CHRONOLAB

CE
IVD

Apolipoprotein C-II

Turbidimetry

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

PACKAGING

Ref.: 101-0548	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-II is present in plasma at a concentration of nearly 4 mg/dL, and its prime metabolic function appears to be associated with its ability to act as a cofactor in activating lipoprotein lipase (LPL), enzyme that hydrolyses the triglycerides in the lipoprotein, liberating fatty acids.

Deficiency or defective apo C-II, reduces the activity of the LPL enzyme, impairs chylomicrons catabolism, and increases plasma tryglicerides (500 – 1000 mg/dL). Those affected by this disorder have less than 10% of normal concentration of apo C-II, the minimum amount needed for normal LPL activity.

Homozygous hereditary deficiency of apo C-II leads to a hyperchylomicronemic syndrome very similar to hereditary LPL deficiency. The heterozygous state for one mutant apo C-II allele, when associated with apo E-IV, also is associated with hypertriglyceridemia.

PRINCIPLE OF THE METHOD

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

Anti- Apo C-II antibodies when mixed with samples containing Apo C-II, form insoluble complexes. These complexes cause an absorbance change, dependent upon the Apo C-II concentration of the patient sample, that can be quantified by comparison from a calibrator of know Apo C-II 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-II, tris 100 mmol/L, pH 7.2. Sodium azide 0.95 g/L.
Optional	APO CAL ref: 101-0499

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 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)	25

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

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

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 1.6 – 4.2 mg/dL.

Each laboratory should establish its own reference range.

PERFORMANCE CHARACTERISTICS

1. Linearity: Up to 10 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.

2.Detection Limit: Values less than 0.4 mg/dL give non-reproducible results.

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3. Prozone effect: No prozone effect was detected upon 28 mg/dL

4.Sensitivity*:* Δ 23.5 mA / mg/dL (4.8 mg/dL).

5.Precision:

	Intra-assay (n=10)			Inter-assay (n=5)	
Mean (mg/dL)	2.6	3.7	8.1	2.6	3.2
SD	0.08	0.05	0.07	0.04	0.03
CV	2.24	1.39	0.92	1.7	0.9

6. **Accuracy:** Results obtained using this reagent (y) were compared to those obtained with single radial immuno diffusion (SRDI) method. 50 samples ranging from 0.5 to 6 mg/dL of Apo C II were assayed. The correlation coefficient (r) was 0.976 and the regression equation $y = 1.040\ x - 0.12$.

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

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