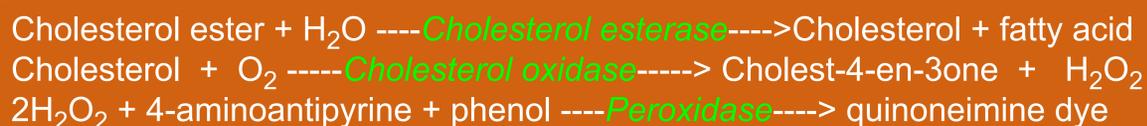


Composition of Reagents for Cholesterol Determination by Enzymatic Endpoint Method

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An elevated plasma cholesterol level may lead to an increased chance of developing either atherosclerosis or cardiovascular diseases. On the other hand, decreased plasma cholesterol and low density lipoprotein cholesterol (LDL-C) and increased high density lipoprotein cholesterol (HDL-C) concentrations reduce the developing of diseases. Therefore the lipid profile including total cholesterol, triglycerides, LDL-C, and HDL-C, is necessary for screening general populations and for monitoring patients who are on cholesterol reducing diets or drugs.

Enzymatic method is the most popular for serum cholesterol determination. It is simple, specific, and uses no corrosive reagents. The analytical reaction is commonly based on the following reaction:



The assay is generally performed as an endpoint or a kinetic method. Advantage of the endpoint method is its insensitivity to minor change in the reaction condition.

Cholesterol Reagents for Enzymatic Endpoint Method

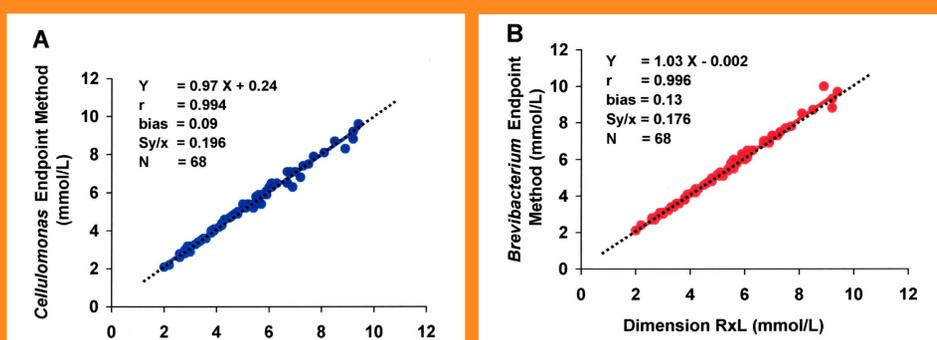
Inventive cholesterol reagents for the enzymatic endpoint method utilize the new cholesterol oxidase derived from *Cellulomonas* or *Brevibacterium*. The cholesterol determination using each enzyme proves the appropriate assay used in routine clinical laboratory because its accuracy and precision meet the currently established analytical performance goals. Linearity of serum cholesterol obtained from each reagent is up to 18.1 mmol/L (700 mg/dL). A method comparison study with the CDC-standardization method shows excellent correlation and acceptable agreement between results.



Analytical Bias

Sample	CDC Cholesterol (mmol/L)*	Bias, % Cellulomonas	Brevibacterium
1	3.92	0.681	0.043
2	4.09	0.312	-1.032
3	4.43	0.614	0.552
4	5.10	-0.306	-0.523
Average		0.33	-0.24

* The reference value was assigned from Abell-Kendall reference method



Correlation of Cholesterol

Advantages of Inventive Cholesterol Reagents

Most commercial enzymatic cholesterol reagents have used cholesterol oxidase isolated from *Nocardia*, and *Streptomyces*. Performance characteristics of *Cellulomonas* and *Brevibacterium* enzymes are essentially analytical equivalent to the generally used sources but are less costly. The cost of *Nocardia* enzyme is expensive while *Streptomyces* requires shipment on ice (-20o C). For this reason, *Cellulomonas* and *Brevibacterium* may represent the best enzyme for use in cholesterol determination by the endpoint method.

The reagents can be applied for 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, porous synthetic, membrane, fiber and electrode.

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