Rate Nephelometric Measurement of Rheumatoid Factor in Serum

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We describe the measurement of rheumatoid factor in human sera with a rate nephelometer. The National Reference Preparation for Rheumatoid Factors is used to calibrate the assay in International Units. We used Hyland Positive Control, Level I, as a secondary standard. The standard curve is exponential, but is linear when plotted on log-log graph paper. Aggregated immune globulin (IgG) is the antigen used to detect rheumatoid factor (IgM-class antibody to IgG). The rate reaction measures the rate of increase in light scatter by the antigen–antibody complexes; the reaction takes place in 17 to 20 s. Precision, linearity, and accuracy are excellent. Results agree well with those for a commonly used latex precipitation test. The advantages of speed, quantification in International Units, and superior discrimination of concentration as compared to serological titration provide a more reliable test for use in the diagnosis and treatment of rheumatoid arthritis.

Additional Keyphrases: diagnostic aids • rheumatoid arthritis • nephelometry • latex precipitation test compared • measurement of IgG and IgA autoantibodies • “Level I” as a substitute for National Reference Preparation • cutoff value

Rheumatoid arthritis (RA) is a systemic disease characterized by muscular pain and stiffness as well as articular inflammation and destruction. Its precise etiology is unknown, but there is evidence that a genetic predisposition (1) and environmental factors (viral infection or other) (2) are co-requisite for its initiation. Data also suggest the existence of a functional defect in T-lymphocytes (3). How the genetic, environmental, and immunologic factors interrelate is unknown. Evidence for altered immune functions include hypergammaglobulinemia, decreased in vivo and in vitro T-lymphocyte reactivity, and the presence of autoantibodies to nucleoproteins and IgG, i.e., “rheumatoid factor” (RF). Autoantibodies synthesized by plasma cells within the inflamed synovium can form immune complexes, which activate complement, and recruit neutrophils, which ingest the immune complexes, causing release of destructive lysosomal enzymes. Toxic lymphokines and activated T-lymphocyte secretory products are also detected in synovial fluid and probably contribute to joint destruction (4, 5). Circulating immune complexes can deposit in the walls of blood vessels, and, as occurs in the joint space, activate complement and produce an acute vasculitis.

RF, the serologic hallmark of rheumatoid arthritis, is an antibody that reacts specifically with the Fc portion of the IgG class of immunoglobulins. Although rheumatoid factors occur in all major immunoglobulin classes, the IgM RF usually predominates and is most easily detected by the commonly used latex precipitation method of Singer and Plotz (6). The principle of all the commonly used methods for demonstrating RF involves use of an indicator system such as latex, bentonite, or erythrocytes, to which human IgG is attached. Heat-aggregated IgG may also be used directly as the antigen in the test system. The presence of RF is recognized by agglutination, flocculation, or precipitation of the various indicator systems (7).

Overall, about 75% of patients with RA will demonstrate a positive RA latex-fixation test (6). Although low-titer (<1:80) RF activity is nonspecific and can be seen during an acute immune response to many infectious agents and with advanced age, the presence of high-titer (1:80 or higher) RF activity is strong supportive evidence for RA if other clinical information is consistent with this diagnosis. The clinical activity of RA in general may be directly related to the RF titer; higher titers usually occur during acute exacerbations of the disease, but this correlation is imperfect. If there were a more nearly accurate and discriminating method for determining RF activity, expressed in the recommended International Units (IU), a closer correspondence might be seen between test results and disease activity, prognosis, and response to therapy.

Materials and Methods

Apparatus

The rate nephelometer (Immuno-Chemistry System “ICS”; Beckman Instruments, Inc., Fullerton CA 92634) that we used has been described in detail (8). In the “Normal Mode” the operator is guided step-wise through operation of the instrument by an alphanumeric display, which also displays the results in the desired concentration units (mg/L, IU/mL, or mg/dL). Forty-two microliters of an appropriately diluted sample and of its corresponding antiserum are successively introduced into a stirred, disposable cell containing 600 μL of a buffered polymer solution and a magnetic stir bar. Introduction of the antiserum containing a dye triggers the measurement sequence, leading to display of the antigen concentration in the sample.

