Determination of Alpha-1 Antitrypsin by a Nephelometric Procedure*  

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ABSTRACT

Several reports exist which link low levels of serum alpha-1 antitrypsin with pulmonary emphysema. Deficiency of this protein may be detected by serum electrophoresis or specifically quantitated by radial immunodiffusion. Recent investigations have led to the development of a number of automated and manual procedures for the quantitation of immunochemical reactions in aqueous media through the use of light-scattering techniques. The present report describes a sensitive microtechnique for the nephelometric measurement of the reaction between human serum alpha-1 antitrypsin and its specific antibody.

Principle

Alpha-1 antitrypsin is quantitated by use of an immunochemical reaction with specific commercial antiserum in aqueous medium. A fluorometer, which has been adapted for nephelometry, is employed to make light-scattering measurements of the antigen-antibody complexes thus formed. In the zone of antibody excess, the net light scattered by the immune complexes is proportional to the amount of alpha-1 antitrypsin present in the serum sample. Standardization is accomplished by use of commercial reference serum in which the content of alpha-1 antitrypsin has been determined by radial immunodiffusion.

Reagents

Saline. Reagent grade sodium chloride (9 g) is dissolved in one l of distilled, deionized water. To this solution are added 15 drops of Triton X-100. The saline is then passed through a filter to remove all particles larger than 0.45 microns. The filtration step is necessary since suspended particulate matter interferes with measurement of light scattering from the immune reaction. The filtered saline is used to make all dilutions of antiserum and standards.

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Antiserum. Goat antiserum specific for alpha-1 antitrypsin is obtained from a commercial source. The antiserum is stored at 5°C and diluted 1:25 with saline prior to use.

Standard Solutions

Alpha-1 Antitrypsin Stock Standard (330 mg per dl). This reference standard is obtained from a commercial source and stored at 5°C.

Working Standards. Dilutions of the stock standard are made to provide working standards of 264, 220, 165, 66, and 33 mg per dl.

Apparatus

A filter fluorometer equipped with a square, quartz micro flow-cell is used to make the light scattering measurements. The fluorometer source lamp gives maximum emission at 350 nm and a filter giving peak transmission at 360 nm is installed on the excitation side of the flow-cell. The fluorometer is not equipped with a secondary filter, since non-specific fluorescence of samples is not observed.

Procedure

A 5 microliter syringe is used to add 1.5 microliter of the standard or unknown sample to 1 ml of diluted antiserum in a 10 x 75 mm test tube. To another 1 ml portion of antiserum is added 0.75 microliter of sample to provide a screen for antigen excess samples. Blank solutions are prepared by the addition of 1.5 microliter of the sample to 1 ml of saline. All tubes are then covered with “Parafilm” and mixed by inversion 10 times. Samples are introduced into the flow-cell with a 3 ml syringe by injection into the inlet port on the fluorometer door. The flow-cell and sample syringe are washed with saline after each measurement. This procedure allows use of the same syringe for standards and all samples, and no carry-over is observed.

The reactions are allowed to stand at room temperature for 45 minutes for completion of the antigen-antibody reaction although shorter reaction times may be used. As shown in figure 1, the reaction is essentially complete at 25 minutes. During the delay period, the blanks may be determined using saline to set the baseline. Blank readings greater than zero, however, are found only with grossly lipemic samples. After the delay period, reactions may be read without regard to time, since the light-scattering response is stable for several hours after completion of the reaction. Light scattering of reaction mixtures is measured after adjusting the fluorometer to zero using the diluted antiserum. Blanks are subtracted from the readings obtained from the reaction mixtures and concentrations of alpha-1 antitrypsin in the serum samples are read from the standard curve (figure 2).

Results and Discussion

In order to elucidate fully the characteristics of the alpha-1 antitrypsin antigen-antibody system, it is necessary to construct a precipitin curve over a wide range of alpha-1 antitrypsin concentrations. Once this is done, the reaction conditions are adjusted to provide antibody excess throughout a desirable range of concentration. The precipitin curve and reaction conditions are unique to a particular batch of antiserum, but obtaining antiserum from a single commercial source provides for a certain amount of consistency in the potency of the reagent. Usually only small changes in reaction conditions will be necessary for different batches of antiserum received from the same manufacturer. In the present procedure, the amounts of serum and antiserum employed achieve antibody excess

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* Meloy Laboratories, Springfield, VA 22151.
† G. K. Turner, Palo Alto, CA 94303, Model 111.
‡ Hellma Cells, Inc., Jamaica, NY 11424, No. 176-QS.
§ Turner, No. 110-850.
|| Hamilton Co., Reno, NV 89502.
DETERMINATION OF ALPHA-1 ANTITRYPsin

up to concentrations of approximately 300 mg per dl of alpha-1 antitrypsin. Samples with concentrations greater than 300 mg per dl can be detected by use of the antigen excess screen. This screen is necessary since samples which fall in the antigen excess region of the precipitin curve give a low light-scattering response. This low response, due to the solubilization of the immune complexes in the presence of excess antigen, would lead one to believe that the sample has a low concentration of alpha-1 antitrypsin. The screen will detect these antigen excess samples since the reaction containing one-half the amount of sample will not show a corresponding decrease in light scattering. Appropriate dilutions can be made of these specimens demonstrating the condition of antigen excess.

Precision of the present method was assessed by performing replicate determinations on a pooled serum sample. The values ranged from 214 to 228 mg per dl with a mean of 223 mg per dl. The standard deviation was ±5.0 mg per dl with a coefficient of variation of 2.22 percent.

A typical standard curve for the procedure is shown in figure 2. It is necessary to construct this curve with each set of determinations. Alpha-1 antitrypsin concentrations of 26 sera from hospital patients were determined by both the present method and radial immunodiffusion. The immunodiffusion determinations were carried out using commercial plates.* The correlation of the nephelometric and immunodiffusion techniques is shown in figure 3 and gave a correlation coefficient of 0.863 with a standard error of estimate of 27.6 mg per dl and a regression line slope of 1.06. The scatter of points around the regression line may be attributed to the semiquantitative nature of radial immunodiffusion.

Sources of Error

Storage of Antiserum

Antiserum should be stored at +2° to +5°C since repeated freezing and thawing can cause loss of potency.

Antiserum Changes

New precipitin curves should be constructed for each batch of antiserum since potency varies between batches.

* Meloy Laboratories, Springfield, VA 22151 and Miles Labs, Kankakee, IL 60901.
Figure 2. Standard curve for the measurement of alpha-1 antitrypsin in serum.

Figure 3. Correlation of alpha-1 antitrypsin concentrations of 26 serum samples determined by both immunodiffusion and the nephelometric technique.
Normal Ranges

Normal ranges include 212 ± 32 mg per dl, heterozygotes — 125 ± 46 mg per dl and homozygotes — 25 ± 6 mg per dl.

Résumé of Clinical Interpretations

An increased incidence of chronic pulmonary emphysema has been shown to be linked with hereditary alpha-1 antitrypsin deficiency. Cirrhosis of the liver has also been demonstrated in infants with very low levels of alpha-1 antitrypsin. In their severe, homozygous forms, both conditions carry a grave prognosis.

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References