

Store at +2 to +8°C

PRINCIPLE

Turbidimetric test for the measurement of apolipoprotein E in human serum or plasma.

Anti- apo E antibodies when mixed with samples containing apo E, form insoluble complexes. These complexes cause an absorbance change, dependent upon the apo E concentration of the patient sample, that can be quantified by comparison from a calibrator of known apo E concentration.

CLINICAL SIGNIFICANCE

Human plasma apolipoprotein E is a 299-aminoacid protein constituted by a single polypeptide chain. It is mainly produced by liver and secreted as a glycoprotein. In humans, apo E presents a genetic polymorphism; apo E is present as 3 major isoforms, apo E2, E3 and E4.

Apo E is an important component of plasma lipoproteins and influences lipoprotein metabolism through its action as a receptor ligand. It is found primarily in chylomicrons, VLDL, HDL, and chylomicron and VLDL remnants. Removal of apo E-bearing lipoproteins is mediated by several different cellular receptors that recognize a cluster of positively charged aminoacids in a specific region of apo E. This apolipoprotein plays a central role in the metabolism of chylomicrons and VLDL remnants, and regulates and facilitates lipoprotein uptake in the liver through interaction of chylomicron remnants with chylomicron remnant receptors, and binding of VLDL remnants to the LDL receptor. Apo E has in addition several other functions, e.g., immunoregulation, nerve regeneration, and the activation of several lipolytic enzymes (hepatic lipase, lipoprotein lipase, and lecithin-cholesterol acyltransferase).

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 E, 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 Internal Reference Material. 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.

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

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)	25

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

Reagent 2 (µl)	250
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7. Mix and read the absorbance (A_2) 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

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

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: 2.7 – 4.5 mg/d L.

Each laboratory should establish its own reference range.

PERFORMANCE CHARACTERISTICS

1. Linearity: up to 12 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 0.5 mg/dL give none-reproducible results.
3. Prozone effect: no prozone effect was detected upon 13 mg/dL.
4. Sensitivity: Δ 44.39 mA/mg/dL (4 mg/dL).
5. Precision:

Mean (mg/dL)	Intra-assay (n=10)			Inter-assay (n=5)	
	2.8	4.2	6.9	3.0	3.7
SD	0.05	0.05	0.06	0.02	0.04
CV	1.70	1.23	0.83	0.80	1.20

6. Accuracy: Results obtained using this reagent (y) were compared to those obtained with single radial immuno diffusion (SRDI) method. 50 samples ranging from 2 to 7 mg/dL of Apo E were assayed. The correlation coefficient (r) was 0.974 and the regression equation $y=1.040x+0.50$.

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

INTERFERENCES

Hemoglobin (up to 1000 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. Rifai N. Arch Pathol Lab Med 1986; 110: 694-701.
3. Itakura K et al. Clinica Chimica Acta 1986; 161: 275-282.
4. Mahley RW et al. J Lipids Res 1984; 25:1277-1294.
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

Ref. 101-0550	Cont.: 1x45 ml / 1x15 ml
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