The instrument may also be operated in the “Manual Mode.” If this mode is used, the operator may select his own conditions for analysis, and he may measure either antigen or antibody. The rate and scatter signals are indicated in units and the measurement sequence is initiated manually by pressing the “Option” button. The two signals may also be traced using a dual-pen recorder. Provided with each system are four manual cards, plus a manual-mode calibration card. The five manual cards are coded M11, M22, M33, M44, and M-CAL. These cards are programmed such that each successively increasing number provides approximately a 2.5-fold increase in sensitivity to the amount of scattered light, and a fourfold increase in sensitivity to the rate of change of scattered light.

Because in the Beckman Immunochemistry Analyzer, as in most optical systems, the signal-to-noise ratio decreases as
the reaction signal to be monitored decreases, the manual cards have been coded to program variable levels of electronic filtering into the instrument for each indicated gain setting. Because higher gain settings are required for the detection of scatter signals, the degree of electronic filtering has been increased to minimize detection of spurious rate signals resulting from system noise. Use of the manual mode card M11 results in the lowest sensitivity gain setting and entails the least electronic filtering. Therefore, the M11 card programs the instrument for a gain of one, and a filtering level of one. In our experiments we used the manual mode of operation and the M22 card.

Light scatter and rate were recorded simultaneously with a dual-pen recorder (Model 385, Linear Instruments Corp., Irvine, CA 92714). Manual Mode Program Cards, including M-Cal and gain card M22, were from Beckman Instruments, as were the cuvettes, magnetic stir bars, and 42-µL pipettes. The 82-µL pipettes (Repetman, Gilson, cat. no. R-200) were from Rainin Instrument Co., Inc., Boston, MA 02155.

Reagents

Aggregated immunoglobulin G (IgG), human (“LAS-R Rheumatoid Factor Antigen, Human”; Hyland Diagnostics, Division of Travenol Laboratories, Inc., Bannockburn, IL 60015), lot no. 8680U004A.

“LAS-R Rheumatoid Factor Positive Control Serum (Human), Level I” (Hyland), lot no. 086-505.

“LAS-R Rheumatoid Factor Positive Control Serum (Human), Level II” (Hyland), lot no. 086-506.

“LAS-R Rheumatoid Factor Negative Control Serum (Human),” (Hyland), lot no. 086-505.

“RA-Test” (Hyland). This is a rapid slide and tube test for qualitative and quantitative determination of rheumatoid factors in serum.

National Reference Preparation for Rheumatoid Factors, lot no. 79-0013 (lyophilized). (Center for Disease Control, Atlanta, GA 30333). The standard, when reconstituted with 0.5 mL of water, contains 1000 IU/mL.

NaCl, 155 mmol/L (9 g/L; isotonic saline) and 50 mmol/L.

“Level I” may be used as a standard (see Results). “Level II” and “Negative Control Serum” may be used as controls.

Procedure

Inactivate the standards, control, and patients’ samples at 56 °C for 30 min. Samples should be tested within 6 h after heating. Never reheat them.

Insert the Manual Program Card (M-CAL) into the reading slot of the nephelometer. The digital display will show the instruction: “READ AB CARD.”

Insert the M22 card. The display will show “EA MEMORY TEST.” then “INSERT CELL STIR.”

Place one magnetic stir-bar into each cuvet. Fill as many as needed with 600 µL of 50 mmol/L NaCl.

Put 84 µL of standard, control, or patient’s sample into successive cuvetas (126 µL and 84 µL of undiluted level I, as well as 84 µL of two-, four-, and eightfold dilutions of Level I are used in preparing the standard curve; see Results—Standard curve). Use 50 mmol/L NaCl as diluent for any samples requiring dilution.

Place the cuvet into the cuvet holder in the instrument. Push the “Option” button before closing the cuvet-holder lid. Read the initial light-scatter signal.

