Ouchterlony Double Diffusion
Specialized Topics Spring 2010

Supplies needed:

Ouchterlony plates
Vacuum flask
eppendorf tubes (1.5 ml)
Antibodies (anti-dog, anti-cat)
known bloods (instructor will provide)
unknown samples (instructor will provide)

1. Prepare Ouchterlony plates for use as follows. Note: the plates will already be made for the students, but the students should know and understand how the plates are made.

This recipe will prepare approximately 30x2 inch disposable plastic petri dishes.

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agar</td>
<td>0.5 grams</td>
</tr>
<tr>
<td>Sodium Chloride</td>
<td>0.85 grams</td>
</tr>
<tr>
<td>Sodium azide</td>
<td>0.01 grams</td>
</tr>
<tr>
<td>distilled water</td>
<td>100 mls</td>
</tr>
</tbody>
</table>

Boil with constant stirring until agar is completely dissolved.

Pipette about 3 ml of agar solution in 30 x 2 inch disposable plastic petri dishes and allow to cool on a level surface. Place inverted at 4°C.

2. Remove plates from 4°C prior to use.
   A. Prepare extracts.
      1. Cut a small amount of blood standard.
      2. Place in 1.5 ml eppendorf tube
      3. Add 10 ul of sterile water to the tube (per sample)
      4. Do this for the unknown as well as the known samples
   B. Each student will need to prepare for two plates.
      1. There will be unknowns available to choose from.
      2. Pick an unknown and cut two small samples from it.
      3. Place each cutting into a labeled tube (x2).
4. The samples for each plate are as follows:
   Plate 1:       unknown versus cat
                   positive control (cat versus cat)
                   negative control (cat versus water)
   Plate 2:       unknown versus dog
                   positive control (dog versus dog)
                   negative control (dog versus water)

Since each student will be doing TWO plates, one for CAT and one for DOG, the samples should be prepared as follows: (all are 5-10 ul per well)
   Make 40 ul of KNOWN CAT
   Make 40 ul of KNOWN DOG
   Make 20 ul of unknown

3. Punch the desired number of wells into the plate (s) in a triangular pattern as demonstrated by the instructor. Be sure and evenly space your wells, and follow the general lay out provided.

4. In the top well, place the antiserum to be used (5-10 ul).

5. In the right well, place the known standard antigen extract or diluted serum (5-10 ul). See diagram below.

Each plate will have THREE SETS of wells:

- antiserum
- unknown
- known
- test
- known
- known
- positive control
- known
- known
- negative control
- water
- known
6. In the left well, place your unknown or questioned stain extract (5-10 ul) (for questioned sample; for positive control you will put dog or cat; for negative control it will be water)

7. Cover the petri dish and leave undisturbed for overnight.

8. Read the plate the next day (or next class meeting). Record results in your notebook. Precipitin bands which form a continuous arc of convergence (identity band) between the antiserum well and the 2 extract wells are considered positive results. Be sure to record the unknown you chose as well as your conclusions on what it is.

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

Why do we run a positive AND negative control for both plates? Shouldn’t one control be good enough? Explain your answer.

What if your unknown did not react with EITHER the cat or dog antiserum? What could conclude from that? Explain your answer.

Why aren’t the plates made up using agarose instead of agar?