive?

Which test did you prefer and why?

Which test do you think is more subjective, and which is more objective? Explain your answer.

Why do we run an unstained sample?

Why do you think you should not handle the Phadebas tablet with your bare fingers?