CHRONOLAB

CE
IVD

Apolipoprotein C-III

Turbidimetry

Quantitative determination of apolipoprotein C-III (APO C-III)

PACKAGING

Ref: 101-0549	Cont.: 1 x 45 / 1 x 15 mL

Store 2 - 8º C.

CLINICAL SIGNIFICANCE[1]

The C apolipoproteins (C-I, C-II and C-III) are surface components of chylomicrons, VLDL and HDL. It appears that the liver is the major site of synthesis of the apo C proteins, with the intestine contributing a minor portion. Apo C-III is the most abundant apolipoproteins C in plasma (≈ 4 mg/dL).

Apo C-III plays an important role in controlling plasma triglyceride metabolism and in determining the plasma concentration of potentially atherogenic triglycerides-rich lipoprotein (TRL). Plasma concentration of apo C-III is positively correlated with the level of plasma triglycerides, and apo C-III production is increased in patients with hypertriglyceridemia. Apo C-III gene polymorphisms are associated with increased levels of plasma apo C-III and hypertrigliceridemia. Liver perfusion studies have demonstrated that apo C-III inhibits the hepatic uptake of TRL and their remnants, and can inhibit the activity of both lipoprotein lipase and hepatic lipase. It therefore modulates the plasma catabolism and clearance of TRL. Statistically speaking, it is very important, as apo C-III is a significant independent predictor of the progression or severity of coronary artery disease.

PRINCIPLE OF THE METHOD

Turbidimetric test for the measurement of apolipoprotein C-III in human serum or plasma.

Anti- apo C-III antibodies when mixed with samples containing Apo C-III, form insoluble complexes. These complexes cause an absorbance change, dependent upon the Apo C-III concentration of the patient sample, that can be quantified by comparison from a calibrator of know Apo C-III concentration.

REAGENTS

Diluent (R1)	Tris buffer 100 mmol/L, PEG 4000, pH 8.5. Sodium azide 0.95 g/L.
Antibody (R2)	Goat serum, anti-human Apo C-III, tris 100 mmol/L, pH 7.2. Sodium azide 0.95 g/L.

CALIBRATION

The assay and the value of the calibrator concentration have been standardized against an Internal Reference Material. 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 - 8º C and contaminations are prevented during their use. Do not use reagents over 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 37º C.
- Spectrophotometer or photometer thermostatable at 37º C with a 340 nm filter.

SAMPLES

Fresh serum or plasma. EDTA or heparin should be used as anticoagulant. Stable 2 weeks at 2 - 8º C or 3 months at –20º C.

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

Do not use highly hemolized or lipemic samples.

PROCEDURE

Notes: CHRONOLAB SYSTEMS has instruction sheets for several automatic analyzers. Instructions for many of them are available on request.

Linearity depends on the calibrator concentration.

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

1. Bring the reagents and the photometer (cuvette holder) to 37º C.
2. Assay conditions:

   Wavelength : 340 nm
   Temperature : 37º C
   Cuvette ligth path : 1cm

3. Adjust the instrument to zero with distilled water.
4. Pipette into a cuvette:

Reagent R1 (µL)	750
Sample or Calibrator (µL)	20

5. Mix and read the absorbance (A_1) after the sample addition.
6. Immediately, pipette into de cuvette:

Reagent R2 (µL)	250

7. Mix and read the absorbance (A_2) of calibrators and sample exactly 5 minutes after the R2 addition.

CALCULATIONS

$$\frac{(A_2\text{-}A_1)_{sample}}{(A_2\text{-}A_1)_{calibrator}} \text{ x Calibrator concentration = mg/dL Apo C-III}$$

QUALITY CONTROL

Control sera are recommended to monitor the performance of manual and automated assay procedures. Chronolab Apolipoprotein Control (Ref.:101-0503) is available. Each laboratory should establish its own Quality Control scheme and corrective actions if controls do not meet the acceptable tolerances.

REFERENCE VALUES[5]

Between 5.5 – 9.5 mg/dL.

Each laboratory should establish its own reference range.

PERFORMANCE CHARACTERISTICS

Linearity*:* Up to 22 mg/dL (Note 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.

Detection Limit: Values less than 0.5 mg/dL give non-reproducible results.

Prozone effect: No prozone effect was detected upon 55 mg/dL.

Sensitivity: Δ 44.8 mA / mg/dL (10.7 mg/dL).

CHRONOLAB SYSTEMS, S.L., C/Diputación 260, 08007 Barcelona, SPAIN
Tel. +34 617722466, www.chronolab.com, e-mail: comercial@chronolab.es

Precision*:*

	Intra-assay (n=10)			Inter-assay (n=5)	
Mean (mg/dL)	6.9	9.7	25.6	7.2	9.0
SD	0.12	0.06	0.16	0.04	0.06
CV	1.72	0.61	0.63	0.5	0.6

Accuracy*:* Results obtained using this reagent (y) were compared to those obtained with single radial immuno diffusion (SRDI) method. 50 samples ranging from 3 to 12 mg/dL of Apo C III were assayed. The correlation coefficient (r) was 0.975 and the regression equation

$y = 0.95 x + 0.57$.

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

INTERFERENCES

Hemoglobin (up to 1000 mg/L), bilirrubin (up to 40 mg/dL), and lipemia (up to 20 g/L), do not interfere. Other substances may interfere [6,7].

BIBLIOGRAPHY

1. Clinical Guide to Laboratory Tests, Edited by NW Tietz W B Saunders Co., Phipladelphia, 483, 1983.
2. Rifai N. Arch Pathol Lab Med 1986; 110: 694-701.
3. Norum RA et al. Precociuos Coronary Disease 1980; 306(25): 1513-1519.
4. Freedman DS et al. N Eng J Med 1986; 315: 721-726.
5. Sakurubayashi I et al. Clin Chim Acta 2001: 312: 87-59.
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.

CHRONOLAB SYSTEMS, S.L., C/Diputación 260, 08007 Barcelona, SPAIN
Tel. +34 617722466, www.chronolab.com, e-mail: comercial@chronolab.es