Determining Bacterial Concentration (Optical Density or OD) using the Spectrophotometer

These steps should be done in the biosafety cabinet with sterile pipet tips to avoid contamination of your sterile media stock and also to protect your cultures from contamination, in case they need to grow longer.

1. Pipet 1 mL of your blank (sterile, uninoculated media – in this case, TY) into a disposable polystyrene cuvette using sterile technique. Label the cuvette on the frosted portion at the top. Do not touch the lower part of the cuvette with your fingers, as it will interfere with the reading.

2. Using sterile technique, pipet 1 mL of each culture into a labeled cuvette. Replace the foam stoppers and foil, preparing the cultures to go back into the incubator of they need to grow longer.

3. Take the cuvettes to room 137 to the spectrophotometer. The multi-sample cuvette holder should be on the machine. If it is not, unscrew the screws holding the single-well holder and remove it. Replace it with the 6-sample cuvette holder and screw in tightly. One side of the holder has "front" printed on it for orientation.

4. On the home screen on the computer, select "fixed wavelength" from the menu at the top. (If it is not on the home screen, click on "quit" in the upper right corner. Unclick all the boxes in the window that pops up and click OK. This will bring you to the home screen).

5. On the next screen, check to make sure the wavelength is 600 nm. If it is not, click on the wavelength number (λ) and click in 600 on the number pad window.

6. In the lower left of the screen, there will be a toggle that says "vis off" or "vis on". If it says "vis on", the spec is ready to use. If it says "vis off" click on it and it will toggle to "vis on". This turns on the visible light lamp you will use to measure the absorbance of your sample.

7. Click on "status devices" at the bottom of the screen. Click on the square that says "home" to set the cuvette holder in the correct position. When it is finished, click on the box that says 1. This will move the cuvette holder to read the first sample.

8. On the upper right, there will be a line that says "sampling device", likely with "one cell" written next to it. Click on this and select "auto sampler". Click on the small number next to this and a window with a number pad will pop up. Click on the number of samples you will be reading. Click OK.

9. Put the cuvettes into the cuvette holder, blank first. The clear triangle at the top of the cuvettes should face the computer screen.

10. In the lower left corner, you will see the word “blank”. With the cuvette holder in position 1, click blank. You will hear a click. This is giving the spec a baseline absorbance of the TY so it can subtract it from the reading of your bacterial samples.

11. On the main screen will be a number 1. Click on it and the spec will read your blank, followed by each of your samples in order. Record the OD’s.

12. If the OD’s are between 0.7 and 1.3, you may proceed with the inoculation protocol. If the OD is lower, you’ll need to put your cultures back on the shaker and allow them to grow longer. If it is higher, you will need to dilute the culture with fresh TY and re-take the spec reading, repeating this until the OD is in the correct range. If the OD is over 2, the culture is overgrown and will have to be restarted.

13. When finished with the spec, click on “vis on” to toggle the lamp off. Click on quit in the upper righthand corner. Unclick all of the boxes in the window that pops up and click OK. This will return the computer to the main screen for the next user.

14. Clean up the biosafety cabinet with 70% ethanol. Return equipment to the lab.

15. For inoculation of caissons, spin 10 mL of culture for 10-15 minutes at 2000 rpm. Resuspend pellet in 10 mL sterile water. Pour inoculum directly into caisson.