APO-B

Apolipoprotein B

Turbidimetry

Quantitative determination of apolipoprotein B (APO B)
IVD

Store 2 - 8ºC.

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 SIGNIFICANCE[1]

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 tryglicerides 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 hiperbetalipoproteinemia 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 abetaliporpteinemia or homozygous hypobetalipoproteinemia.

REAGENTS

Diluent (R1)	Tris buffer 20 mmol/L, PEG , pH 8.3. Sodium azide 0.95 g/L.
Antibody (R2)	Goat serum, anti-human Apo B, tris 50 mmol/L, pH 7.5. Sodium azide 0.95 g/L.
Optional	APO CAL ref: 93005

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. The reagent (both monoreagent and bireagent) should be recalibrated every three weeks, when the controls are out of specifications, and when changing the reagent lot or the instrument settings. For monoreagent, a reagent blank should be run daily before sample analysis.

PREPARATION

Reagents: Ready to use.
Calibration Curve: Prepare the following APO CAL Calibrator dilutions in NaCl 9 g/L as diluent. Multiply the concentration of the Apo B calibrator by the corresponding factor stated in table bellow to obtain the Apo B concentration of each dilution.

Calibrator dilution	1	2	3	4	5	6
Calibrator (µL)	--	10	25	50	75	100
NaCl 9 g/L (µL)	100	90	75	50	25	-
Factor	0	0.1	0.25	0.5	0.75	1.0

STORAGE AND STABILITY

All the components of the kit are stable until the expiration date on the label when stored tightly closed at 2-8ºC 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 37ºC.
- Spectrophotometer or photometer thermostatable at 37ºC with a 340 nm filter.

SAMPLES

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

The samples with presence of fibrin should be centrifuged before testing.
Do not use highly hemolized or lipemic samples.

PROCEDURE

1. Bring the reagents and the photometer (cuvette holder) to 37ºC.
2. Assay conditions:
   Wavelength : 340 nm
   Temperature : 37 ºC
   Cuvette ligth path : 1cm
3. Adjust the instrument to zero with distilled water.
4. Pipette into a cuvette:

Reagent R1 (µL)	800
Sample or Calibrator (µL)	7

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

Reagent R2 (µL)	200

7. Mix and read the absorbance (A_2) of calibrators and sample exactly 2 minutes after the R2 addition.

Spinreact has instruction sheets for several automatic analyzers. Instructions for many of them are available on request.

CALCULATIONS

Calculate the absorbance difference (A_2-A_1) of each point of the calibration curve and plot the values obtained against the Apo B concentration of each calibrator dilution. Apo B concentration in the sample is calculated by interpolation of its (A_2-A_1) in the calibration curve.

QUALITY CONTROL

Control sera are recommended to monitor the performance of manual and automated assay procedures. Spinreact Apolipoprotein Control (Ref.:93006) 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 69 – 105 mg/dL.
Each laboratory should establish its own reference range.

PERFORMANCE CHARACTERISTICS

1. **Measurement range:** Up to 250 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 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 1,91 mg/dL give non-reproducible results.
3. **Precision:** The reagent has been tested for 20 days, using three levels of serum in a EP5-based study (NCCLS).

EP5	CV (%)		
	23.92 mg/dL	59.08 mg/dL	119.07 mg/dL
Total	7.4%	4.3%	3.6%
Within Run	2.0%	1.4%	1.0%
Between Run	3.7%	2.2%	1.8%
Between Day	6.1%	3.4%	3.0%

4. **Accuracy:** Results obtained using this reagent (y) were compared to those obtained with a Daiichi immunoturbidimetric method. 48 samples ranging from 50 to 200 mg/dL of Apo B were assayed. The correlation coefficient (r) was 0.965 and the regression equation y = 0.996x + 5.112.

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

INTERFERENCES

Hemoglobin (20 g/L), bilirrubin (40 mg/dL), lipemia (5 g/L), and rheumatoid factor (800 UI/mL) do not interfere. Other substances may interfere [6,7].

NOTES

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., Phipladelphia, 483, 1983.
2. Mahley RW et al. J Lipids Res 1984; 25: 1277-1294.
3. Brown MS at 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, 3tn ed. AACC Pres, 1997.

PACKAGING

Ref.: 1003013	Cont.	R1. Diluent: 1 x 40 mL R2. Antibody: 1 x 10 mL

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