

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 know Apo A-I concentration.

CLINICAL SIGNIFICANCE¹

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-acyl-transferase (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/L are associated to an increased CHD risk, while concentrations ≥ 160 mg/L 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) concentration, 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 -II. Current evidence suggests that these patients have increased incidence of CHD.

REAGENTS

R1	Tris buffer 100 mmol/L, PEG 4000, pH 7.2. Sodium azide 0.95 g/L.
R2	Goat serum, anti-human Apo A-I, tris 100 mmol/L, pH 7.2. Sodium azide 0.95 g/L.
Saline Solution	NaCl 9% (for samples predilution)
Optional	APO A-I/B Dual Control ref: ACC16-014

CALIBRATION

The assay and the value of the calibrator concentration have been standardized against the Certified Reference Material WHO/IFCC SP1-01 (CDC, USA).

PREPARATION

Working Reagent: Swirl the R2 vial gently before use. Prepare the necessary amount as follows:

- 50 µL R2 + 2 mL R1 (1:41 dilution)

Samples and Controls Pre-Dilution: Before the use, samples and/or controls must be diluted in this way:

- 25 µL sample or control + 500 µL NaCl 9 g/L = Dilution 1
- 25 µL Dilution 1 + 100 µL NaCl 9 g/L

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 contamination are prevented during their use. Do not use reagents over the expiration date.

Reagent deterioration: The presence of particles and turbidity.

Working Reagent: The working reagent is stable 2 weeks at 2-8 °C.

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

ADDITIONAL EQUIPMENT

- Pipette 100 - 1000 µL
- Pipette 10 - 100 µL
- Cuvettes and microstirrers (ref. code ACC16-037)

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 hemolyzed or lipemic samples.

TEST PARAMETER

Filter: set filter on position A

Reading time: 300 seconds

PROCEDURE

1. Pipette into an appropriate number of cuvettes:

kind of reagent	µL
Working Reagent	500

2. Incubate the cuvettes in positions 1-4 for at least 5 minute
3. Transfer each cuvette in the reading channel and, when requested on the display, add 50 µL of prediluted sample or control.
4. Read the result which will appear automatically on the reader's display after 300 sec

QUALITY CONTROL

Control sera are recommended to monitor the performance of the assay procedure. Electa Lab Apolipoprotein A1/B dual Control (Ref.ACC16-014) is available. Each laboratory should establish its own Quality Control scheme and corrective actions if controls do not meet the acceptable tolerances.

REFERENCE VALUES⁵

Between 122 – 161 mg/dL.

Each laboratory should establish its own reference range.

PERFORMANCE CHARACTERISTICS

1. Linearity: Up to 500 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:

	Mean	SD	CV%
Intra-assay (n=10)	105.3	0.8	0.76
Inter-assay (n=10)	153.9	1.1	3.4

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 $y = 0.9997x + 1.70$.

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 ^{6,7}.

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

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2. Mahley RW et al. J Lipids Res 1984; 25: 1277-1294.
3. Rifai N Arch Pathol Lab Med 1986; 110: 694-701.
4. Freedman DS et al. N Eng J Med 1986; 315: 721-726.
5. Sakurabayashi I et al. Clinica Chimica Acta 2001; 312: 87-95.
6. Young DS. Effects of disease on clinical laboratory tests, 3th ed. AACC Pres, 1997.
7. Friedman and Young. Effects of disease on clinical laboratory tests, 3tn ed. AACC Pres, 1997.

PACKAGING

	: 1 x 80 mL R1	
Ref.: ACC16-001	: 1 x 2 mL R2	Cont.
	: 1 x 80 mL Saline Solution	