

PRINCIPLE:

The apolipoproteins contained in the human serum form immunocomplexes with their corresponding antibodies. The concentrations are determined by turbidimetry assay. The results are evaluated using a reference curve prepared with the aid of dilutions of calibrator.

CLINICAL SIGNIFICANCE:

In the study of cardiovascular diseases, the apolipoproteins A₁ and B determination represents a more discriminate parameters that the normal lipid determination. Lipoprotein A₁ and B are respectively the markers from HDL and LDL lipoproteins. From an individual level, the risk is increased by a higher Apo B concentration and a lower Apo A₁ concentration.

REAGENTS:

1. Reagent 1 (1x80 ml)

TRIS/PEG. buffer pH 7.5

2. Reagent 2 (1x2 ml)

Antiserum Apolipoprotein B

Optional: 101-0499 Apo A₁/Apo B calibrator liquid serum

PREPARATION AND STABILITY:

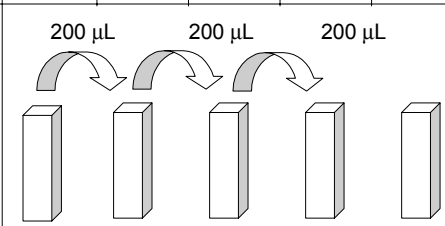
R.1: Ready to use. Stable at 2-8°C up to the expiration date.

R.2: Must be diluted with buffer solution. The dilutions depends on the analyser (Inquire).

Stable, at 2-8°C, up to the expiration date.

Calibrator; Ready to use.

Calibration curve: Prepare dilutions of the calibrator APO A1/B using 9 g/L NaCl as diluent:

Std N°	1	2	3	4	5
Dilution	1/5	1/10	1/20	1/40	0
NaCl (µL) Calibrator (µL)	400 100	200 --	200 --	200 --	200 --
					
Factor	3.00	1.5	0.75	0.37	0

Multiply the Apo B calibrator concentration by the corresponding dilution factor indicated in the table to obtain the Apo B concentration of the different calibrators.

SAMPLES:

Fresh serum. Apolipoproteins in serum are stable 8 days at 2-8°C. Do not use haemolized or lipemic samples.

The controls and samples will dilute manually or automatically with saline solution. (NaCl 0.9%).

PROCEDURE:

Wavelength: 340 nm
 Cuvette: 1 cm light path
 Temperature: 37 °C
 Zero: distilled water

1. Dilute Antiserum Apo-B (R.2) 1:41 with buffer solution R.1. The working reagent is stable 2 weeks at 2-8°C.
2. Dilute samples and controls 1:15 with saline solution. (NaCl 0.9%)
3. Pipette into a cuvette:

	Blank	Calibrator	Sample
NaCl 9 g/L (µL)	20	--	--
Calibrator (µL)	--	20	--
Dil. Sample (µL)	--	--	20
Work. Reag. (mL)	1.0	1.0	1.0

4. Mix and read the absorbance (A) against blank after 10 minutes of the working reagent addition.

CALCULATION:

Calculate the absorbance for each calibrator and plot the values found against the concentration in a calibration curve. Apo B concentration in the sample is calculated by interpolation its A value on the calibration curve.

Chronolab has instruction sheets available for several automatic analyzers.

REFERENCE VALUES:

Between 55 – 159 mg/dL.
 Each laboratory should establish its own reference range.

PERFORMANCE CHARACTERISTICS:

1. *Measurement interval:* 20-600 mg/dL, under the described assay conditions.

REFERENCES:

Alaupovic P., Lee D.M., Mc. Conathy W.J., Biochem, Byophys Acca 260, 289 (1972)
 Fruchart J.L., Kora I., Cachera C. Duthilleul P., Clin. Chem. 28,59 (1982)