

Store at +2 to +8°C

PRINCIPLE

Turbidimetric test for the measurement of Apo A₁ in human serum or plasma. Anti- Apo A₁ antibodies when mixed with samples containing Apo A₁, form insoluble complexes. These complexes cause an absorbance change, dependent upon the Apo A₁ concentration of the patient sample, that can be quantified by comparison from a calibrator of known Apo A₁ concentration.

CLINICAL SIGNIFICANCE

Apo A₁ is the major structural apolipoprotein in HDL and constitutes about 70% of the total protein. Apo A₁ 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 A₁ concentration is specially important in detecting coronary heart disease risk (CHD) as well as in the diagnosis 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 A₁ synthesis may highly increase the CHD risk.

Tanger disease, a consequence of an Apo A₁ 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 A₁ concentrations in homozygotes are very low, while Apo A₂ concentration is less than 10% of its normal concentration. Heterozygotes are characterized by half-normal concentration of HDL-c, Apo A₁ and Apo A₂. Current evidence suggests that these patients have increased incidence of CHD.

REAGENTS

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 A₁, tris 100 mmol/l,
pH 7.2. Sodium azide 0.95 g/l

Optional: 101-0499 Apolipoproteins Calibrator
101-0503 Apolipoproteins Control

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 Apolipoprotein 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 to 8 °C and contaminations prevented during their use. Do not use reagents over the expiration date. Reagent deterioration: Presence of particles and turbidity.

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 to +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.

PROCEDURE

1. Bring the reagents and the photometer (cuvette holder) to 37°C.
2. Assay conditions:
Wavelength: 600 nm
Temperature: 37°C
Cuvette light path: 1 cm
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 immediately (A₁) after the sample addition.
6. Immediately, pipette into de cuvette:
Reagent 2 (µl) 250
7. Mix and read the absorbance of calibrators and sample exactly 5 minutes after the Reagent 2 addition.

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

CALCULATION

$$\frac{(A_2 - A_1)_{\text{sample}}}{(A_2 - A_1)_{\text{calibrator}}} \times \text{Calibrator concentration} = \text{mg/dL Apo A}_1$$

QUALITY CONTROL

Serum controls are recommended to monitor the performance of manual and automated assay procedures.

Chronolab Apolipoprotein Control 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, 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 ration, as well as analyzer used. 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 none-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 (mg/dL)	Intra-assay (n=10)			Inter-assay (n=5)	
	105.3	113.3	153.6	153.9	216.8
SD	0.80	0.70	1.27	1.09	1.95
CV	0.76	0.66	0.83	0.71	0.90

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 A₁ 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.

NOTES

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

REFERENCES

1. Clinical Guide to Laboratory Tests, Edited by NW Tietz W B Saunders Co., Philadelphia, 483,1983.
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, 3th ed. AACC, Pres, 1997.

PACKAGING

Ref. 101-0546 Cont.: 1x45 ml / 1x15 ml