Rate Nephelometry of Human IgE in Serum

Oscar E. Beck1 and Paul E. Kaiser

We have developed an immunoassay method for the determination of human IgE concentration in serum by rate nephelometry. Using the Beckman Immunochemistry Analyzer, human IgE antiserum, and a human IgE standard, we analyzed 150 serum samples from normal and allergic patients. We report these results and those from comparison studies involving PRIST and Phadebas kits (both from Pharmacia), and Enzygnost (Behringwerke) kits. We also discuss diagnosis of allergy patients and their classification according to their concentrations of IgE and IgG4.

Additional Keyphrases: allergy, classification and monitoring treatment - immunoglobulins - immunoassay - "kit" methods - reference interval

Since the initial reports describing a previously unknown class of immunoglobulins in 1986 (1), the structure (2) and some of the functions of IgE (3) have been elucidated. Quantitative determination of IgE in serum has heretofore been carried out by single radial immunodiffusion assays (4, 5), radioimmunosorbent tests (6), solid-phase sandwich tests (7), radioallergosorbent tests (8), and even erythrocyte-linked antigen-antiglobulin reactions (9).

The IgE concentration in human serum is in the microgram per liter range, and the World Health Organization (WHO) has arbitrarily set 2.4 ng of their reference material equal to 1 int. unit. The WHO IgE standard reference 69/204, a high-titer preparation, has the assigned, standardized value of 10 948 int. units/mL. However, the manufacturers of test kits have developed their own internal standards and references, which vary somewhat from this defined value. This must be kept in mind in reviewing and evaluating results derived from serum samples in the clinical setting. Fortunately, variations of 100 int. units/mL do not significantly alter the mode of treatment chosen by the allergist or clinician.

Previous success in adapting the Beckman Immunochemistry Analyzer to IgG subclass rate nephelometry (10) led us to believe that IgE concentration could be determined in a similar manner. Here, we describe a rate-nephelometric method and report results obtained from 150 serum samples with IgE content ranging from 10 to 4000 int. units/mL. The results of parallel studies by the PRIST, Phadebas, and Enzygnost methods for the same serum samples are presented for comparison. The serum samples were selected on the basis of their preliminary mean concentration as determined by PRIST and were grouped in 100 int. units/mL increments.

Materials and Methods

The Immunochemistry Analyzer and nephelometric-grade reagents (diluent and buffer) were purchased from Beckman Instruments, Munich, F.R.G. The diluent was phosphate-buffered isotonic saline, and the buffer was phosphate-buffered saline with polymer enhancer, polyethylene glycol.

Recrystallized human albumin was purchased from Serva, Heidelberg, F.R.G.

Rabbit anti-human IgE (ε chain, 492 273 titer-int. units/mL) and an IgE standard series (10, 25, 50, and 100 int. units/mL) were provided by Behringwerke, Marburg, F.R.G., and confirmed by immunoelectrophoresis for specificity.

Serum. Serum samples, selected from a normal clinical setting, were kindly provided by Dr. Grossmann, Frankfurt; Dr. Kienholz, Aschaffenburg; and numerous donors from the Paul Ehrlich Institut. All sera were collected without preservatives and allowed to clot before centrifugation at 357 × g for 15 min. Preliminary trials, involving the removal of serum lipids by Frigen (Freon) 113-TR-T treatment, did not improve the accuracy of determination of IgE.

Comparison methods. IgE concentration of all samples were also determined with the PRIST and Phadebas kits (Pharma
cia, F.R.G.) and Enzygnost kits (Behringwerke).

Manual mode rate nephelometry. Using previously reported methods (10, 11) as guidelines, we calculated the optimal sample and antibody dilutions (see manual for manual-mode operation, Beckman) and experimentally verified them nephelometrically by using manual mode card M-44. The nephelometer was adjusted to a scatter value of 120 by using the manufacturer’s reference in the simulated scatter program. This assures a photomultiplier gain of 40 rate units per second, which produces a range of 200 to 700 peak rate units for the lowest (10 int. units/mL) and highest (100 int. units/mL) calibrators, respectively.

To establish a standard curve, we added 42 μL of each of the respective undiluted standards to reaction cells containing 600 μL of buffer and 42 μL of the diluted antiserum. We diluted the serum samples 10- to 100-fold in nephelometric-grade buffer to minimize the high nonspecific background turbidity caused by the other serum proteins.

The most reliable dilutions of the highly concentrated rabbit antiserum lots were 1296- to 2592-fold in 40 g/L human albumin; these were then sterile-filtered (0.22-μm pore size filter) to reduce excess scatter. At times excess scatter, which blocks the data processing, must be overridden by removing the reaction cell and then replacing it in the holder.

Results

Analytical variables. Rate nephelometry of human IgE concentration in serum requires optimal proportionality of antigen and antibody throughout the measuring range of the standard or reference curve. By limiting the measuring range and using high dilutions of samples and antiserum to reduce background turbidity, the kinetics of immune-complex formation can be precisely registered by a rate nephelometer that is adjusted to maximum gain.

Preliminary studies with the Behring standard series, and others, showed that rate curves for standards exceeding 100 int. units/mL were not reliable references, but that those below this concentration rendered dependable, reproducible results. Therefore, all samples were diluted accordingly, to bring them within the measuring range of the reference curve of 10 to 100 int. units/mL, as shown in Figure 1.

