

Quantitative determination of Apolipoprotein B (APO B) IVD

Store 2 - 8°C.

PRINCIPLE OF THE METHOD

Turbidimetric test for the measurement of apolipoprotein 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 SIGNIFICANCE¹

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 A-I 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

R1	Tris buffer 100 mmol/L, PEG 4000, pH 7.2. Sodium azide 0.95 g/L.
R2	Goat serum, anti-human Apo B, tris 100 mmol/L, pH 7.2. Sodium azide 0.95 g/L.
Saline Solution	NaCl 9% (for samples predilution)
Optional	APO A-I/B Dual Control ref: ACC16-014

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 APO CAL Calibrator for calibration.

PREPARATION

Working Reagent: Swirl the R2 vial gently before use. Prepare the necessary amount as follows:

- 50 µL R2 + 2 mL R1 (1:41 dilution)

Samples and Controls Pre-Dilution: Before the use, samples and/or controls must be diluted in this way:

- 25 µL sample or control + 350 µL NaCl 9 g/L (1:15)

STORAGE AND STABILITY

All the components of the kit are stable until the expiration date on the label when stored tightly closed at 2-8°C and contaminations are prevented during their use. Do not use reagents over the expiration date.

Reagent deterioration: The presence of particles and turbidity.

Working Reagent: The working reagent solution is stable 2 week at 2-8°C

Do not freeze; frozen Antibody or Diluent could change the functionality of the test.

ADDITIONAL EQUIPMENT

- Pipette 100 - 1000 µL
- Pipette 10 - 100 µL
- Cuvettes and microstirrers (ref. code ACC16-037)

SAMPLES

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 hemolyzed or lipemic samples.

TEST PARAMETER

Filter: set filter on position B

Reading time: 300 seconds

PROCEDURE

1. Pipette into an appropriate number of cuvettes:

kind of reagent	µL
Working Reagent	500

2. Incubate the cuvettes in positions 1-4 for at least 5 minute
3. Transfer each cuvette in the reading channel and, when requested on the display, add 50 µL of prediluted sample or control.
4. Read the result which will appear automatically on the reader's display after 300 sec

QUALITY CONTROL

Control sera are recommended to monitor the performance of the assay procedures. Vital Diagnostics Apolipoprotein A1/B dual Control (Ref.ACC16-014) is available. Each laboratory should establish its own Quality Control scheme and corrective actions if controls do not meet the acceptable tolerances.

REFERENCE VALUES⁵

Between 69 – 105 mg/dL.

Each laboratory should establish its own reference range.

PERFORMANCE CHARACTERISTICS

1. Linearity: Up to 500 mg/dL (Nota 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 2 mg/dL give non-reproducible results.
3. Prozone effect: No prozone effect was detected upon 280 mg/dL
4. Sensitivity: Δ 4.48 mA / mg/dL (107 mg/dL).
5. Precision:

	Mean	SD	CV%
Intra-assay (n=10)	58.4	0.45	0.82
Inter-assay (n=10)	82.3	0.65	0.79

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 ^{6,7}.

NOTES

1. Linearity depends on the calibrator concentration.
2. Clinical diagnosis should not be made on findings of a single test result, but should integrate both clinical and laboratory data.

BIBLIOGRAPHY

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. AACCPres, 1997.
7. Friedman and Young. Effects of disease on clinical laboratory tests, 3th ed. AACCPres, 1997.

PACKAGING

Ref.: ACC16-002	Cont.	: 1 x 80 mL R1
		: 1 x 2 mL R2
		: 1 x 80 mL Saline Solution