INTENDED USE

FOR IN VITRO DIAGNOSTIC USE

APO A-1 Reagent is intended for the quantitative determination of apolipoprotein A-1 (apo A-1) in human serum by immuno-turbidimetric analysis.

SUMMARY

The distribution of cholesterol between high density lipoprotein (HDL), very low density lipoprotein (VLDL), and low density lipoprotein (LDL) is important in determining risk for coronary artery disease (CAD) 1-3. Also, measurements of apo A-1, the major protein of HDL, in combination with measurements of apo B, the major protein of LDL, have been useful in identifying individuals who are at risk for developing CAD 4-6 and in the diagnosis of patients at risk for premature CAD (familial apo A-1 deficiency and Tangier Disease) 7. Individuals with CAD consistently have lower blood levels of apo A-1 than control subjects without the disease 8.

PRINCIPLE OF PROCEDURE

An insoluble turbid immunoprecipitate is formed by the reaction between the apo A-1 antigen in human serum and the specific antibody in the antibody reagent. Maximum exposure of antigenic sites to which the antibody will bind is achieved through the use of surfactants contained in the antibody reagent. The resulting turbidity is measured spectrophotometrically at 340nm 9,10 and the apo A-1 in the serum is determined from a calibration curve obtained by using the five level calibrator set provided.

The Antigen Reagent of this method was prepared from antiserum produced in goats against purified human apo A-1 derived from the HDL fraction (d=1.063-1.210) of pooled human serum. The antiserum was found to be monospecific when tested by immunoelectrophoresis against whole human serum.

The liquid calibrators provided were prepared from pooled human serum and contain apo A-1 levels sufficient for quantitation and quality control of normal and abnormal samples. The apo A-1 protein in these sera has been assigned concentration values with the use of the IFCC/W.H.O. Standard Reference Material, SRM 1, and by participation in the IFCC/CDC directed International Standardization Program 5.

REAGENTS

APO A-1 Antibody Reagent

Reactive Ingredients
- Sodium azide, 0.1%
- APO A-1 Calibrators 1-5

Reactive Ingredients
- Sodium azide, 0.1%
- Non-reactive Ingredients
- Stabilizers

STORAGE AND STABILITY

Store the reagents and calibrators at 2-8 °C. The reagents are stable until the expiration date on the label. Reagent and calibrators must not be stored frozen and should not be allowed to stand for repeatedly long periods of time (up to 8 hours) at room temperature. Once opened the reagent and calibrator is good for 7 days.

WARNINGS AND PRECAUTIONS

1. Good laboratory safety practices should be followed when handling any reagents. Refer to a recognized laboratory safety program for additional information.

2. Results should be interpreted considering all other test results and clinical status of the patient.

3. Contains Sodium azide as preservative. Upon disposal, flush with large amounts of water to avoid formation of explosive metal azides in copper or lead plumbing.

SPECIMEN COLLECTION

Use a fasting serum sample to minimize the production of nonspecific turbidity in the assay reaction mixture. Allow blood to clot at room temperature; rim the clot if required and centrifuge at least 10 minutes at approximately 900 rcf. Aspirate at least 0.5ml of cell-free serum promptly to avoid excess hemolysis.

INTERFERING SUBSTANCES

Elevated concentration levels of hemoglobin (50 g/l) and bilirubin (150 mg/dl) do not interfere with the turbidimetric determination of apo A-1 (10.7). Although elevated triglyceride concentrations were not found to interfere with this method, serum samples which are visibly lipemic or contain triglyceride concentrations greater than 1000 mg/dl be diluted before assaying.

Young 11 has published a comprehensive list of drugs and substances, which may interfere with in vitro diagnostic assays.

PROCEDURE

Materials provided
- 85490 Reagent
- 2 x 25 mL
- Calibrators 1-5
- 5 x 1 mL

Materials required but not provided
- 1. Automated chemistry analyzer.

TEST PROCEDURE

Allow all reagents to equilibrate to room temperature prior to use. Filter antibody reagent if turbid.

1. Add 1ml of antibody reagent to the calibrator and patient test tubes.
2. Add 1ml of distilled water to the calibrator and patient blank test tubes.
3. Add 5 µl of patient samples to the patient and patient blank tubes. Add 5 µl of calibrator sample to the calibrator and calibrator blank tubes. Mix well by inversion.
4. Incubate at room temperature (15-30°C) for 15 minutes.
5. Set the spectrophotometer wavelength at 340nm and the absorbance reading to zero with water as reference.
6. Read and record absorbance of all tubes at 340nm. REACTION MIXTURES MUST BE READ WITHIN 30 MINUTES FROM SETUP TIME.
7. Subtract the blank absorbance value from the respective test value to remove absorbance caused by sample turbidity (corrected absorbance).
8. Determine apo A-1 concentration (mg/dl) in sample.
9. Plot values on linear graph paper, plot the concentrations of Calibrators 1-5 on the x-axis and the corrected absorbance of the calibrators on the y-axis.
10. Determine the concentration for the apo A-1 for serum samples and control using the prepared calibration curve.

CALIBRATION

Use the Apo A-1 calibrator provided with the kit. The use of other Apo A-1 calibrators is not recommended. Calibrate in accordance with the instrument manufacturer’s specifications. Calibration is required daily or when one of the following occur:

1. Change in the reagent lot number.
2. Preventative maintenance is performed on the analyzer.
3. A critical element of the analyzer is replaced.
4. Control material results have shifted or are out of range and the use of a freshly reconstituted vial of control does not correct the situation.

Each laboratory should establish its own procedures for corrective action if calibration is not acceptable.

QUALITY CONTROL

Controls are recommended to monitor the performance of manual and automated assay procedures, providing a continued screening of the instrument, reagents and techniques. Raichem Assayed Control, Level 1 (R83082) and Assayed Control, Level 2 (R83083) is recommended for this purpose. Each laboratory should establish its own control schedule.

PROCEDURAL LIMITATIONS

1. Serum samples that are visibly lipemic or contain triglyceride concentrations of more than 1,000 mg/dl should be diluted with an equal volume of isotonic saline. Multiply the final assay value by 2 to compensate for dilution.
2. The antibody reagent in this kit has been optimized to measure apo A-1 typically found in human serum. Samples with apo A-1 concentrations which exceed the value of the high calibrator (225 to 235 mg/dl) must be diluted with an equal volume of isotonic saline and reassayed.

PERFORMANCE CHARACTERISTICS

Precision

Precision serum studies were performed on an automated analyzer according to the NCCLS Publication EP5-A1.

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<th>Within Run</th>
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EXPECTED VALUE

The range of expected values is:
- Male 115-206 mg/dl
- Female 107-187 mg/dl

It is recommended that each laboratory establish its own reference range.
REFERENCES

For in vitro diagnostic use

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RE-ORDER INFORMATION
APO A-1 Reagent
Catalog No. REF 85490
Made in the USA