GLUCOSE ASSAY

1. Mark a paper towel with labels for each sample you will be analyzing. (We will use this to store temporarily some of the pipetting tips.)

2. Remove and spin-down a sample from each flask.
   - Set a P-1000 micropipetter dial to 50 (0.5 ml). Attach a blue tip.
   - Homogenize the sample by first swirling by hand then use the Vortex shaker set at *Shake 3* for 15 seconds.
   - Without touching the sides or edge of the flask extract 0.5 ml from the flask and expell it into the plastic microcentrifuge tupes (with caps).

   - Label the cuvettes, and put them balanced in the microcentrifuge. If the number of cuvettes is odd; add one with water. Spin according to attached directions. Remove and set aside for later use.

3. Put 2 ml of glucose reagent in each Spec-20 cuvette (blank, standard, samples).

   - The reagent is kept in the refrigerator along the NORTH wall.
   - Use the bottle with the black dot on the lid so we can best monitor usage for re-ordering.
   - Use the P-1000 micropipette with the dial set to 100 and a blue pipette tip. Without touching the sides of the flask, extract 1 ml twice and deposit each in the cuvette.

4. Blank: add nothing more.
5. Standard: add 0.02 ml of glucose standard.
   - Find the dropper bottle of the standard and squeeze 1 small drop into cap.

   - Attach a plastic tip to the P-20 micropipetter. Set the dial to 200. Extract 0.02 mL of glucose standard into the tip.

   - Deposit the tip contents near the surface of the reagent in the standard's cuvette. Depress the liquid expelling plunger firmly. Touch the tip to the inside of cuvette to remove any traces. Remove the pipette tip and place it on the proper place on the paper towel.

6. Sample: Repeat the above procedure using a new tip for 0.02 ml of the sample in its cuvette.

7. Mix each cuvette using the test-tube Vortex set at Vortex-1 for about 5 seconds. Then let everything sit for 10 min. After incubation, read absorbance on the Spec-20 using the attached instructions. Readings must be done within 60 minutes. The contents of the standard cuvette should be a *dark pink*.

8. If the sample glucose is too concentrated (absorbance greater than 0.7), dilute with distilled water using a pipette until the absorbance is less than 0.7. Note the dilution factor on your data sheet.

9. Record absorbance, dilution factor, and glucose concentration. Glucose concentration (mg/L) is: (Sample absorbance)/(Standard absorbance) x 1000.

James Powell
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