Press the “Option” button. Remove the cuvet briefly, then replace it into position and close the lid. The display will read, “INJECT SAMPLE M22.” The instrument is now in the rate mode.

Inject 42 µL of antigen (aggregated immune globulin). At the same time, press the “Option” button, to activate the circuit for rate reading.

The display will show the Rate Units and time elapsed to reach the maximum rate (usual elapsed time, 17 to 20 s)

If the final scatter reading is desired, remove the cuvet briefly, put it back into the holder, press the “Option” button, and close the lid. The final scatter reading will appear in the display.

Plot the rate units on a previously prepared calibration curve (see Results—Standard curve), and calculate the concentration in International Units.

Results

In preliminary experiments we determined the best combinations of sample volume, antigen volume, and gain card. If the patient’s sample (antibody) is used as the final trigger of the reaction, results are erratic and inaccurate, and the initial light-scatter of the sample cannot be measured. If instead the antigen (aggregated immunoglobulin) is used as the trigger, the results are reproducible and predictable, and the initial scatter of the sample can be noted—this measurement is important. We found the scatter of the antigen to be very consistent from day to day, 40 ± 4 (2 SD) scatter units. The usual order of addition in the Normal Mode is buffer—antigen (patient’s sample)—antibody trigger, for example, in the assay for IgG. In our system the reverse order, with antigen (aggregated immunoglobulin) serving as the trigger, is far more efficient if antibody concentration is to be determined.

Figure 1 shows recorder tracings of the reaction (rate and scatter) for three samples with low (but abnormal), medium, and high concentrations of RF. Sample 1 shows that the scatter of a sample (70 rate units, equivalent to 60 IU/mL and a titer of 1:160) with a rather low but abnormal concentration reaches a steady state of scatter, which is maintained for at least 7 min. The rate signal reaches its maximum at about 17–20 s, at which time the instrument quickly verifies that the peak is valid, and the rate units and time are then displayed. When the concentration of RF is higher (Figure 1, samples 2 and 3), the rate of scatter change reaches its maximum at the same time, but the scatter begins to decrease within 1 to 2 min. If the cuvet is examined at this time (after 2 min), aggregates are visible. The decreased scatter at this time is probably ascribable to larger amounts of light being passed directly through the increased spaces between the large particles. At the time of peak rate (20 s), the antigen—antibody complexes are evenly dispersed and no aggregates are visible. Because of the general dispersion with very little clear space, the light is greatly scattered. When the particles finally (after several minutes) aggregate sufficiently, the light is not scattered. The higher the concentration, the more rapidly the scatter de-

![Fig. 1. Recorder tracings of the reaction (rate and scatter) of three patients' samples with low (70 rate units), medium (300 rate units), and high (650 rate units) concentrations of rheumatoid factor.](image-url)
were compared. The maximum rate signal is reached so quickly (20 s), however, that the larger aggregates formed later do not interfere with measurement of the rate of increase in scatter.

**Standard curve.** A standard curve was constructed with use of the National Reference Preparation. This material has been compared by the Communicable Disease Center (CDC) with the WHO's International Reference Preparation of Rheumatoid Arthritis Serum (9). When reconstituted with 0.5 mL of water, it contains 1000 IU/mL. It and the Hyland control serum, Level I, produced identical curves when the samples were assayed (dilutions were made with 50 mmol/L NaCl) in a cuvet containing 50 mmol/L NaCl (Figure 2). Repeated comparisons of Level I and dilutions of it with the curve described by the CDC Reference Preparation have established that Level I contains 250 IU/mL and Level II contains 62.5 IU/mL. Hyland Control Level I also has been compared with the International Standard by Hyland Laboratories, and has been found to contain 250 IU/mL. The pools are rigorously controlled from lot to lot, and have been judged to be consistent over a period of several years (personal communication, Dr. Clarence Jones, Hyland Laboratories).

