A New Automated Turbidimetric Immunoassay for Quantifying \( \alpha_1 \)-Antitrypsin in Serum

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This rapid, sensitive equilibrium turbidimetric immunoassay for quantification of \( \alpha_1 \)-antitrypsin involves a monospecific antibody, polyethylene glycol 6000 to accelerate and enhance the immunoprecipitation reaction, and Tween 20 surfactant to decrease and stabilize the sample-blank values. Turbidity at 334 nm is measured by an automated discrete analyzer. Grossly lipemic, icteric, or hemolyzed samples can be assayed. Correlation with results by radial immunodiffusion (RID) was excellent \((r = 0.97, n = 84)\). Analytical recovery averaged 97.7 (SD 2.9)%. Within-run CVs ranged from 1.6 to 1.9%, between-day CVs from 2.0 to 3.5%. Reference values for healthy adults \((n = 147)\) were determined by parametric estimation \((r)\) for an assumed normal distribution of untransformed data. The lower limit \((g/L)\) with its 0.90 confidence interval is 1.23 \((range 1.18–1.28)\), the upper limit is 2.15 \((2.10–2.20)\), and the mean is 1.69 g/L.

Additional Keyphrases: glycoprotein • protease inhibitors • acute-phase reactant • pregnancy • inflammation • reference interval • radial immunodiffusion compared

\( \alpha_1 \)-Antitrypsin (A1AT) is a glycoprotein synthesized in the hepatic parenchymal cells. Its molecular mass is 52 000 to 56 000 Da and it exhibits considerable electrophoretic and genetic polymorphism \((I)\). One of the principal extracellular protease inhibitors in plasma \((2)\), A1AT is an acute-phase reactant protein in human serum. Under a variety of conditions, such as inflammation, pregnancy, and after the administration of estrogens, its concentration in serum may increase by two- to threefold \((3)\).

A deficiency of A1AT is closely related to earlier obstructive lung disease. Severe deficiency is associated with a high risk of developing pulmonary emphysema \((4)\). Inherited deficiency of A1AT is associated with infantile cirrhosis of the liver, most commonly after neonatal cholestasis \((5)\).

A1AT can be quantified by immunochromographic or functional methods, the former including radial immunodiffusion \((RID)\) \((6)\), electroimmunoassay \((7)\), nephelometry \((8–10)\), and immunoturbidimetry \((11, 12)\). Here we describe an immunoturbidimetric method that is more rapid \((220 \text{ samples/ hour})\), accurate \((CV < 2\%)\), simple, and economical \((costs 5\% \text{ as much})\) than RID.

Materials and Methods

Instrumentation

We used an EPOS Analyzer 5060 178 V2.4 (Eppendorf Gerätebau Netheler Hinz GmbH, 2000 Hamburg 63, F.R.G.) \((13)\). All dilutions of the samples, controls, and standards, were made with the STATUS System Diluter/Dispenser (American Dade Division, American Hospital Supply Corp., Miami, FL 33152).

Serum Samples

We studied serum from healthy and sick subjects. The sera were either freshly collected or stored frozen at –20 °C until assay \((no longer than one month)\).

Reagents

Antiserum diluting buffer: Phosphate-buffered saline \((PBS)\), pH 7.30, containing, per liter, 50 mmol of phosphate, 150 mmol of NaCl, 40 g of polyethylene glycol 6000 \((PEG)\), 0.2 mL of Tween 20 polyoxyethylene \((20)\) sorbitan monolaurate surfactant, and 1 g of NaN3.

Samples \((standards)\) diluent: Saline solution with Tween-20, nephelometric grade \((no. T01-0494-10; Technicon Instruments Corp., Tarrytown NY 10591)\).

Antiserum and standards: Goat anti-human A1AT antisera was obtained from Technicon \((no. T-21-0458-51)\); A1AT Reference Serum was used as calibrator \((no. T-03-495; Technicon)\).

Reagent auxiliary: Lipoclean* \((no. OUCI 02/03; Behringwerke)\). We measured A1AT by RID as specified by the manufacturer.

Immunoturbidimetric Assay

Preparations. Dilute goat antiserum to human A1AT four-fold with PBS-PEG solution, and mix; let stand at room temperature for at least 30 min, then centrifuge until the supernate is clear. Dilute with saline–TWEEN 20 solution the A1AT Reference Serum containing A1AT to obtain standards for the calibration curve covering 0.24 to 8.86 g/L \((six points)\). Dilute serum samples 20-fold with saline–TWEEN 20 solution.

