LAB LECTURE NOTES FOR WEEK 12

EXPERIMENT 11A – PERIOD 5

(ISOLATION OF STREPTOMYCES)

1. Look at the control culture first. It will demonstrate antibiotic production in that two or three of the test organisms will show no growth near the culture.
2. Do not expect any of the isolates to show the same results, as we isolated quite a few other species of *Streptomyces*. Many may not show inhibition at all.

EXPERIMENT 14A – PERIOD 3

(ISOLATION AND IDENTIFICATION OF "ENTERICS")

1. KIA (Kligler Iron Agar) should confirm what we saw for each of our isolates when they were colonies on the Modified MacConkey Agar, and KIA will also tell us if the organism is a fermenter – that is, able to ferment at least glucose. The following shows how this medium is used as a **screening medium**:
   a. If **no fermentation** is noted, that means it is **not an enteric** and we can set it aside and work with it no longer if our goal is to isolate only enterics.
   b. Out in the real world, if one is after a specific physiological type of enteric that is discernable with KIA, then tubes with a particular combination of reactions will be chosen for further study. For example, *Salmonella* would generally show a lactose-negative and hydrogen sulfide-positive appearance in KIA.
2. The introduction to Experiment 14 goes into KIA further, and a whiteboard summary is shown here: http://www.jlindquist.com/microbiology102/M102images/NewKIA-5tubes.jpg One should add a fifth tube to this diagram that shows **both** lactose fermentation and H_2S production (yellow slant and butt with additional black coloriation due to H_2S reacting with the iron in the medium).
3. From **one** of the KIA tubes, we are inoculating the test media detailed in the procedure in the lab manual. We must choose a KIA tube which shows fermentation, as we do not want to carry on the experiment with the non-fermenting non-enteric in the mixture which happens to be *Pseudomonas*.

EXPERIMENT 15A – PERIOD 4

(ENRICHMENT AND ISOLATION OF COLIFORMS)

1. We are finishing up the "completed test" to tell something about the identity of the coliforms we have isolated from the original water sample.
   a. Look at your EMB Agar plates for **dark** colonies which are our **coliforms**. The dark color is from the pH indicator which shows acid production from the fermentation of lactose. There are two classical types of coliform colonies which we may see, and they are called the "coli-type" and the "aerogenes type." Note the descriptions in the manual and also the demonstration. A summary is shown here: http://www.jlindquist.com/generalmicro/dfemb.html . Actually, there can be "intermediate" types of colonies. But as long as we pick something **dark**, it is likely to be a **coliform**.
   b. To be a coliform we should show that it is a **gram-negative** rod and also able to **ferment lactose to acid and gas**. We have the lactose fermentation broth, and growth on EMB Agar was probably enough to show gram-negativity, so we are skipping the Gram stain.
   c. Only **well-isolated colonies** can be inoculated into the differential media provided. We will perform the IMViC Tests **except for the Voges-Proskauer test**. The IMViC tests stand for **I**ndole, **M**ethyl-Red, **V**oges-Proskauer, and **C**itrate, and they have been used for around 100 years to help classify coliforms. To add a bit more precision to our identification process, we are also doing tests for motility and ornithine decarboxylation which are tested for (along with indole production) in Motility Indole Ornithine (MIO) Medium (as also done in Experiment 14A).

EXPERIMENT 17A – PERIOD 1
(THE LAST ISOLATION EXPERIMENT)

1. This is basically a more general exercise to see what we can pick up from natural samples that grow on an all purpose medium (like Heart Infusion Agar) and also on MacConkey Agar. We will not be doing any specific identification, but we will ultimately test our isolates for basic characteristics as indicated in the table on the last page

VIRTUAL EXPERIMENT 10 – A QUICK REVIEW OF THE ANTIBIOTIC DISK SENSITIVITY TEST

1. We can test a pure culture of an organism of interest (such as one isolated from a patient who is suffering from an infection caused by the organism) to see what antibiotics might be used to inhibit this organism. The following goes along with the diagram shown here: http://www.jlindquist.com/microbiology102/M102images/virtual10diagram.jpg

   a. After inoculating a plate to obtain a "lawn" of the organism, we place some disks on the plate, each containing a certain amount of an antibiotic. As the culture grows on the plate, it may be inhibited around one or more of the disks, as the antibiotic in each disk is diffusing into the medium such that it may inhibit sensitive organisms around it.

   b. When we want to find out if any particular antibiotic is hitting the organism at the "target site" – which is what we expect an antibiotic to do to an organism truly sensitive to it – we should see a zone of inhibition around the disk of a certain **minimum** size, which is why we **measure the diameters** of the zones and compare them to a table such as what you see for Virtual Exp. 10.

   c. Whatever we find out about the zone around Antibiotic Disk A, we also have to take into account that there are colonies of resistant cells present. So, the use of Antibiotic A in a patient suffering from an infection (caused by this organism) may not be helpful, as the resistant cells could continue the infection such that another antibiotic would have to be found. **The use of Antibiotic A would make the patient a selective-enrichment medium for cells resistant to Antibiotic A!**

   d. Antibiotic D looks like a better choice, and if the zone of inhibition is of a certain minimum size (diameter) to show that the organism is truly sensitive to Antibiotic D, then it would be the antibiotic of choice.