The standard curve is exponential; on log-log paper it is linear between 20 and 330 IU/L (approximately 20 and 680 rate units, respectively). The use of linear plots allows inspection of the slopes (see Discussion). If NaCl (155 mmol/L) is used as the diluent and in the reaction cuvet, a different slope results (Figure 2). We have established that the use of 50 rather than 155 mmol/L NaCl results in a more nearly accurate analysis. Hyland Level II (a fourfold dilution of Level I), when tested with use of 50 mmol/L NaCl, yielded results identical to those for a fourfold dilution of Level I, whereas it does not when 155 mmol/L NaCl is used. The National Reference Preparation is in short supply, but Level I may be confidently used as a standard for routine measurements.

**Precision.** Within-day precision was assessed by assaying three patients' samples 20 times during the same day. The samples represented low, medium, and high concentrations of RF. The results (in IU/mL) were, respectively: mean, 35 (SD, 1.6; CV, 4.6%); mean, 168 (SD, 6.5; CV, 3.9%); and mean, 269 (SD, 10.2; CV, 3.8%). Between-day precision was tested by assaying Level II (Hyland) and three patients' samples on 20 separate days during a month. Results (in IU/mL) were:

Hyland Level II, mean, 62 (SD, 3.1; CV, 5%); patients' samples, range of means 33 to 305 IU/mL, CV's 4.8 to 2.6%. We did the between-day study by using aliquots of serum that had been stored at −20 °C, because we established that at 4 °C samples were not stable (see below).

**Sample stability.** Serum samples may validly be stored for as long as 24 h at 4 °C, but longer storage is not recommended because serum samples kept at 4 °C for longer than 24 h gave erratic, inconsistent, and imprecise results (usually increasing with time). Further, serum should be used rather than plasma, which showed substantial light-scattering effects, especially if stored at 4 °C for several days. Sample stability is excellent at −20 °C for two weeks. Samples should be frozen if the assay cannot be performed on the day the sample is received. Samples should not be reheated. If a repeat analysis is required, a new aliquot that has not been heated previously should be used. Repeated heat inactivation causes increased rate and scatter signals.

**Accuracy.** We added sera with known concentrations of RF to negative and positive sera and assayed them. Analytical recoveries ranged from 86 to 105%. Linearity was repeatedly examined by serially diluting all abnormal samples and Level I. The upper limit of linear analytical response is about 330 IU/mL. If a sample with a high concentration is diluted, the response of the system becomes linear between 330 and 20 IU/mL, but is nonlinear when the concentration falls within the normal (negative) range—i.e., less than 20 IU/mL or 20 rate units. Figure 3 depicts the dilution curves for three patients' samples. Sample A (titer = 1:320, IU/mL = 80) becomes nonlinear at a fourfold dilution (approximately 20 IU/mL). Sample C becomes nonlinear above 620 rate units (320 IU/mL).

If the sample concentration exceeds 333 IU/mL (about 680 rate units), we make dilutions in 50 mmol/L NaCl. Table 1 shows an example of dilutions of three samples with high concentration of rheumatoid factor. Sample 1, at a twofold dilution, showed a rate and concentration above the linear limit of the assay. The corrected rate (for a twofold dilution) was not consonant with the corrected rates for the greater dilutions, but dilutions that give values that fall within the linear range show remarkable agreement. Sample 2 (Table 1) is nonlinear at a fourfold dilution. Both the rate and scatter are above the acceptable linear range. Further dilutions show excellent agreement. The actual titer of sample 3 (Table 1) by
"RA-Test" was 1:81 920. We tested the sample again in the rate nephelometer, using a twofold (168 μL) and threefold (252 μL) volume of serum. The serum concentrations represented about 12 000 and 18 000 IU/mL, respectively. The rate responses (1150 and 1290) were well above the accepted linear limit of the system (680 rate units), and the rates, when calculated, would be nonlinear. Evidently there is little possibility of a false-negative result caused by antibody (RF) excess in this system. This contrasts with the conventional nephelometry of proteins, where antigen or antibody excess is always a possibility.

