

Lipid Profile (total cholesterol, HDL-cholesterol, triglyceride, calculated LDL)

These assays are run at the Clinical Chemistry Laboratory at Fletcher Allen Health Care, an affiliate of the University of Vermont. The Ortho Vitros Clinical Chemistry System 950IRC instrument (Johnson & Johnson Clinical Diagnostics, Rochester, NY), which uses thin film technology, is used to quantitatively measure lipid levels via a colorimetric reaction.

Cholesterol is measured using a colorimetric reflectance spectrophotometric method. Normal ranges for adults are Desirable : < 200 mg/dL; Borderline: 200 – 239 mg/dL; High: => 240 mg/dL. The reportable range for this assay is 50-325mg/dl. The expected CV of this assay is <2%.

Direct HDL Cholesterol is assayed by colorimetric reflectance spectrophotometry after samples are treated with phosphitungstic acid/magnesium chloride to precipitate HDLs and non-HDLs. Normal ranges for adults are Highly Desirable: > 60 mg/dL; Desirable: 35-60 mg/dL; High Risk: <40 mg/dL. The reportable range for this assay is 5.0-110.0 mg/dL. The expected CV of this assay is approximately 7%.

LDL Cholesterol is calculated: $\text{Total Cholesterol} - \{\text{HDL} + (\text{Triglycerides}/5)\}$. Normal ranges for adults are Desirable: < 130 mg/dL; Borderline: 130-159 mg/dL; High Risk: >= 160 mg/dL.

Triglyceride is measured by colorimetric reflectance spectrophotometry. Normal ranges for adults are Normal:<150 mg/dL; Borderline High: 150-199 mg/dL; High; Very High: >=150mg/dL . The reportable range for this assay is 10.0-525.0 mg/dL. The expected CV of this assay is <2%.

INSTRUCTIONS FOR USE

dHDL

VITROS Chemistry Products dHDL Slides

Direct HDL Cholesterol

REF 680 1895
680 2469

Intended Use

For *in vitro* diagnostic use only.

VITROS dHDL Slides are used to quantitatively measure HDL cholesterol (HDLC) concentration in serum and plasma. High Density Lipoprotein (HDL) cholesterol is used to evaluate the risk of developing coronary heart disease (CHD). The risk of CHD increases with lower HDL cholesterol concentrations.

Summary and Explanation of the Test

High Density Lipoprotein (HDL) cholesterol is used to evaluate the risk of developing coronary heart disease (CHD). The risk of CHD increases with lower HDL cholesterol concentrations.

Principles of the Procedure

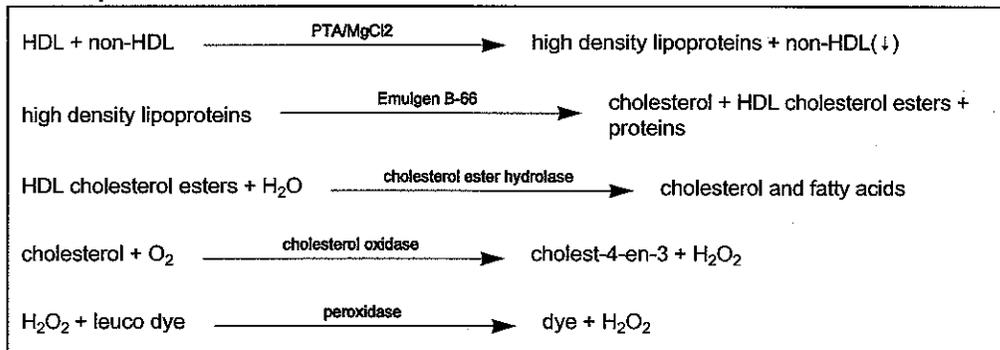
The VITROS dHDL Slide method is performed using the VITROS dHDL Slides and the VITROS Chemistry Products Calibrator Kit 25 on VITROS Chemistry Systems.

