

Intended Use

For *in vitro* use only. This reagent is intended for use in the quantitative determination of apolipoprotein A1 (apo A1) in human serum by immunoturbidimetric analysis as an aid in the assessment of individuals who are at risk for developing coronary artery disease.

Clinical Significance

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).^{1,2} Also, measurements of apo A1, 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.^{3,4,5,6} and in the diagnosis of patients at risk for premature CAD (familial apo A1 deficiency and Tangier Disease).^{7,8} Individuals with CAD consistently have lower blood levels of apo A1 than control subjects without the disease.⁹

Principle

An insoluble turbid immunoprecipitate is formed by the reaction between the apo A1 antigen in human serum and the specific antibody in the R2 reagent. Maximum exposure of antigenic sites to which the antibody will bind is achieved by using the R1 reagent. The resulting turbidity is measured spectrophotometrically at 340 nm^{8,10} and the apo A1 in the serum is determined from a calibration curve obtained by using the four level calibrator set used to calibrate the chemistry analyzer (See Procedure).

The R2 reagent of this method was prepared from antiserum produced in goats against purified human apo A1 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 R1 reagent was prepared from specific surfactants dissolved in a buffered solution containing sodium azide as a preservative.

The liquid calibrators were prepared from pooled human serum and contain apo A1 levels sufficient for quantitative and quality control of normal and abnormal samples. The apo A1 protein in these sera has been assigned concentration values with the use of the International Federation of Clinical Chemistry (IFCC) proposed Standard Reference Material, SP1, and by participation in the IFCC/CDC directed Standardization Program.

Reagents

1. R1 Reagent: Tris (pH 7.0) 100mmol/L, Polyethylene glycol (PEG), detergents, stabilizers.
2. R2 Reagent: Tris (pH 7.0) 100mmol/L, anti-human apolipoprotein A1 antibody (goat) with stabilizers.

NOTE: See Precautions section for information regarding handling of kit components.

Reagent Preparation

Reagents are supplied in ready-to-use liquid form. Allow both reagents to equilibrate to room temperature prior to use.

Reagent Storage and Stability

1. The apo A1 reagents are stable until the expiration date shown on the labels. Store the kit in a refrigerator at 2-8°C. Do Not Freeze.
2. Keep reagent vials tightly capped to avoid microbial contamination and evaporation.

Precautions

1. Avoid ingestion of reagent and contact with skin. The toxicity of these reagents has not been established.
2. The reagent contains 0.1% sodium azide which can react with lead and copper plumbing to form highly explosive metal azides. On disposal, flush with a large volume of water.¹¹
3. Reagents and calibrators intended for use with this reagent kit must not be stored frozen and should not be allowed to stand for repeatedly long periods of time (over 8 hours) at room temperature. Keep reagents and calibrator vials tightly capped at all times when not in use to avoid microbial contamination and evaporation.

Specimen Collection

Serum, heparinized plasma or EDTA plasma

Stability : 5 days at 15-25°C

14 days at 2-8°C

90 days at -20°C

Avoid repeated freezing and thawing as this may result in lipoprotein denaturation. Discard contaminated specimens.

Materials Required but not Provided

1. Suitable chemistry analyzer. See Procedure and the specific instrument Application Guide for required reagent volumes and instrument parameters. Contact the manufacturer's Technical Service Department for available application guides.
2. **Apo A1/B Serum Calibrators.** This four level human serum calibrator set must be used with the reagents of this kit. **The manufacturer does not recommend the use of calibrators from other sources with the reagents of this kit.**
3. Controls with known apolipoprotein values.
4. 0.9% NaCl solution

Materials Provided

1. Apo A1 Reagent 1 (1 x 120ml)
2. Apo A1 Reagent 2 (1 x 30ml)

Procedure (Hitachi 717)

| | |
|---------------------|---------------------|
| TEST NAME | [APOA1] |
| ASSAY CODE | [2-POINT]:[23]-[50] |
| SAMPLE VOLUME | [3] [2] |
| R1 VOLUME | [300] [100] [NO] |
| R2 VOLUME | [75] [20] [No] |
| WAVELENGTH | [700] [340] |
| CALIBRATION | [NONLINEAR] [1] [5] |
| STD (1) CONC-POS | [0] [1] |
| STD (2) CONC-POS | [*] [*] |
| STD (3) CONC-POS | [*] [*] |
| STD (4) CONC-POS | [*] [*] |
| STD (5) CONC-POS | [*] [*] |
| STD (6) CONC-POS | [*] [*] |
| SD LIMIT | [300] |
| DUPLICATE LIMIT | [500] |
| SENSITIVITY LIMIT | [0] |
| ABS LIMIT (INC/DEC) | [32000] [INCREASE] |
| PROZONE LIMIT | [0] [LOWER] |

Apolipoprotein A1 Reagent Set

| | |
|-------------------|---------|
| EXPECTED VALUE | [*] [*] |
| PANIC VALUE | [*] [*] |
| INSTRUMENT FACTOR | [1.0] |

1. Allow reagents, calibrator sera, control sera, and samples to equilibrate to room temperature prior to use.
2. Calibrate the clinical chemistry analyzer in accordance with the instrument user's manual and the above parameters.

