Glucose Assay; Single Cuvette (Pointe Scientific)

**Materials Required:**
1. Pipettes: 5uL – 1000uL
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 340nm (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 8-10x immediately following collection, let sit 30min at RT then centrifuge at 1000g’s (2.6-3.0 RPM) for 15min.
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 minutes.
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 1000uL of hexokinase reagent into each tube.
3. Pipette 5uL of standard/control/sample into tube and vortex using low setting.
4. Let stand at room temperature for 3 minutes.
   (Refer to ‘Cary Spectrophotometer_Single Cuvette’ instructions if needed).
5. Zero spectrophotometer using water at 340nm.
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 minutes 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 1000uL saline and 5uL of the sample. Read against water and subtract this value from sample Abs.
Reportable Range (linearity) is 0.6 – 600mg/dL. If a sample exceeds the upper linearity limit it needs to be diluted with isotonic saline and rerun. Multiply results by 2.

**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 (≤15%), rerun if necessary.