Plasma Triglyceride Quantitation

Version: 1.0
Replaced by version: N/A
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Summary:.

Reagents and Materials:

Reagent/Material	Quantity Required	Vendor	Stock Number
Triglyceride GPO	4 x 100ml	Wako	995-86108
Wako Control Serum I	10 x 5 ml	Wako	410-00101
Wako Control Serum II	10 x 5 ml	Wako	416-00201
Triglyceride Standard Solution A (300 mg/dl)	4 x 10ml	Wako	272-56799
Phosphate Buffered Saline			
Repeat pipette			
Humidifying Chamber			
Spectra Max 250		Molecular Devices	

Protocol:

Reagent: Triglyceride GPO, 4 x 100ml (Wako 995-86108)
Consists of color reagent and 100 ml buffer container
Controls: Wako Control Serum I, 10 x 5 ml (Wako 410-00101), low control
Wako Control Serum II, 10 x 5 ml (Wako 416-00201), high control
Standard: Triglyceride Standard Solution A, 4 x 10ml (272-56799), 300 mg/dl

1. Prepare the triglyceride reagent by adding the color reagent powder to the 100 ml buffer container. Mix thoroughly to dissolve all the powder. The reagent is stable for 10 days at 4°C.
2. Prepare standards of 0, 100, 200, and 300 mg/dl using PBS and Wako's Triglyceride Standard Solution A, 300 mg/dl (272-56799). The standards are stable for at least 3 months when stored in tightly closed tubes at 4°C.
3. Prepare a work sheet with 8 rows and 12 columns to correspond to the wells of a 96 well plate. Enter places for standards, controls, and unknown samples in duplicate.
4. If values greater than 300mg/dl are expected, dilute the unknown samples by transferring 10 µl of the unknown to a new tube containing 30 µl PBS.
5. Vortex and spin the standards, controls and unknown samples.
6. According to the places indicated on the work sheet add 10 µl of the standards, controls and unknown samples to the wells of a clear 96 well flat-bottom micro-titer plate.
7. Add 250 µl of reagent to each of the wells to be tested using a repeater pipette.
8. Place the plate in a humidified chamber consisting of a covered Tupper Ware box with wet paper towels on the bottom. Place the Tupper Ware box on an orbital shaker at room temperature for 2 minutes and then in a 37 °C incubator for 45 minutes.
9. Program the location of the standards, controls, and unknown samples on the work sheet into an absorption plate reader (such as Spectra Max 250 made by Molecular Devices).
10. Set the wavelength to 600 nm.
11. Read the plate.
12. Save the results and print the information. The machine will calculate the concentration of triglycerides in the unknown samples. If the unknowns have been diluted, multiply the results by the dilution factor (4 in this case).

Quality control:

13. Check linearity of the standard curve.
14. Check if the concentrations of the controls agree with the expected values.
15. Check the duplicates and if they differ by more than 10% re-assay the sample.
16. Samples that exceed the highest standard (300mg/dl) should be re-assayed after dilution (see 4 above).