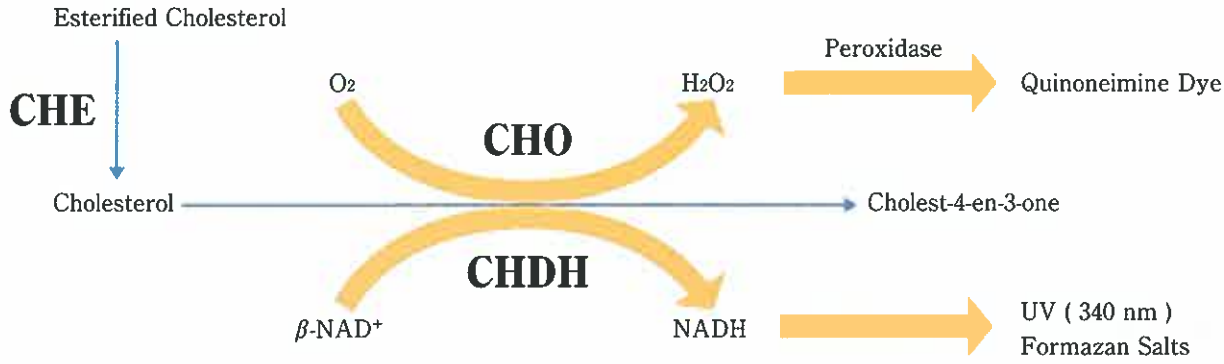

Figure 1. Enzymatic Determination of Cholesterol.
CHE , Cholesterol esterase ; CHO , Cholesterol oxidase ; CHDH , Cholesterol dehydrogenase.

Arteriosclerotic diseases such as cerebral infarction and ischemic heart diseases (myocardial infarction and angina pectoris) are one of the primary causes of death not only in European and American countries but also in Japan.
A large-scale epidemiological study indicated that obesity, aging, smoking, hyperlipidemia, and diabetes are the risk factors for arteriosclerotic heart diseases. A number of studies enabled us to draw a rough picture representing the process of arteriosclerosis from the accumulation of cholesterol in the wall of blood vessels to the manifestation of arteriosclerotic diseases.

Since the discovery of the relationship between blood cholesterol concentration and arteriosclerotic diseases, measuring cholesterol has been one of the most widely performed medical tests. This article will focus on how to measure blood cholesterol.

Transportation of blood cholesterol

Cholesterol is a component of the biomembrane and serves as a precursor for bile acid, steroid hormones, and vitamins. Cholesterol is absorbed from the diet through the small intestine or synthesized in the liver and transported to peripheral tissues via the blood stream. Since cholesterol is almost completely insoluble in water, it is transported via lipoproteins.

Lipoproteins are spherical granules encapsulated with a monolayer membrane consisting of phospholipids, cholesterol, and proteins. Cholesterol esterified with fatty acid and triglyceride constitutes the hydrophobic core of the sphere. The surface of the granule is composed of proteins, phospholipids, and the hydrophilic part of cholesterol, which makes the entire granule a blood-soluble, transportable lipid.