All of the samples tested by dilution gave linear curves, all of which had identical slopes (Figure 3). Repeated preparation of dilution curves of Level I also demonstrated that its slope was identical to those for all the patients' samples. Moreover, the slopes for the National Reference Standard were the same as those for the patients' samples and Level I. We therefore could, with confidence, calculate values for unknown samples by comparison with the secondary standard curve described by Level I.

Interferences. Bilirubin in concentrations up to 30 mg/L does not affect results. There is apparent suppression of the calculated concentration (from the rate response) of 20% at 40 mg/L, 30% at 50 mg/L, and 50% at 80 mg/L. In our series of seropositive and seronegative patients there were no samples containing bilirubin in abnormal concentration.

Hemoglobin concentrations up to 1.8 g/L do not interfere. Above this concentration the following apparent suppression was noted: 2.0 g/L, 10%; 2.5 g/L, 15%; and 5.0 g/L, 30%.

Lipemic samples should be avoided because of the light-scattering effects of such sera. If the initial light-scatter reading (before antigen injection) exceeds 800, results may be inaccurate. If the final light scatter exceeds 900, the instrument will indicate that the scatter is in excess, and no rate signal will be displayed, or if it is it cannot be assumed to be accurate. Negative samples usually show an increase of scatter (after antigen injection) of less than 50 scatter units, and thus may be analyzed even if the initial scatter is about 800. However, samples (with initial scatter of 800) containing RF will usually demonstrate enough increase in scatter to go into the "Excess Scatter" mode.

The problem may be avoided by diluting the sample (if the concentration is high enough) to bring the initial light scatter down to <400 units. Several dilutions should be made to ensure that there is a linear response, and that the slope of the dilution line is the same as that of the standard. Or the sample can be delipidated; we used the same method as that of Finley et al. (10), following their technique for precipitating lipoproteins (except the high-density lipoproteins) with dextran sulfate and MgCl₂. The light scatter of all samples can be decreased 50 to 80% in this way. We tested 98 negative samples before and after delipidation, and found no difference in rate response. Also 32 samples from patients with rheumatoid arthritis were tested, and again the rate responses were unaltered by the delipidation procedure. The precipitation neither alters the rate of reaction nor changes the concentration of total protein or of immunoglobulins IgG, IgA, and IgM.

The need for this technique, however, rarely arises. The usual initial scatter for our samples ranged from 50 to 400 light scatter units.

Moreover, the use of 50 mmol/L NaCl instead of 155 mmol/L NaCl increases the rate of reaction enough that the reaction is complete before excess scatter occurs. For example, a sample assayed in 50 mmol/L NaCl may show the same change of scatter (initial to final) as when assayed in 155 mmol/L NaCl, but the actual rate of increase of scatter may be two- to threefold greater. The slope is also steeper when 50 mmol/L NaCl is used (Figure 4); therefore, the reaction is much less likely to go into "excess scatter" and invalidate the rate reaction.

Clinical samples. We obtained 487 serum samples (30 Red Cross donor samples and 457 patients' samples) from individuals known to be free of symptoms attributable to RA or clinically related diseases such as systemic lupus erythematosus, Sjögren's syndrome, sarcoidosis, and other collagen or immunologic disease. We obtained 62 samples from patients judged to be affected by RA in various stages of clinical activity, five samples from patients with systemic lupus erythematosus, and two from patients with osteoarthritis.

All of the 487 samples from individuals with no evidence of RA showed rates of less than 20 rate units (<20 IU/mL). All of these samples were also negative by the latex test on heated samples. Of the negative samples, 95% had rates from 1 to 14 rate units. The Hyland "RA-Test" (latex) is intended to be performed on unheated serum in the usual assay, according to the instructions of the manufacturer; the incidence of false positives by this method ranges from 7 to 15% with no heat inactivation. In the original latex method of Singer and Plotz (6) it was recommended that the sample be heat inactivated, to eliminate the influence of complement component C1q, which enhances the reaction. Cheng and Persellin (11) found
that if the sera were heated at 56 °C for 30 min, the incidence of weak false-positive tests in the slide latex method (in patients with no evidence of RA) decreased from 45% to 9%.

