Lipoprotein Cholesterol Distribution Assay by FPLC

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Abstract

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Protocol

Step 1.

- Blood is collected from mice in EDTA coated tubes by cardiac puncture and plasma was isolated by centrifugation.

Step 2.

- An aliquot of plasma is diluted to 0.5 µg total cholesterol/µL in 0.9% NaCl, 0.05% EDTA/NaN_3 and centrifuged at 2000xg for 10 minutes to remove any particulate debris.

Step 3.

- The supernatant is transferred to a glass insert contained in a GC vial.

Step 4.

- After loading the vial into an autosampler set at 4°C (Agilent Technologies, G1329A), 40 µL of sample is injected onto a Superose 6 10/300 (GE Healthcare Life Sciences) chromatography column.

Step 5.

- Under control of an isocratic pump (Agilent Technologies, G1310A/B), the sample is separated at a flow rate of 0.4 ml/min with eluent containing 0.9% NaCl, 0.05% EDTA/NaN_3.

Step 6.

- Column effluent is mixed with total cholesterol enzymatic reagent (Pointe Scientific) running at a flow rate of 0.125 mL/min and the mixture is passed through a knitted reaction coil (Aura Industries Inc., EPOCOD) in a 37°C H_2O jacket.

Step 7.

- The absorbance of the reaction mixture is read at 500 nm using a variable wavelength detector (Agilent Technologies, G1314F).

Step 8.

- The signal is subsequently integrated using Agilent Open LAB Software Suite (Agilent Technologies).

Step 9.

- VLDL, LDL, and HDL cholesterol concentrations are calculated by multiplying the total plasma cholesterol concentration by the cholesterol percentage within the elution region for each lipoprotein class.