



Store at: +2+8°C.

Presentation:

Cod. IT010 CONT: R1 1 x 40 mL. + R2 1 x 10 mL .

Procedure

Diagnostic reagent for quantitative determination of human apolipoprotein A1 (Apo A1).

Only for *in vitro* use in clinical laboratory (IVD)

TEST SUMMARY

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.

REAGENTS COMPOSITION

Diluent (R1)	Tris buffer 20 mmol/L, PEG , pH 8.3. Sodium azide 0.95 g/L.
Antibody (R2)	Goat serum, anti-human Apo A-I, tris 50 mmol/L pH 7.5. Sodium azide 0.95 g/L.
Optional	APO-CAL IT230

REAGENT PREPARATION AND STABILITY

Reagents: Ready to use.

Do not freeze; frozen reagents could change the functionality of the test.

Signs of reagent deterioration:

- Particles and turbidity indicates contamination or reagents deterioration.

All the reagents of the kit are stable up to the end of the indicated month and year of expiry. Store tightly closed at 2-8°C. Do not use reagents over the expiration date.

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. The reagent (both monoreagent and bireagent) should be recalibrated every three weeks, when the controls are out of specifications, and when changing the reagent lot or the instrument settings. For monoreagent, a reagent blank should be run daily before sample analysis.

SPECIMEN

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.

Discard contaminated specimen.

MATERIAL REQUIRED BUT NOT PROVIDED

- Thermostatic bath at 37°C.

- Spectrophotometer or photometer thermostatable at 37°C with a 340 nm filter.

General laboratory equipment

TEST PROCEDURE

CALIBRATION CURVE:

Prepare the following APO CAL Calibrator dilutions in NaCl 9 g/L as diluent. Multiply the concentration of the Apo A-I calibrator by the corresponding factor stated in table below to obtain the Apo A-I concentration of each dilution.

Calibrator dilution	1	2	3	4	5	6
Calibrator (µL)	--	10	25	50	75	100
NaCl 9 g/L (µL)	100	90	75	50	25	-
Factor	0	0.1	0.25	0.5	0.75	1.0

PROCEDURE

1. Bring the reagents and the photometer (cuvette holder) to 37°C.

2. Assay conditions:

- Wavelength : 340 nm

- Temperature : 37 °C

- Cuvette lighth path : 1cm

3. Adjust the instrument to zero with distilled water.

4. Pipette into a cuvette:

Reagent R1 (µL)	800
Sample or Calibrator (µL)	7

5. Mix and read the absorbance (A₁) after the sample addition.

6. Immediately, pipette into de cuvette:

Reagent R2 (µL)	200
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7. Mix and read the absorbance (A₂) of calibrators and sample exactly 2 minutes after the R2 addition.

CALCULATIONS

Calculate the absorbance difference (A₂-A₁) of each point of the calibration curve and plot the values obtained against the Apo A-I concentration of each calibrator dilution. Apo A-I concentration in the sample is calculated by interpolation of its (A₂-A₁) in the calibration curve.

QUALITY CONTROL

Control Serum APO Ref.: IT240. Control sera are recommended to monitor the performance of manual and automated assay procedures.

Serum Controls are recommended for internal quality control. 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.

It is suggested that each laboratory establish its own reference range.

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/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-I 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.

Clinical diagnosis should not be made on a single test result; it should integrate clinical and other laboratory data.

REAGENT PERFORMANCE

- **Measurement range:** Up to 250 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 ratio. It will be higher by decreasing the sample volume, although the sensitivity of the test will be proportionally decreased.

- **Limit detection:** Values less than 0.31 mg/dL give non-reproducible results.

- **Precision:** The reagent has been tested for 20 days, using three levels of serum in an EP5-based study (NCCLS).

EP5	CV (%)		
	27.22mg/dL	65.74 mg/dL	131.07 mg/dL
Total	4%	3.7%	4.8%
Within Run	2.2%	0.8%	1.1%
Between Run	2.3%	1.3%	1.4%
Between Day	2.4%	3.3%	4.5%

- **Accuracy:** Results obtained using this reagent (y) were compared to those obtained with a Bayer immunoturbidimetric method. 39 samples ranging from 50 to 200 mg/dL of Apo A-I were assayed. The correlation coefficient (r) was 0.92 and the regression equation y = 1.18x - 37.8.

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

INTERFERING SUBSTANCES

Hemoglobin (20 g/L), bilirubin (40 mg/dL), lipemia (< 5 g/L), and rheumatoid factor (800 IU/mL) do not interfere. Other substances may interfere^{6,7}.

NOTES

1. Clinical diagnosis should not be made on findings of a single test result, but should integrate both clinical and laboratory data.

2. GPL have instructions for many automatic instruments, available on request.

BIBLIOGRAPHY

- Clinical Guide to Laboratory Tests, Edited by NW Tietz W B Saunders Co., Philadelphia, 483, 1983.
- Mahley RW et al. J Lipids Res 1984; 25: 1277-1294.
- Rifai N Arch Pathol Lab Med 1986; 110: 694-701.
- Freedman DS et al. N Eng J Med 1986; 315: 721-726.
- Sakurabayashi I et al. Clinica Chimica Acta 2001; 312: 87-95.
- Young DS. Effects of disease on clinical laboratory tests, 3th ed. AACC Press, 1997.
- Friedman and Young. Effects of disease on clinical laboratory tests, 3th ed. AACC Press, 1997.

