

Composition of Reagents for Cholesterol Determination by Enzymatic Kinetic Method

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Enzymatic method is the most popular for serum cholesterol determination. It is specific, does not require corrosive chemicals and is easily adapted for automation. The assay is generally performed as an endpoint or a kinetic method. The main advantage of the kinetic method is shorter analysis time, reduced effects of interfering substances and elimination of sample blank measurement.

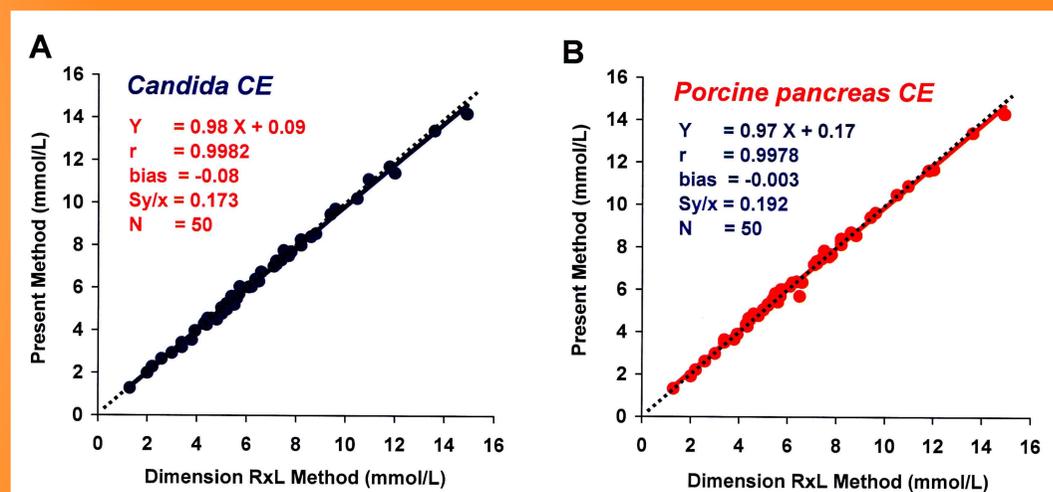
The enzymatic cholesterol reagent consists of three enzymes, cholesterol esterase (CE), cholesterol oxidase (COD) and peroxidase. The reagents for the enzymatic kinetic method have used COD isolated from *Streptomyces* or mutant *Nocardia* and CE from *Pseudomonas fluorescens*. Nowadays, the kinetic cholesterol determination is not a popular method because of using **very high amount of enzymes**. Moreover, the cost of enzymes is expensive especially *Streptomyces* COD.

Cholesterol Reagents for Enzymatic Kinetic Method

Inventive cholesterol reagents for the kinetic cholesterol method utilize the new COD derived from *Brevibacterium* and CE from *Candida* and *porcine pancreas*. The cholesterol determination using each reagent proves the appropriate assay used in routine clinical laboratory because its accuracy and precision meet the currently established analytical performance goals. The analytical bias are less than 3%. A method comparison study with the CDC-standardization method shows excellent correlation and acceptable agreement between results.



Cholesterol Reagents



Correlation of serum cholesterol

Prominent point of the inventive reagent is the requirement for **less amount of enzymes** than normally used in a prior art test composition as shown following

Test composition	Inventive reagent	Other reagent
COD (U/L)	100	1000
CE (U/L)	100	400

As COD and CE are the most expensive ingredient in the test composition, **inventive cholesterol reagents** will provide **a more cost effective** cholesterol method.

Advantages of Inventive Cholesterol Reagents

The cost of *Brevibacterium* cholesterol oxidase and *Candida* or *porcine pancreas* cholesterol esterase used in the present inventive reagents is 6-8 fold less expensive than *Streptomyces* cholesterol oxidase and *Pseudomonas fluorescens* cholesterol esterase. Moreover, the reagent cost for the kinetic method is still lower than that for the endpoint method.

All inventive reagents can be applied for the determination of total cholesterol, free cholesterol and cholesterol content in LDL and HDL particles. These reagents may be adapted for the widely use such as manual method, clinical laboratory analyzers, and point-of-care testing using impregnated reagent onto a solid carrier e.g. filter paper, cellulose, porous synthetic, resin membrane, fiber and electrode.

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