

IN VITRO DIAGNOSTIC USE



|     |       |             |               |               |                      |
|-----|-------|-------------|---------------|---------------|----------------------|
| REF | 23018 | (200 Tests) | R1: 2 x 30 ml | R2: 2 x 10 ml | R3: 1 x 1 ml (Lyoph) |
| REF | 23025 | (100 Tests) | R1: 1 x 30 ml | R2: 1 x 10 ml | R3: 1 x 1 ml (Lyoph) |

CLINICAL SIGNIFICANCE

The principal role of high density lipoprotein (HDL) in lipid metabolism is the uptake and transport of cholesterol from peripheral tissue to the liver through a process known as reverse cholesterol transport. Low HDL cholesterol levels are strongly associated with an increased risk of coronary heart disease and coronary artery disease. Hence, the determination of serum HDL-Cholesterol is a useful tool in identifying high-risk patients. Increased Total Cholesterol/HDL-Cholesterol ratio is significant of an increased risk of atherosclerosis.

PRINCIPLE

“Selective detergent and accelerator» methodology  
Direct method, without pre-treatment of the specimen.  
During the first phase, LDL, VLDL, and chylomicron particles release free cholesterol which undergoes an enzymatic reaction, producing hydrogen peroxide, which is degraded by the reaction with POD and DSSmT. No coloured derivatives are formed.  
During the second phase, a specific detergent solubilises the HDL cholesterol. Under the combined action of CO and CE, the POD + 4- AAP couple develops a coloured reaction proportional to the HDL cholesterol concentration. The reading is taken at 600 nm.  
**LDL**= Low Density Lipoproteins  
**HDL** = High Density Lipoprotein  
**VLDL**= Very Low Density Lipoproteins - **POD** = Peroxidase  
**CO** = Cholesterol Oxidase - **CE** = Cholesterol Esterase – 4  
**AAP** = 4-Aminoantipyrine - **AAO** = Ascorbate Oxidase  
**DSSmT** = N, N-bis(sulphobutyl)-m-toluidine-disodium

REAGENT COMPOSITION

|           |   |   |
|-----------|---|---|
| Reagent 1 | GOOD<br>Cholesterol oxidase<br>Peroxidase<br>DSBmT                                  | pH = 7<br>< 1000 U/l<br>< 1300 U/l<br>< 1mM         |
| Reagent 2 | GOOD<br>Cholestérol oxydase<br>4 Amino Antipyrine<br>Detergent<br>Ascorbate oxidase | pH = 7<br>< 1500 U/l<br>< 1mM<br>< 2%<br>< 3000 U/l |
| Reagent 3 | HDLc/LDLc calibrator: freeze-dried human serum                                      |   |

SAFETY CAUTIONS

Biomaghreb reagents are intended for use by qualified personnel for in vitro use (do not pipette by mouth).  
- Refer to the current SDS available on request or at www.biomaghreb.com;  
- Verify the integrity of the reagents before use; and  
- Disposal of waste: comply with the legislation in force.

For safety reasons, treat any specimen or reagent of biological origin as potentially infectious. Respect the legislation in force.

REAGENT PREPARATION

Reagents R1 and R2 are ready for use.  
- Reconstitute the vial of the HDLc/LDLc calibrator with 1 ml of distilled water, then homogenize the contents of the bottle gently.  
- Wait 30 minutes before use.  
*For safety reasons, treat the calibrator as potentially infectious.*

SAMPLE PREPARATION

The patient must be taken after at least 12h -14h fasting  
-Plasma : collected on EDTA or sodium heparinate or lithium ; citrate must not be used.  
Separate plasma from blood cells by centrifugation within 3 hours after collection.  
-Serum: Centrifugally separate the Serum from the blood cells within 3 hours after collection.  
Sera and plasma should not be left at room temperature for more than 14 hours.  
HDL-cholesterol is stable in the specimen:  
- 7 days at 2-8°C ;  
- 1 month at -20°C.

PRESERVATION AND STABILITY

Store at 2-8°C, in the original bottle, tightly stoppered and protected from light.  
• **Before opening** : if stored under the recommended conditions, the reagents are stable until the expiry date indicated on the label.  
• **After opening and in the absence of contamination**: Reagents R1 and R2 are stable for 8 weeks at 2-8°C.  
• **After reconstitution**: The calibrator is stable 2 weeks at 2-8°C and 3 months at -20°C.

LIMITS

Do not use the reagents if they are cloudy or after the expiry date.

ADDITIONAL EQUIPMENT

- Basic equipment of the medical analysis laboratory ;
- Spectrophotometer or Clinical Biochemistry Analyzer.

LINEARITY

The reaction is linear from 25mg/dl up to 200mg/dl.  
Above this concentration, dilute the sample 1+1 with a 9 g/l NaCl solution and repeat the determination.  
Multiply the result by 2.

PROCEDURE

Wavelength.....600-700 nm  
Tank: .....1 cm thick  
Temperature.....37°C  
Adjusting the spectrophotometer zero with distilled water

|            | Blank  | Calibrator | Dosage |
|------------|--------|------------|--------|
| Reagent R1 | 300 µl | 300 µl     | 300 µl |
| Calibrator | - -    | 3 µl       | - -    |
| Sample     | - -    | - -        | 3 µl   |

|  |        |            |        |
|--|--------|------------|--------|
| Stir well, leave to stand for 5 minutes at 37°C.<br>Record absorbances A1 at 600 nm against the reagent blank. |        |            |        |
| Add  | Blank  | Calibrator | Dosage |
| Reagent R2   | 100 µl | 100 µl     | 100 µl |
| Mix well, let stand 5 minutes at 37°C.<br>Record absorbances A2 against the reagent blank                      |        |            |        |

CALCULATION

Calculating the increase in absorbance A = A2-A1

$$\frac{A \text{ sample}}{A \text{ calibrator}} \times \text{Calibrator Concentration}$$

= mg/dl of HDL direct

with mg/dl x 0,0259 = mmol/l

REFERENCE VALUES

|               | Men        | Women      |
|---------------|------------|------------|
| Low risk      | > 50mg/dl  | > 60mg/dl  |
| Moderate risk | 35-50mg/dl | 45-60mg/dl |
| High risk     | <35mg/dl   | <45mg/dl   |

-Concentrations tested (mg/dl) without significant interference (+10%):

|                       |            |
|-----------------------|------------|
| Conjugated bilirubin: | 60 mg/dl   |
| Total Bilirubin:      | 60 mg/dl   |
| Hemoglobin :          | 1000 mg/dl |
| Ascorbic Acid:        | 100 mg/dl  |
| Fat (intralipid)      | 1800 mg/dl |

-The reagent may interfere with the magnesium determination.

REFERENCES

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Manufacturer



Use by



In Vitro Diagnostic



Temperature  
Limitation



Catalogue number



See insert



Store away from light



Sufficient  
for < n > essays



Batch number