

Critically discuss the measurement and standardisation of serum cholesterol

## **Introduction**

Serum cholesterol

Free and esterified – hydrolysis step needed

Heterogeneity and complexity of lipoprotein structure and composition

Main cholesterol carrying particles LDL and HDL

Measurement and standardisation involves reagents (and calibrators) in one phase and the analyte in a second phase as lipids are not truly in aqueous solution.

Matrix effects exhibited by reference materials

Importance of standardisation as treatment goals and thresholds set internationally.  
'Field' methods and calibrators should be traceable back to CDC methods.

## **1. Standardisation**

Primary reference material for cholesterol is provided by NIST (pure cholesterol).

Note primary reference material is not available for HDL and LDL cholesterol.

Secondary reference material for cholesterol - CDC pools

'Calibrators' should be certified by CDC cholesterol reference method laboratory network.

## **2. Total cholesterol measurement**

(a) Definitive, 1<sup>o</sup> reference method

- ID-MS developed by NIST (CV typically <0.5%)
- Labour intensive
- Expensive and specialised equipment required

(b) 2<sup>o</sup> Reference method

- CDC modification of Abell-Kendall method (CV typically <1.5%)
- Hydrolysis of cholesterol esters (alcoholic KOH)
- Organic extraction (hexane)
- Liebermann-Burchard reagent (acetic anhydride/sulphuric acid/acetic acid)

On average values are 1.6% higher than definitive method.

Less demanding technically and cheaper than definitive method but still not suitable for routine use.

(c) Enzymic methods

- Adapted for automated analysers
- No extraction (purification) step, so methods are more likely to suffer from interference (turbidity, haemolysis, icterus)

Typical method uses lipase/detergent to breakdown lipoproteins and cholesterol esterase (bacterial origin), cholesterol oxidase and Trinder type reaction to measure hydrogen peroxide produced. Note that cholesterol oxidase is specific for free cholesterol.

Then brief mention of HDL/LDL methods as routine methods measure 'cholesterol' after pre-treatment of serum.

### **3. HDL cholesterol measurement and standardisation**

- 1<sup>o</sup> reference method and standardisation materials are not available
- CDC 2<sup>o</sup> reference method is ultracentrifugation, followed by heparin-MnCl<sub>2</sub> precipitation and then modified Abell-Kendall procedure
- Older routine methods involve removing non-HDL cholesterol by precipitation
- Newer methods are homogenous assays using blocking agents to prevent non-HDL cholesterol reacting in the cholesterol assay.

### **4. LDL cholesterol measurement and standardisation**

- 1<sup>o</sup> reference method and standardisation materials are not available
- Ultracentrifugation and calculation of LDL by beta quantification method.
- Calculated using Friedewald formula (requires measurement of TC, HDL and TG and subject to restrictions on use eg Tg<4.5 mmol/L, genetic hyperlipidaemias etc)
- 'Direct' methods using antibodies to block reactions before measuring LDL cholesterol – still requires further evaluation/development