Glucose Assay; Single Cuvette (Pointe Scientific)

Materials Required:

1. Pipettes: 10 – 1000 uL
2. Test tubes and racks
3. Timer
4. Hexokinase Reagent (Pointe Scientific Cat #G7517)
5. Glucose Standard (Pointe Scientific Cat #G7518-STD)
6. Quality Controls (Multi-analyte Cholestech Controls #88773)
7. Cary Spectrophotometer set at 340 nm (see 'Cary Spectrophotometer_Single Cuvette' instructions)
8. Cuvettes

Samples Collection/ Storage:

1. Clear, unhemolyzed serum or plasma can be used.
2. Serum (gold-topped tube): Gently invert vial 8-10 times immediately following collection, let sit 30 min at RT then centrifuge at 1000g's (2.6-3.0 RPM) for 15 min.
3. Plasma (purple-topped tube): Gently invert vial 8-10 times immediately following collection then centrifuge at 1000g's (2.6-3.0 RPM) for 10 min.
4. Following centrifugation, immediately pipette serum or plasma into appropriately labeled micro-centrifuge tubes. Separate serum or plasma from red cells as soon as possible to minimize glucose decomposition by glycolysis.
5. Samples should be run immediately or frozen for batch analyses.

Procedure:

1. Label test tubes, in duplicate, and place in rack (e.g. 'Standard, Control, Subject ID').
2. Pipette 1000 uL of hexokinase reagent into each tube.
3. Pipette 10 uL of standard/control/sample into tube and vortex using low setting.
   NOTE: A ratio of 1:100 is required for sample and reagent. If you have less sample you can use 5 ul of sample and 500 ul of reagent.
4. Let stand at room temperature for 3 min.
   (Refer to 'Cary Spectrophotometer_Single Cuvette' instructions if needed).
5. Zero spectrophotometer using water at 340 nm.
6. Read the absorbance of all the tubes and enter in excel template (Glucose Assay_Single Cuvette_Results Template).

Notes: Final color is only stable for 15 min following the incubation period so only a limited number of samples can be run at one time.

If samples are extremely lipemic they may give falsely elevated values. Prepare a 'blank' using 1000 uL saline and 10 uL of the sample. Read against water and subtract this value from sample Abs.

Reportable Range (linearity) is 0.6 – 600 mg/dL. If a sample exceeds the upper linearity limit it needs to be diluted with isotonic saline and rerun. Multiply results by dilution factor.

Verifying Results:

1. Make sure your controls are within range.
2. Verify that your coefficient of variation for your sample duplicates are within acceptable limits ($\leq$15%), rerun if necessary.