We performed the latex test on both heated and unheated samples (180 nonrheumatoid individuals). The incidence of false positives (latex) in this series was 10%. All of these samples, when retested (latex) after heating were negative, and all the samples were negative by nephelometry. Three of the samples were from pregnant women; a fourth sample from a pregnant woman remained weakly positive after heating (by both latex and rate testing). Activation of rheumatoid factor has been reported in pregnancy (12) with use of an unpublished solid-phase radioimmunomassay method, but this view is disputed by Amino et al. (13), who could find no evidence of this phenomenon when they used the Hyland latex test. Our proposed nephelometric assay, when performed on unheated sera, produced much higher rates on negative sera (up to 10-fold) and made the distinction between normal and abnormal sera more difficult. When 10 heated negative samples were tested, with a twofold (168 µL) and threefold (252 µL) volume of serum, the rates, in all cases, remained in the negative range, suggesting that a false-positive reaction in our system would be unlikely.

The 62 samples from individuals judged to be afflicted with clinical RA gave results that agreed with the latex test. In this series there were no conflicting results for the two methods. The latex test was performed on both heated and unheated specimens; the results occasionally differed for the two, but were always clearly positive and of significant titer. Our final comparisons were made on heated specimens with both methods. Five specimens (8%) were negative both by latex and rate nephelometry. Four of the five patients had classical RA that was mild, stable, quiescent, required no therapy, and in one case was localized to the wrists and elbows. One patient (of the five seronegative) had severe, active disease. The incidence of false-negatives for the conventional latex testing reportedly is about 25% (14). If the mild and inactive cases are included in our series, we found a rate of 8%, both by latex (after heating) and rate nephelometry. It should be noted that the data quoted from the inserts of the Hyland Company refer, in the case of latex testing, to unheated samples whose titers are 1:40 or greater, and in the case of the Hyland LAS-R end-point nephelometric test, to heated samples with "relative light scatter" greater than 3 units on their instrument ("PDQ" Nephelometer). We have established that 3 relative light-scatter units in the Hyland nephelometer method is equivalent to about 20 rate units (20 IU/mL) by our method.

Table 2 shows the relationships between IU/mL, rate units, and titer (latex). The table was constructed by using the assay results of many samples. Samples with concentrations greater than 333 IU/mL are corrected for dilution. The cutoff value between negative and positive sera is 20 IU/mL. The routine dilution in the latex (RA-Test) test is a 20-fold dilution of serum, hence the designation, "1:20, weak positive," for the concentration of 25 IU/mL. If the latex test is positive for the usual 20-fold dilution, the analyst makes serial dilutions of the samples, and retests all the dilutions. The end-point is the highest dilution for which agglutination is visible.

Table 2 shows an approximation of titers to a representative curve of rates vs IU/mL. The titers always represent twofold increases and therefore cannot measure subtle gradations in change of concentration of rheumatoid factor. Further, the imprecision of serological titrations adds to the inevitability that measurement of a quantifiable protein will not be very accurate.

The seronegative patients may represent cases of rheumatoid arthritis with a predominance of circulating antiglobulins of IgG or IgA class, rather than the usual IgM class of anti-IgG protein. Torrigiani et al. (14, 15) have described a technique that involves absorbing the antiglobulins from the serum with an insolubilized gamma-globulin preparation, eluting the absorbed antiglobulins, and measuring the IgG, IgM, and IgA concentrations in the eluate by radial immunodiffusion. In this way, 50% of patients' samples that were seronegative by the conventional latex tests demonstrated an increased concentration of rheumatoid factor of the IgG or IgA class. If aggregated IgG was used as the antigen, some samples containing IgG or IgA autoantibodies did not react in the system. The bivalent IgG and IgA antibodies are much less efficient agglutinators than the more usually present IgM class autoantibody.