Interferences / Limitations

1. Apolipoprotein A1 is a specific immunoassay for Apo A1 through its use of specific antibodies for Apo A1. No interference was observed by ascorbic acid up to 20mg/dl, bilirubin up to 17.5 mg/dl, hemoglobin up to 500 mg/dl and triglycerides up to 900 mg/dl.
2. No cross reaction with apolipoprotein A2 or apolipoprotein B was observed under test conditions.

Quality Control

The reliability of test results must be monitored by routine use of control sera containing assayed levels of apo A1. These QC materials should be assayed in every run and treated as patient samples. For interpretation of results refer to target values and limits of performance provided with the controls. If QC results are not expected: 1) Review relevant applications guide to ensure that the test was performed in accordance with the prescribed procedure, 2) Check to see that the materials used have not expired, 3) If necessary, rerun the controls. For further assistance contact the manufacturer.

Results

The concentration of apo A1 for unknown serum samples is obtained by non-linear data reduction which is automatically calculated by the clinical chemistry analyzer. Results are then printed by the analyzer in appropriate units (mg/dL or g/L). For instrument specific calculations, see the appropriate Operator's Manual concerning information on the required non-linear math model for calibration curve fit.

Assay Range

The test has been developed to determine the concentrations of apolipoprotein A1 within a measuring range from 2 – 250 mg/dl. When values exceed this range, samples should be diluted 1:1 with 0.9% saline solution and the result multiplied by 2.

The assay range for this reagent is 2 - 250 mg/dl

Prozone Limit

No prozone effect was observed up to an apolipoprotein A1 value of 400 mg/dl.

Sensitivity / Limit of Detection

The lower limit of detection is 2 mg/dl.

Expected Values

The reported reference ranges for apo A1 are: 115-206 mg/dL for females and 107-187 mg/dL for males.¹² It is recommended that each laboratory establish its own "normal" range.

Performance Characteristics

The following categories list typical data obtained when using the Hitachi 717 instrument system. The actual results obtained may vary when other automated instrument procedures are employed.

Comparisons

A comparison between this apolipoprotein A1 (y) and a commercially available test (x) using 72 samples gave the following results: $y = 0.923x + 11.27$. $r = 0.955$

Precision

Two of the serum samples used in the method comparison study above were analyzed for precision of apo A1. Apo A1, mg/dl:

| Within Run | | | | Run to Run | | | |
|------------|------|-------|----|------------|------|-------|----|
| Mean | S.D. | C.V.% | N | Mean | S.D. | C.V.% | N |
| 99.8 | 1.6 | 1.6 | 20 | 105.0 | 1.8 | 1.8 | 21 |
| 288.8 | 4.6 | 1.6 | 20 | 298.0 | 5.7 | 1.9 | 21 |

References

1. Gordon, T., W.P. Castelli, M.C. Hjortland, W.B. Kannel, & T.R. Dawber, Am. J. Med. 62:707-714, (1977).
2. Levy, R.I. Clin. Chem. 27:653-662 (1981).
3. Avogaro, P.G., Cazzolato, G.B. Bittolo, & G.B. Quinci. Lancet 1:901-903 (1979).
4. Kladetzky, R.G., G. Assman, S. Walgenbach, P. Tavchert & H.D. Helb. Artery 7:191-205 (1980).
5. Parra, H., C. Fieuet, B. Boniface, M. Bertrand, P. Duthilleul & F.C. Fruchart. In latent dyslipoproteinemia and atherosclerosis. J.L. de Gennes, J. Polonovski & R. Paoletti, Eds: (1984) p. 187-197. Raven Press. New York.
6. Sniderman, A., S. Shapiro, D. Marpole, B. Skinner, B. Teng & P.O. Kwitervish, Jr. Proc. Natl. Acad. Sci USA 77: 604-608, (1980).
7. Naito, H.K. J. Clin. Immunoassay 9:11-20, (1986).
8. Rifia, N. and M.E. King. Clin. Chem. 32:957-961, (1986).
9. Kottke, B.A., A.R. Zinsmeister, D.R. Holmes Jr., R.B. Kneller, B.J. Hallaway, S.J.T. Mao. Mayo Clin. Proc. 61:313-320, (1986).
10. Brustolin, D., M. Maierna, F. Aquzzi, F. Zoppi, G. Tarnghi, G. Berti. Clin. Chem. 37:742-747 (1991).
11. Center for Disease Control, HEW Manual Guide – Safety Management, No. CDC-22, CDC Tn-761, 4-30-76.
12. Albers, J.J., Northwest Lipid Research Laboratories, Seattle, WA 98103 (USA), (1992) Personal Communications.

Manufactured for Pointe Scientific, Inc.
5449 Research Drive, Canton, MI 48188

"European Authorized Representative"
(O.E.A.R.C.) Av. De Tervueren 34 bte
44 B-1040 Brussels, Belgium



Rev. 5/03 P803-A7544-01