The VITROS dHDL Slide is a multilayered analytical element coated on a polyester support. The method is based on a non-HDL precipitation method similar to one used by Burstein et al.¹, followed by an enzymatic detection similar to that proposed by Allain et al.²

A drop of patient sample is deposited on the slide and is evenly distributed by the spreading layer to the underlying layers. HDL is separated by the precipitation of non-High Density Lipoproteins (non-HDL) using phosphotungstic acid (PTA) and magnesium chloride (MgCl₂) in the spreading layer. The Emulgen B-66 surfactant in the spreading layer aids in the selective dissociation of the cholesterol and cholesterol esters from the HDL lipoprotein complexes present in the sample. Hydrolysis of the HDL-derived cholesterol ester to cholesterol is catalyzed by a selective cholesterol ester hydrolase. Free cholesterol is then oxidized in the presence of cholesterol oxidase to form cholestenone and hydrogen peroxide. Finally, hydrogen peroxide oxidizes a leuco dye in the presence of peroxidase to generate a colored dye.

The density of dye formed is proportional to the HDL cholesterol concentration present in the sample and is measured by reflectance spectrophotometry.

Reaction Sequence



Test Type and Conditions

Test Type and Conditions for dHDL

Test Type	VITROS System	Approximate Incubation Time	Temperature	Wavelength	Sample Drop Volume
Colorimetric	250, 350, 950, 5,1 FS	5 minutes	37 °C (98.6 °F)	670 nm	10 µL

dHDL

Direct HDL Cholesterol

INSTRUCTIONS FOR USE

Warnings and Precautions

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For *in vitro* diagnostic use only.

Take care when handling materials and samples of human origin. Since no test method can offer complete assurance that infectious agents are absent, consider all clinical specimens, controls, and calibrators potentially infectious. Handle specimens, solid and liquid waste, and test components in accordance with local regulations and CLSI Guideline M29³ or other published biohazard safety guidelines.

For specific warnings and precautions for calibrators, quality control materials, and other components, refer to the Instructions for Use for the appropriate VITROS product, or to other manufacturer's product literature.

Reagents

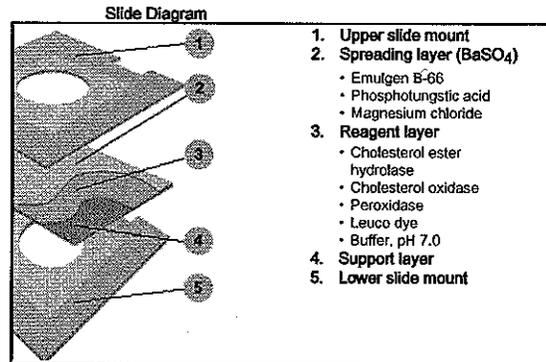
Slide Ingredients

Reactive Ingredients per cm²

Emulgen B-66 0.7 mg; phosphotungstic acid 0.3 mg; magnesium chloride 0.2 mg, cholesterol oxidase (*Cellulomonas*, E.C.1.1.3.6) 0.8 U; cholesterol ester hydrolase (*Candida rugosa*, E.C.3.1.1.3) 1.2 U; peroxidase (horseradish root, E.C.1.11.1.7) 2.2 U; and 2-(3,5-dimethoxy-4-hydroxyphenyl)-4,5-bis-(4-dimethylaminophenyl) imidazole (leuco dye) 0.02 mg.

Other Ingredients

Pigment, binders, buffer, surfactants, stabilizers, scavenger, and cross-linking agent.



Reagent Handling

Caution: Do not use slide cartridges with damaged or incompletely sealed packaging.

- Inspect the packaging for signs of damage.
- Be careful when opening the outer packaging with a sharp instrument so as to avoid damage to the individual product packaging.

Reagent Preparation

IMPORTANT: The slide cartridge must reach room temperature, 18–28 °C (64–82 °F), before it is unwrapped and loaded into the slide supply.

IMPORTANT: dHDL cartridges contain cartridge clips that enhance long term storage. These must be removed prior to being loaded into the slide supply. Do not remove the clip from the cartridge until ready to load the cartridge on the system.