

Store at +2 to +8°C

PRINCIPLE

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

CLINICAL SIGNIFICANCE

Apo B is the major structural apolipoprotein in VLDL (Very Low Density Lipids), LDL (Low Density Lipids) lipoproteins and chylomicron. The most important function is the transport of rich triglycerides lipoproteins from liver and intestine to other tissues. Apo B exists in two forms: Apo B-100 and Apo B-48. Apo B-100, the most important component of LDL, is synthesized in the liver and excreted in plasma as part of VLDL. Apo B-48, the most important component of chylomicrons, is synthesized in the intestine. Several studies demonstrated that in people with coronary heart disease (CHD), changes in the serum concentrations of Apo A₁ and Apo B are similar to those for HDL and LDL, respectively and whereas, are somewhat better discriminators of people with CHD than the LDL and HDL cholesterol concentrations.

The hyperbetalipoproteinemia is characterized by increased LDL Apo B-100 concentrations with normal or moderately increased concentrations of LDL cholesterol. The ratio of LDL cholesterol to Apo B-100 is therefore reduced in these patients.

Defects in the Apo B structure or lipoproteins containing Apo B prevent the secretion of triglycerides rich intestinal and hepatic lipoproteins; this disorder occurs in abetalipoproteinemia or homozygous hypobetalipoproteinemia.

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 SP3-07 (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 340 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: 340 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

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

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 69 – 105 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 26 mg/dL give none-reproducible results.
3. Prozone effect: no prozone effect was detected upon 280 mg/dL.
4. Sensitivity: Δ 4.44 mA/mg/dL (107 mg/dL).
5. Precision:

| Mean (mg/dL) | Intra-assay (n=10) | | | Inter-assay (n=5) | |
|--------------|--------------------|------|-------|-------------------|-------|
| | 54.8 | 86.4 | 116.7 | 82.3 | 170.6 |
| SD | 0.45 | 0.60 | 1.40 | 0.65 | 1.10 |
| CV | 0.82 | 0.76 | 1.21 | 0.79 | 0.64 |

6. Accuracy: Results obtained using this reagent (y) were compared to those obtained with single radial immuno diffusion (SRDI) method. 50 samples ranging from 40 to 160 mg/dL of Apo B were assayed. The correlation coefficient (r) was 0.980 and the regression equation y=0.927x+5.96.

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. Brown MS et al. Science 1986; 232:34-47.
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

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| Ref. 101-0547 | Cont.: 1x45 ml / 1x15 ml |
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