Figure 1 represents a typical reference curve prepared from data on the Behring series of standards (undiluted) and antiserum diluted 1296-fold. As shown, the reference curve is
Fig. 1. Beckman ICS standard curve for IgE, prepared from anti-human IgE diluted 1296-fold with human albumin (40 g/L) and IgE standards of 10, 25, 50, and 100 int. units/mL in the manual mode of rate nephelometry.

Sterile filtration of the antiserum dilution and adjustment of the dilution with human albumin not only decreases excess scatter but also stabilizes the reaction so that the nephelometer and recorder more smoothly register the kinetics.

Either fresh or frozen samples may be used; i.e., freezing and thawing of samples did not alter the results. Samples and antiserum should, however, be diluted just before the run.

The within-run coefficient of variation (CV) for rate nephelometry over various ranges of IgE concentration were as follows: 0–100 int. units/mL = 7.5%; 200–499 = 9.0%; 500–999 = 2.5%; and 1000–4000 = 2.3%. We performed no day-to-day studies on these samples.

WHO IgE standard 69/204, determined in the rate nephelometer with use of the Behring standard series as reference, produced a mean value of 9477 int. units/mL as compared with the assigned value of 10948 int. units/mL, a difference of approximately 13.5%, or 3.53 μg of IgE.

Although sterile filtration was necessary for rate nephelometry, the mean values ascertained for unfiltered samples with the kit methods were decidedly lower than the assigned value.

Comparison with other methods. Table 1 shows a compilation of results of serum samples ranging from 20 to 4000 int. units/mL, as determined with the rate nephelometer and with the PRIST, Phadebas, and Enzygnost kits.

The rate-nephelometric method repeatedly gave higher values for the same sample within any given range. Specifically, within the 200–300 int. units/mL range, samples clustered near the upper-limit values, i.e., towards 300 int. units/mL, rather than the lower limit.

Discussion

The concentration of IgE in human serum can range from 24 ng (10 int. units) to 9.6 μg (4000 int. units) or more, per milliliter, especially in specific allergies and in parasitic and helminthic infections. Therefore, all of the present methods as well as a recent new one (12) used to determine IgE concentrations >1000 int. units/mL depend on dilution of samples so that results fall within the measuring range of the reference curves.

The rate-nephelometric standard curve shown in Figure 1 is nearly linear, the concentration of the sample being virtually proportional to the magnitude of maximum rate units of the sample in reference to those of the standard times the dilution factor.

The mean values for one sample for each given range, selected without conscious bias from preliminary PRIST determinations, as shown in Table 1, and compared with mean values derived by rate nephelometry, clearly demonstrate that the latter is a dependable method. All of the values derived by all the methods lie within a tolerable variation for biological analytes, and supply clinically useful information regarding IgE concentration.

The methods with the least standard deviation within each range of IgE concentration investigated are given in Table 1. All are suitable in some range or other. The primary advantage

<table>
<thead>
<tr>
<th>Range, int. units/mL</th>
<th>Method with least SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–100</td>
<td>14</td>
</tr>
<tr>
<td>101–200</td>
<td>184</td>
</tr>
<tr>
<td>201–300</td>
<td>156</td>
</tr>
<tr>
<td>301–400</td>
<td>358</td>
</tr>
<tr>
<td>401–500</td>
<td>490</td>
</tr>
<tr>
<td>501–600</td>
<td>528</td>
</tr>
<tr>
<td>601–700</td>
<td>542</td>
</tr>
<tr>
<td>701–800</td>
<td>792</td>
</tr>
<tr>
<td>801–900</td>
<td>894</td>
</tr>
<tr>
<td>901–1000</td>
<td>981</td>
</tr>
<tr>
<td>1001–1500</td>
<td>1144</td>
</tr>
<tr>
<td>1500–1600</td>
<td>1556</td>
</tr>
<tr>
<td>1600–1700</td>
<td>1672</td>
</tr>
<tr>
<td>1701–2000</td>
<td>1750</td>
</tr>
<tr>
<td>2001–4000</td>
<td>4075</td>
</tr>
</tbody>
</table>

* Data shown are the mean of two determinations for a sample chosen without conscious bias from 10 samples measured within that range by each method.

Within each respective range.
of rate nephelometry, in contrast to the other established methods, is immediate availability of results; this permits the most nearly accurate determination, because samples with values beyond the measuring range of the reference can be appropriately diluted on the same day.

We believe we have determined with this method the probable range of IgE concentrations that occur in normal and allergy patients in the clinical setting. Our motive for developing this method was that, by determining IgG subclass concentration and percentile distribution by rate nephelometry (10), especially IgG4, as well as IgE concentration and histamine-release values, we could provide information that would aid the allergist in determining the type of allergic reaction and course of treatment. These values could also help to classify the patient (13–15) for extended preventive or maintenance therapy. One can argue that the clinician requires only information concerning an increase of IgE and not its magnitude, but we contend that once IgG4 determinations become routine, the clinician will be able not only to classify the type of allergy patient, but also to monitor more closely the course of treatment regimen.

We owe a special debt of gratitude to Dr. Heinrich M. Menzel at Paul Ehrlich Institut for his expertise and suggestions during the development of this method. Drs. Grossmann and Kienholz receive our thanks for providing serum samples from the clinical setting. Also, the donors from Paul Ehrlich Institut deserve a note of appreciation for their cooperation.

References