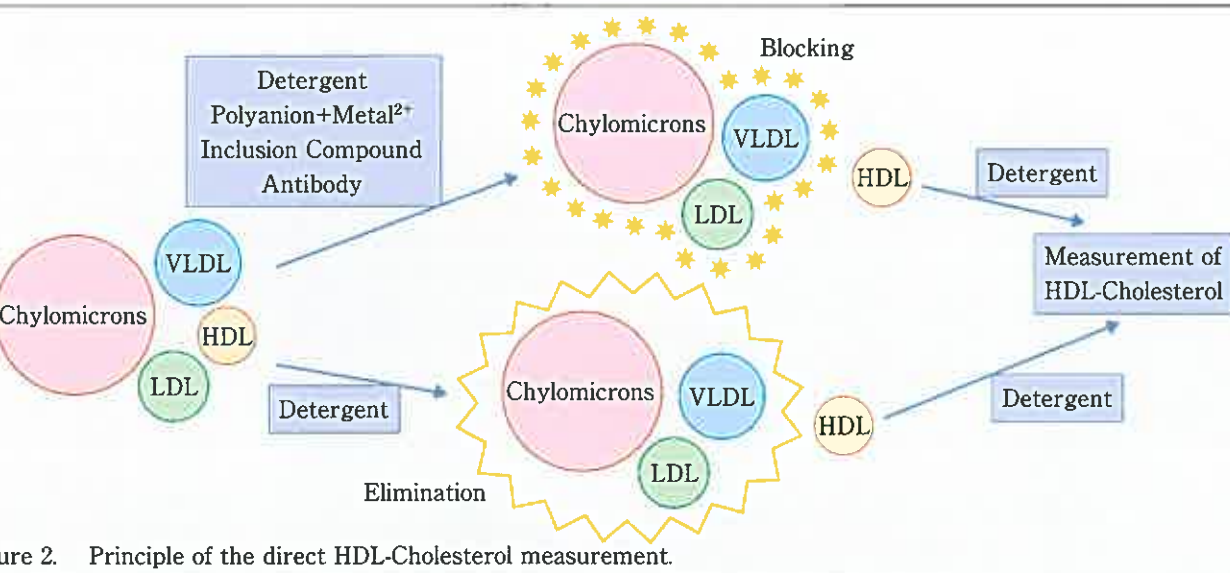

Figure 2. Principle of the direct HDL-Cholesterol measurement.
HDL, High-density lipoprotein ; LDL, Low-density lipoprotein ; VLDL, Very low-density lipoprotein.

Among lipoproteins, high-density lipoprotein (HDL) protects against atherosclerosis, but low-density lipoprotein (LDL) induces atherosclerosis. Therefore, to evaluate risk for atherosclerosis, it is necessary to separate HDL or LDL from other lipoproteins so that the concentrations of cholesterol contained in HDL and LDL can be measured separately.

Quantification of blood cholesterol

There are two methods for enzymatic measurements of cholesterol: the system using cholesterol oxidase (CHO) and the system using cholesterol dehydrogenase (CHDH). In the CHO system, hydrogen peroxide generated by the reaction catalyzed by CHO is mixed with a hydrogen donor and coupler to create color (quinoneimine pigment) by the action of peroxidase. In the CHDH system, NADH generated by the reaction catalyzed by CHDH is measured by absorption in the ultraviolet part of the spectrum or is coupled with electron mediators such as diaphorase and phenazinium compounds to form formazan salts, which can be measured by absorbance in the visible part of the spectrum **(Figure 1)**.

Distinguishing between cholesterol in HDL or LDL relies on 1) surfactants, 2) a variety of additives, and 3) specificity of enzymes used [cholesterol esterase (CHE), CHO, CHDH]. We optimize these factors individually when we measure cholesterols within HDL or LDL that possess various molecular sizes, surface characteristics, and molecular ingredients.

Blood total cholesterol concentration is measured by performing an enzymatic reaction after solubilizing all lipoproteins with surfactants. Esterified cholesterol is converted to cholesterol by the action of CHE to remove the fatty acids bound to cholesterol, and then cholesterol concentration is determined by the enzymatic reaction as described above.

The concentration of cholesterol within HDL is determined by the direct measuring method. In the direct measuring method, either the surface of the lipoproteins other than HDL are blocked by surfactants, clathrate compounds (cyclodextrin sulfate, calixarene), chemical modification enzymes, antibodies, etc. to inhibit enzymatic cholesterol oxidation, or cholesterol contained in the lipoproteins other than HDL is degraded. As the next step, a surfactant that can exclusively solubilize HDL is added so that cholesterol within HDL is measured. However, the above-described blocking method cannot completely block the cholesterol existing at the surface of the lipoproteins other than HDL; we therefore add polyanions and Mg^{2+} for complete blocking **(Figure 2)**.

Concluding remarks

Management of the risk factors described previously is essential to prevent arteriosclerotic diseases. To manage hyperlipidemia, especially hypercholesterolemia, it is necessary to monitor continuously. We believe our method to measure cholesterol within lipoproteins, especially HDL and LDL, as described in this article can contribute in some way to the possible future self-monitoring of blood cholesterol.

Table Amano's Diagnostic Enzymes for Cholesterol Determination

Product name	Origin	Activity
Cholesterol esterase "AMANO" 3	*Pseudomonas* sp.	≧ 15 u/mg
Cholesterol oxidase "AMANO" 1	*Burkholderia* sp.	≧ 1.5 u/mg
Cholesterol oxidase "AMANO" 6	*Microorganism*	≧ 10 u/mg
Cholesterol dehydrogenase "AMANO" 5	*Nocardia* sp.	≧ 5 u/mg