APO A-I

# Apolipoprotein A-I

Turbidimetry

## Quantitative determination of apolipoprotein A-I (APO A-I) IVD

Store 2 - 8ºC.

### PRINCIPLE OF THE METHOD

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

### CLINICAL SIGNIFICANCE[1]

Apo A-I is the major structural apolipoprotein in HDL and constitutes about 70% of the total protein. Apo A-I is a cofactor for lecithin-cholesterol-acyl-transferase (LCAT), the enzyme responsible for forming cholesteryl esters in plasma and plays and important role in the transport of cholesterol from peripheral tissues to the liver, to be finally excreted. Measurements of Apo A-I concentration is specially important in detecting coronary heart disease risk (CHD) as well as in the diagnostic of hyperlipoproteinemia. Concentrations < 120 mg/L are associated to an increased CHD risk, while concentrations ≥ 160 mg/L may even protect from the same risk. Patients with deficiencies in Apo A-I synthesis may highly increase the CHD risk.
Tanger disease, a consequence of an Apo A-I catabolism defect, is characterized by several reduced plasma HDL cholesterol (HDL-c) concentration, abnormal HDL composition and accumulation of cholesteryl esters in many body tissues. Plasma HDL-c and Apo A-I concentrations in homozygotes are very low, while Apo A-II concentration is less than 10% of its normal concentration. Heterozygotes are characterized by half-normal concentration of HDL-c, Apo AI and Apo –II. Current evidence suggests that these patients have increased incidence of CHD.

### 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 A-I, 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 SP1-01 (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 A-I calibrator by the corresponding factor stated in table bellow to obtain the Apo A-I 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 A-I concentration of each calibrator dilution. Apo A-I 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 122 – 161 mg/dL.
Each laboratory should establish its own reference range.

### PERFORMANCE CHARACTERISTICS

**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.
**Detection Limit:** Values less than 0,1 mg/dL give non-reproducible results.
**Precision:** The reagent has been tested for 20 days, using three levels of serum in a EP5-based study (NCCLS).

| EP5 | CV (%) | | |
|---|---|---|---|
| | 27.22mg/dL | 65.74 mg/dL | 131.07 mg/dL |
| Total | 4% | 3.7% | 4.8% |
| Within Run | 2.2% | 0.8% | 1.1% |
| Between Run | 2.3% | 1.3% | 1.4% |
| Between Day | 2.4% | 3.3% | 4.5% |

**Accuracy:** Results obtained using this reagent (y) were compared to those obtained with a Bayer immunoturbidimetric method. 39 samples ranging from 50 to 200 mg/dL of Apo A-I were assayed. The correlation coefficient (r) was 0.92 and the regression equation y = 1.18x - 37.8.
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 IU/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


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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.
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### PACKAGING

| | | |
|---|---|---|
| Ref.: 1003012 | Cont. | R1. Diluent: 1 x 40 mL<br>R2. Antibody: 1 x 10 mL |



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