Cholesterol / Triglyceride Measurement

Preparation

a) Use a disposable non-sterile assay plate, 96 well, flat bottom
b) Cholesterol or triglyceride calibrator (note dilution in mg/dl)
c) Cholesterol or triglyceride reagent

Dilution of cholesterol/triglyceride calibrator (indicator or standard)

Add distilled H_2O (or PBS) to the indicator to obtain a dilution factor of two. Example if the indicator's dilution is 400 mg/dl the next dilutions would be: 400, 200, 100, 50, 25, 12.5, 6.25. The last sample should be a blank with just distilled H_2O.

Using small Eppendorf tubes, fill the first tube with 20 ul of indicator. The next six tubes should contain 10 ul dH_2O and the last tube should contain 20 ul dH_2O. Remove 10 ul from the first tube, pipet that into the next tube, mix. With a new tip continue with the rest of the tubes. Do not mix anything with the blank.

Apply the standard and samples

a) The plasma needs to be diluted. Dilution should be proportional to the concentration of the standard. If you are using a standard that is 400 mg/dl, use a 1:2 plasma dilution (10 ul plasma + 20 ul dH_2O). If using a 1:3 dilution, 5ul plasma + 15 ul dH_2O
b) Use 2 ul of each standard and sample
c) Standard should be placed in A1-H1
d) Samples should be placed in A2-H12 (depending on how many samples)
e) Add 200 ul of either cholesterol or triglyceride reagent to each well

Measure cholesterol/triglyceride

a) Use Bio-Rad plate reader in N820
b) Always select end-point reading
c) When setting up the plate template, select "#", enter 2 (duplicate readings, will take average)
d) While the template page is still open edit standards and concentrations
e) Pick reports: unknown, curve, absorbance report, raw data
f) Select dual wavelengths: 492nm and 600nm