Assay procedure. We used the following settings for the immunoturbidimetric: diluted sample, 12 \(\mu L\); PBS-PEG \((R1), 225 \mu L\); preincubation, 36 s; temp, 30 °C; wavelength, 334 nm. The first reading at 36 s corresponded to a blank for the final reading \((sample blank)\); therefore, we did not include separate blanks. The starting reagent \((R2)\) was antibody \((25 \mu L)\). The turbidity was measured at 334 nm after 180 s of incubation.

Calculation of the results. The standard curve was determined from six different standard concentrations, in duplicate. The concentration of the samples were calculated by the instrument with the aid of spline interpolation.

When EPOS is not used, but rather a different type of instrument or a manual spectrophotometer, plot the mean absorbance of the six calibrators against their concentrations and connect the points. Derive the concentration for each sample from its absorbance, reading the concentration directly from this calibration curve. Alternatively, the regression equation can be calculated from each line between
two points and used for calculation of the concentration of the samples.

Results

Standard curve. Figure 1 illustrates a typical standard curve for A1AT determination. No antigen excess appeared at A1AT concentrations up to about 8.86 g/L. The limit selected is 4.40 g/L; if the result calculated exceeds this value, it is noted, diluted, and remeasured.

Linearity. The standard curve is not linear but gives accurate results based on spline interpolation. Linearity of the method between 0.12 and 4.40 g/L was established by using serial dilutions of the AIP Reference Serum. The correlation within values calculated and values obtained was excellent ($r = 0.999956$, n = 10) and the equation of the regression line was $y = 0.998x + (0.926 \times 10^{-2})$.

Precision. Table 1 shows the precision of the method at various A1AT concentrations. Within-run assay precision was estimated from the data on 10 replicate analyses of three different serum pools with low, medium, and high concentrations; day-to-day precision was assessed from the data for seven replicate analyses of five different serum pools with newly prepared working antiserum on seven consecutive days.

Interfering substances. Turbid and grossly lipemic sera were cleared with Lipoclean. Addition of increasing quantities of hemoglobin (up to 1200 mg/L) or bilirubin (up to 100 mg/L) to three serum samples containing high, medium, or low concentrations of A1AT did not interfere in the values of A1AT.

Analytical recovery. Table 2 summarizes the results we obtained for three sets of serum with essentially quantitative (mean 97.7%, SD 2.9%) analytical recovery.

Comparison of methods. Sera from 84 healthy or ill subjects were analyzed by both the proposed assay (y) and by radial immunodiffusion (x). The values obtained by the two methods correlated well, $r = 0.97$ (Figure 2).

Statistical analysis. Figure 3 shows the distribution of

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<th>Table 2. Analytical Recovery of A1AT Added to Serum</th>
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Fig. 1. Standard curve (each point represents the mean value of two measurements)

Fig. 2. Correlation between the results by turbidimetric immunoassay and by radial immunodiffusion: $y = 0.4493x + 0.7167$ ($r = 0.97$, n = 84)
frequencies of the values of A1AT in 147 healthy adults. We used the statistical test of D'Agostino (14) to confirm the gaussian distribution of the untransformed data. The reference interval, obtained by parametric estimation (15), was 1.23 to 2.15 g/L (mean 1.69 g/L).

**Discussion**

Immunochemical quantification of A1AT by RID requires prolonged incubation time and subjective quantification. Other disadvantages include: differences in diffusion rates secondary to disulfide complexing, trypsin–antitrypsin complexing, catalysis, and other secondary phenomena. Quantification by electroimmunoassay requires trained personnel and an electrophoresis time of 4–6 h. Nephelometric and immunofluorescent methods present major potential advantages but require special instrumentation.

The automated turbidimetric method described here allows a fast and accurate quantification of A1AT with a routine clinical chemistry analyzer. However, results by the immunoturbidimetric method were approximately 20 to 30% lower than those by RID. This bias is in part due to the significant variation of reactivity of different antisera sources. Standardization of results between laboratories will require common methods, common calibrators, and common reagents.

The proposed method for quantifying A1AT is rapid (220 samples per hour), accurate (CV <2%), simple (no special instrumentation is required), and economical.

**References**