Dialab Cholesterol HDL Direct is a homogeneous method for HDL-cholesterol measurement without centrifugation steps. It uses immobilized Anti-human ß-lipoprotein antibodies to immobilize the lipoproteins in the sample. The reagent contains Cholesterol Oxidase, Peroxidase, Ascorbate Oxidase, and 4-Aminoantipyrine as reaction substrates. The measurement is carried out in two incubation steps, with reagent 1 and reagent 2, followed by absorbance measurement.

### TEST PRINCIPLE

Dialab Cholesterol HDL Direct is a homogeneous method for HDL-cholesterol measurement without centrifugation steps. Antibodies against human lipoproteins form antigen-antibody complexes with LDL, VLDL, and chylomicrons in a way that only HDL is responsible for the HDL-cholesterol uptake from the cells. The four different lipoprotein classes show distinct relationship to coronary atherosclerosis. HDL-cholesterol has a protective effect impending plaque formation and shows an inverse relationship to CHD prevalence. In fact, low HDL-cholesterol values constitute an independent risk factor.

### IMMUNOINHIBITION

Diagnostic reagent for quantitative in vitro determination of high density lipoprotein cholesterol (HDL-C) in human serum or plasma on photometric systems.

### SUMMARY [1, 2]

Cholesterol is transported in plasma via lipoproteins, namely complexes between lipids and apolipoproteins. There are four classes of lipoproteins: high density lipoproteins (HDL), low density lipoproteins (LDL), very low density lipoproteins (VLDL), and chylomicrons. While LDL is involved in the cholesterol transport to the peripheral cells, HDL is responsible for the cholesterol uptake from the cells. The four different lipoprotein classes show distinct relationship to coronary atherosclerosis. HDL-cholesterol has a protective effect impending plaque formation and shows an inverse relationship to CHD prevalence. In fact, low HDL-cholesterol values constitute an independent risk factor.

### TEST PRINCIPLE

Dialab Cholesterol HDL Direct is a homogeneous method for HDL-cholesterol measurement without centrifugation steps. Antibodies against human lipoproteins form antigen-antibody complexes with LDL, VLDL, and chylomicrons in a way that only HDL-cholesterol is selectively determined by an enzymatic cholesterol measurement [4].

### REAGENT PREPARATION

Substrate Start:
- Good’s Buffer pH 7.0
- Cholesterol Esterase
- Anti human ß-lipoprotein Ab. (sheep)

Sample Start:
- Good’s Buffer pH 7.0
- Cholesterol Esterase
- F-DAOs

### MANUAL TEST PROCEDURE

**Blind Sample/Cal.**

- Sample/Calibrator
- Reagent 1
- Reagent 2

**Mix. Incubate for 5 min. at 37°C**

- Read absorbance (A1) & (A2)

**Add:**

- Reagent 2
- Reagent 1

**Calculate:**

\[
\text{H}_{2}\text{O} + \text{F-DAOs} + 4\text{-Aminoantipyrine} \xrightarrow{\text{POD}} \text{blue colored complex + H}_{2}\text{O}
\]

### CALCULATION

**UNIT CONVERSION**

\[
\text{mg/dL x 0.02586 = mmol/L}
\]

**REFERENCE RANGE [7]**

\[
\geq 35 \text{ mg/dL (0.9 mmol/L)}
\]

* Each laboratory should check if reference ranges are transferable to its own patient population and determine own reference ranges as necessary.
Clinical Interpretation
Epidemiological studies have observed that low HDL-
cholesterol concentrations < 39 mg/dL (0.9 mmol/L) in men and
< 43 mg/dL in women, especially if associated with fasting
triglycerides > 180 mg/dL (2 mmol/L), predict a high risk of
coronary heart disease [2].

PERFORMANCE CHARACTERISTICS
LINEARITY, MEASURING RANGE
The test has been developed to determine HDL Cholesterol
concentrations within a measuring range from 1 –180 mg/dL
(0.03 – 4.66 mmol/L). If concentration exceeds 180 mg/dL,
samples should be diluted 1 + 2 with NaCl (9 g/L sodium
chloride in water) and results multiplied by 3.

SENSITIVITY/LIMIT OF DETECTION
The lower limit of detection is 1 mg/dL (0.03 mmol/L).

PRECISION

<table>
<thead>
<tr>
<th>Intra-assay, n = 20</th>
<th>Mean [mg/dL]</th>
<th>SD [mg/dL]</th>
<th>CV [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 1</td>
<td>24.0</td>
<td>0.31</td>
<td>1.27</td>
</tr>
<tr>
<td>Sample 2</td>
<td>49.0</td>
<td>0.26</td>
<td>0.52</td>
</tr>
<tr>
<td>Sample 3</td>
<td>97.7</td>
<td>0.64</td>
<td>0.65</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Inter-assay, n = 20</th>
<th>Mean [mg/dL]</th>
<th>SD [mg/dL]</th>
<th>CV [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 1</td>
<td>27.3</td>
<td>0.54</td>
<td>2.00</td>
</tr>
<tr>
<td>Sample 2</td>
<td>58.0</td>
<td>0.57</td>
<td>0.98</td>
</tr>
<tr>
<td>Sample 3</td>
<td>98.6</td>
<td>1.34</td>
<td>1.36</td>
</tr>
</tbody>
</table>

SPECIFICITY/INTERFERENCES
no interference up to:
Ascorbic acid 50 mg/dL
Bilirubin 50 mg/dL
Bilirubin conjugated 40 mg/dL
hemoglobin 500 mg/dL
triglycerides 1200 mg/dL
For further information on interfering substances refer to Young
DS [6].

METHOD COMPARISON
A comparison of Dialab HDL Cholesterol (y) with a
commercially available test (x) using 100 samples gave
following results:
y = 1.05 x + 0.571 mg/dL; r = 0.995.

CALIBRATION
The assay requires the use of a HDL Cholesterol Calibrator.
We recommend the Dialab HDL-Cholesterol Calibrator or the
lipid calibration plasma Diacal Lipids.
The value in the the HDL-Cholesterol Calibrator is traceable to
the CDC reference method Ultracentrifugation/Heparin-Mn, and
in Diacal Lipids to NIST SRM® 1951 Level 2.

QUALITY CONTROL
All control sera with HDL Cholesterol values determined by this
method can be used.
We recommend the Dialab lipid control sera Diacon Lipids and
Diacon Lipids High and the Dialab multi control sera Diacon N
(with values in the normal range) and Diacon P (with values in
the pathological range).
Each laboratory should establish corrective action in case of
deviations in control recovery.

AUTOMATION
Special applications for automated analyzers can be made on
request.

WARNINGS AND PRECAUTIONS
1. Reagent 1: Warning
H317: May cause an allergic skin reaction.
P280: Wear protective gloves/protective clothing/eye
protection/face protection.
P302+P352: IF ON SKIN: Wash with plenty of water/soap.
P333+P313: If skin irritation or rash occurs. Get medical
advice/attention.

2. In very rare cases, samples of patients with gammopathy
might give falsified results [8].

3. N-acetylcysteine (NAC), acetaminophen and metamizole
medication leads to falsely low results in patient samples.

4. When using enzymatic methods for the determination of
cholesterol, esters, contamination and interference to other
clinical chemistry assays on the same instrument in
principle cannot be excluded. In the event of such a problem
occurring, please refer to the instrument’s manual for
channel setting and washing procedure options.

5. Please refer to the safety data sheets and take the
necessary precautions for the use of laboratory reagents.

6. For diagnostic purposes, the results should always be
assessed with the patient’s medical history, clinical
examinations and other findings.

7. For professional use only!

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