Singh and Francis (16) recently described a technique for quantitation of the antigenic RF irrespective of its immunoglobulin class. Papain Fc fragment of human IgG is labeled with fluorescein isothiocyanate and incubated with serum. Bound Fc is separated from free Fc by precipitation with polyethylene glycol and measured by the fluorescence of the precipitate. Sixty percent of seronegative and 96% of seropositive rheumatoid sera gave results more than 2 SD above the mean value for normal sera.

Four of the five patients with systemic lupus erythematosus had normal rate reactions and one showed a concentration of 330 IU/mL (titer, 1:5120). Some patients with this disease will show circulating RF. Six patients with multiple myeloma were tested; four were negative, and two showed concentrations of 90 and 125 IU/mL, respectively. Also, one patient who had had a heart transplant and was receiving rabbit anti-thymocyte globulin had a concentration of 50 IU/mL. A case of polymyalgia rheumatica showed an RF value of 290 IU/mL. All of these cases represent valid instances of false-positive—circulating RF is present—but the disease state can usually be distinguished from true RA. Two patients with osteoarthritis were seronegative, as would be expected.

**Discussion**

The proposed method is the first in which rate nephelometry is used to quantify RF in human serum in the recommended WHO International Units. The end-point technique of nephelometry (Hyland Laboratories, LAS-R Rheumatoid Factor, and "PDQ" nephelometer) requires 20 to 90 min to complete. Also, blank readings of the buffer, antigen, and each control and patient sample must be taken, and the readings of multiple tubes must be timed exactly. In contrast, because
of the kinetic reaction in the rate nephelometer, no initial blank readings are needed, the reaction is complete within 17 to 20 s, and the concentration of the sample may be calculated in the recommended International Units.

The latex–IgG agglutination technique of Singer and Plotz (6) has become the most popular method for detecting RF. Most of the available commercial preparations are modifications of that method. The various latex methods suffer from lack of sensitivity (25% of patients with RA will have negative results) and specificity (7–25% of people without RA will have positive results). Further, it is not possible to quantify positive samples except by titer, a notoriously inaccurate technique (95% confidence usually ± 1–2 tubes).

The proposed method is rapid and reproducible, produces a result in the recommended International Units, requires no blank tubes, has high sensitivity and specificity, and can be performed with available commercial reagents. The actual concentration of rheumatoid factor in IU/mL may more accurately represent the extent of the disease process and may be helpful in monitoring the disease and the effects of therapy more closely. The rate-nephelometric assay should be adaptable to techniques measuring IgG and IgA class autoantibodies. The method allows great flexibility in such investigations and is limited only by the suitability of the antigens and antibodies.

In the proposed method, there is little possibility of a false-negative result due to antibody (RF) excess. A concentration of 18 000 IU/mL (approximately a titer of 1:243 000) in a sample gave a positive rate response well above the stated upper limit of linearity.

We recommend that a standard curve be constructed with each analysis, using Hyland Level I Positive Control. The highest points on the curve are obtained by using 126 μL of standard; it and the other points are plotted on log-log paper. On the y-axis is plotted IU/mL (126 μL = 333 IU, 84 μL = 250 IU, twofold dilution = 125 IU, fourfold dilution = 62.5 IU, and eightfold dilution = 31.75 IU), and on the x-axis is plotted the rate units (Figure 2). We have also plotted the curve using a cubic least-squares fit on our computer. The rates vary slightly from day to day, but the resulting slopes are invariably parallel. Different rate nephelometers may demonstrate somewhat different rates (owing to variations in response of the photomultiplier tubes), but the slopes of the standard curves will be identical, and patients' samples tested on different instruments will show comparable results. We have established that the Hyland Control, Level I, contains 250 IU/mL, by comparing it with the National Reference Preparation for Rheumatoid Factors, when 84 μL of the undiluted standard is used in the test procedure.

The latex titer correlates roughly with severity of disease (6) but the inherent inaccuracy and imprecision of serological titration have made the use of sample titers inadequate to judge the clinical course of rheumatoid arthritis. The proposed method should provide a more reliable guide to the diagnosis, prognosis, and treatment of the disease, and should be adaptable to investigation of autoantibodies of the IgG and IgA class.

References