Quantitative determination of apolipoprotein A-I (APO A-I)

IVD

Store 2 - 8°C.

PRINCIPLE OF THE METHOD

Turbidimetric test for the measurement of apolipoprotein A-I in human serum or plasma.

ANTI- APO A-I antibodies when mixed with samples containing APO A-I, form insoluble complexes. These complexes cause an absorbance change, dependent upon the APO A-I concentration of the patient sample, that can be quantified by comparison from a calibrator of known APO A-I concentration.

CLINICAL SIGNIFICANCE

Apo A-I is the major structural apolipoprotein in HDL and constitutes about 70% of the total protein. Apo A-I is a cofactor for lecithin-cholesterol-acyltransferase (LCAT), the enzyme responsible for forming cholesteryl esters in plasma and plays an important role in the transport of cholesterol from peripheral tissues to the liver, to be finally excreted. Measurements of Apo A-I concentration is specially important in detecting coronary heart disease risk (CHD) as well as in the diagnostic of hyperlipoproteinemia. Concentrations < 120 mg/dL are associated to an increased CHD risk, while concentrations ≥ 160 mg/dL may even protect from the same risk. Patients with deficiencies in Apo A-I synthesis may highly increase the CHD risk.

Tanger disease, a consequence of an Apo A-I catabolism defect, is characterized by several reduced plasma HDL cholesterol (HDL-c) concentrations, abnormal HDL composition and accumulation of cholesteryl esters in many body tissues. Plasma HDL-c and Apo A-I concentrations in homozygotes are very low, while Apo A-II concentration is less than 10% of its normal concentration. Heterozygotes are characterized by half-normal concentration of HDL-c, Apo AI and Apo A-II. Current evidence suggests that these patients have increased incidence of CHD.

RELENTS

Diluent (R1) Tris buffer 100 mmol/L, PEG 4000, pH 7.2. Sodium azide 0.95 g/L.

Antibody (R2) Goat serum, anti-human Apo A-I, tris 100 mmol/L, pH 7.2. Sodium azide 0.95 g/L.

Optional APO CAL ref: 93005

CALIBRATION

The assay and the value of the calibrator concentration have been standardized against the Certified Reference Material WHO/IFCC SP1-01 (CDC, USA). It is recommended the use of the APO CAL Calibrator for calibration.

PREPARATION

Reagents: Ready to use.

STORAGE AND STABILITY

All the components of the kit are stable until the expiration date on the label when stored tightly closed at 2-8°C and contaminations are prevented during their use. Do not use reagents after the expiration date.

Reagent deterioration: The presence of particles and turbidity.

Do not freeze; frozen Antibody or Diluent could change the functionality of the test.

ADDITIONAL EQUIPMENT

- Thermostatic bath at 37°C.
- Spectrophotometer or photometer thermostatable at 37°C with a 600 nm filter.

SAMPLES

Fresh serum or plasma. EDTA or heparin should be used as anticoagulant.

Stable 2 weeks at 2-8°C or 3 months at –20°C.

The samples with presence of fibrin should be centrifuged before testing.

Do not use highly hemolized or lipemic samples.

PROCEDURE

1. Bring the reagents and the photometer (cuvette holder) to 37°C.
2. Assay conditions:
   - Wavelength: 600 nm
   - Temperature: 37 ºC
   - Cuvette light path: 1cm
3. Adjust the instrument to zero with distilled water.
4. Pipette into a cuvette:
   - Reagent R1 (µL) 750
   - Sample or Calibrator (µL) 8
5. Mix and read the absorbance (A1) after the sample addition.
6. Immediately, pipette into de cuvette:
   - Reagent R2 (µL) 250
7. Mix and read the absorbance (A2) of calibrators and sample exactly 5 minutes after the R2 addition.

Spinreact has instruction sheets for several automatic analyzers. Instructions for many of them are available on request.

CALCULATIONS

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\frac{A_2 - A_1}{\text{calibrator}} \times \text{Calibrator concentration} = \text{mg/dL Apo A-I}
\]

QUALITY CONTROL

Control sera are recommended to monitor the performance of manual and automated assay procedures. Spinreact Apolipoprotein Control (Ref: 93006) is available. Each laboratory should establish its own Quality Control scheme and corrective actions if controls do not meet the acceptable tolerances.

REFERENCE VALUES

Between 122 – 161 mg/dL. Each laboratory should establish its own reference range.

PERFORMANCE CHARACTERISTICS

1. Linearity: Up to 200 mg/dL (Nota 1), under the described assay conditions. Samples with higher concentrations, should be diluted 1/5 in NaCl 9 g/L and retested again. The linearity limit depends on the sample / reagent ratio. It will be higher by decreasing the sample volume, although the sensitivity of the test will be proportionally decreased.
2. Detection Limit: Values less than 0.76 mg/dL give non-reproducible results.
3. Prozone effect: No prozone effect was detected upon 280 mg/dL.
4. Sensitivity: Δ 2.84 mA / mg/dL (148 mg/dL).
5. Precision:

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean (mg/dL)</th>
<th>SD</th>
<th>CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intra-assay (n=10)</td>
<td>105.3</td>
<td>0.88</td>
<td>0.76</td>
</tr>
<tr>
<td>Inter-assay (n=5)</td>
<td>103.3</td>
<td>0.70</td>
<td>0.66</td>
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</tbody>
</table>

6. Accuracy: Results obtained using this reagent (y) were compared to those obtained with single radial immuno diffusion (SRDI) method. 50 samples ranging from 60 to 180 mg/dL of Apo A-I were assayed. The correlation coefficient (r) was 0.956 and the regression equation y = 0.9997x + 1.70.

The results of the performance characteristics depend on the used analyzer.

INTERFERENCES

Hemoglobin (up to 500 mg/L), bilirubin (up to 40 mg/dL), and lipemia (up to 20 g/L), do not interfere. Other substances may interfere 3, 4, 5.

NOTES

1. Linearity depends on the calibrator concentration.
2. Clinical diagnosis should not be made on findings of a single test result, but should integrate both clinical and laboratory data.

BIBLIOGRAPHY