Qualitative Testing for Circulating Immune Complexes by Use of Zone Electrophoresis on Agarose

R. H. Kelly, Margot A. Scholl, V. Susan Harvey, and A. G. Devenyi

On binding of antibody to antigen an immune complex is formed that has a net surface charge different from that of either of the two components. This, together with clonal restriction of the antibody response, gives rise to distinctive patterns that are readily apparent in stained agarose gels after routine zone electrophoresis. Most circulating immune complexes appear as a rectangular pattern, with well-defined edges, located in the γ-region. The identity of the material responsible for these patterns has been established by three different experimental approaches: analysis of tetanus/anti-tetanus complexes formed in vitro, analysis of sera from rabbits with experimental immune complex disease, and analysis of human type II and type III cryoglobulins. Studies of reproducibility, interfering substances, and correlation with other assays for detecting immune complexes indicate that zone electrophoresis in agarose gel is a sensitive, highly specific technique for immune complexes. The identity of zone electrophoresis in agarose gel is a sensitive, highly specific technique for immune complex detection, of potential value as a screening tool.

Additional Keyphrases: immunochemistry • immune complex disease • immunoglobulins • acute-phase reactants • experimental serum sickness in rabbits as a laboratory model • immune complex formation as a "normal" process • screening

Immune complexes are formed by the specific binding of one or more antibody molecules to sites on an eliciting antigen. Such complexes possess a remarkable spectrum of biological activities—e.g., they can augment or suppress immune reactivity (1, 2); potentiate tumor growth (3); deposit in renal glomeruli, blood-vessel walls, and other sites where tissue damage secondary to activation of the complement cascade may ensue (4); and, in the normal situation, facilitate immune clearance of food antigens (5) and invading microorganisms (6). Thus, immune complex formation is a physiological process that is central to humoral immunity (i.e., ubiquitous distribution of immune complex amongst humans), but formation and/or persistence of some types of immune complexes in the circulation can lead to pathological sequelae irrespective of the original antigenic stimuli. Because of this, an increasing number of clinical laboratories are examining methods for detecting and measuring circulating immune complexes (es). These methods, like those for measurement of complement components and acute-phase reactants, are useful, not because they show disease specificity, but rather because they aid in investigation of a wide range of diseases for which the etiology and pathogenesis are unclear. Furthermore, in some cases, they provide an important means for assessing the efficacy of therapy (7).

More than two dozen indirect methods for detecting presumed circulating immune complex(es) have appeared in the literature during the past few years, and recently the World Health Organization completed a study comparing the more prominent methodologies (8). It was clear from the results of this study that none of the techniques is entirely suitable for use in clinical laboratories owing to problems with methodological complexity, sensitivity, and restrictions in the types of circulating immune complex(es) detected by the individual tests (i.e., tests currently available are influenced by the class and subclass of Ig within the complex, the size of the complex, the antigen:antibody ratio, and the ability of an immune complex to fix complement). Thus, use of these tests has largely confined to research laboratories where, because of variability amongst assays and amongst standards used as positive controls, may now search for circulating immune complex(es) by two or three different procedures.

A method of screening for circulating immune complex(es) by use of standard zone electrophoresis in agarose gel is reported here. The approach has been validated by analysis of serum globulin changes in a well-known laboratory model of an acute immune complex-mediated disease, serum sickness of rabbits (9); comparison of results of zone electrophoresis and Raji cell (10) assay of sera of patients clinically suspected of having circulating immune complex(es); and analysis of human type II and type III cryoglobulins.

Materials and Methods

Zone Electrophoresis

We used conventional materials and procedures for zone electrophoresis in agarose gel (11). The buffer was barbituric acid/sodium barburitate (pH 8.6, 50 mmol/L) containing, per liter, 0.4 g of calcium lactate and 1 g of sodium azide. The potential gradient was 10-12 V/cm within the gels. Sample volumes of 1.5 μL were applied to the gel and the run was stopped when albumin had migrated about 4 cm from the point of application. The gels were stained with a dye mixture containing, per liter, 1.6 g of Naphthol Blue Black (color index no. 20470) and 0.5 g of Coomassie Brilliant Blue (color index no. 42660).

Counterimmunoelctrophoresis

Serum globulin fractions obtained by ammonium sulfate fractionation (12) were run against goat anti-bovine serum albumin antisera (N. L. Cappel, Labs., Inc., Cochranville, PA 19330) before and after dissociation of immune complexes by acidification. Wells were spaced about 5 mm apart in an equivalent mixture of 6 g/L solutions of agar (Noble agar; Difco, Detroit, MI 48223) and agarose (Seakem ME; Marine Colloids, Rockland, ME 04841) in barbital buffer as described above. Electrophoresis was carried out overnight at 25 V

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(constant voltage). The slides were examined for precipitin lines; negative ones were soaked in a 10 g/L solution of tannic acid for 5 to 10 min and re-examined.

Tetanus Toxoid/Antitoxoid Complexes

Fluid tetanus toxoid (Massachusetts Department of Health, lot no. LP 403, 1100 Lf/mL) was titered against ultracentrifuged (2 h at 100,000 X g), affinity-column purified (13) human anti-tetanus immune globulin (Cutter Laboratories, Berkeley, CA 94710) by the quantitative precipitin procedure (14). Soluble complexes were prepared in three-fold molar excess of antigen or antibody, assuming a relative molecular mass of 67,000 for the toxoid and 150,000 for the antibody.

Complement Measurement

C₃ in rabbit serum was measured by radial immunodiffusion (15) and the values were expressed as a percentage of the value found for each animal's serum before antigen inoculation (i.e., the pre-experimental test bleeding). A four-point standard curve was run for each of the normal test bleedings, with use of goat anti-rabbit C₃ (Cappel). Values lower than the control were read directly from the dilution curve; higher values were either estimated by interpolation or the serum samples were diluted until they fell within the range of the standard curve.

Serum-Sickness Experiments

Adult New Zealand white rabbits were bled before immunization, then (on day 0) given a single intravenous inoculation of 250 mg of bovine serum albumin (Sigma Chemical Co., St. Louis, MO 63178) in phosphate-buffered isotonic saline. Blood was sampled daily from the marginal ear vein, from day 3 to day 21, inclusive. Thereafter, animals were bled on days 28, 35, 42, and 61. Urine samples were obtained before antigen inoculation and on days 5, 11, 14, 16, and 19 thereafter. Serum was separated and urine clarified by centrifugation and stored at 4 °C. Total protein of serum was determined by refractometry and of urine by turbidimetry after addition of tri-chloroacetic acid (16). Serum and urine samples were separated by zone electrophoresis in agarose gel as described above. In some experiments, serum pH was lowered to 3 by adding dilute (1 mol/L) HCl and held at room temperature for 30 min before electrophoresis, to dissociate immune complexes.

Control Studies and Potentially Interfering Substances

We assessed the incidence of circulating immune complexes detectable by zone electrophoresis in healthy individuals by examining 100 sera (fresh, or frozen and stored at -20 °C) submitted either for routine pre-marital syphilis serology or for serological testing after submission of an application for hospital employment. We determined the incidence of circulating immune complexes in a hospitalized population by examining 100 successive patients' sera (fresh, or frozen and stored at -20 °C) that had given negative results to rapid plasma reagin (RPR) testing. We determined the effect of some materials known to interfere with other immune-complex assays on the outcome of the zone electrophoresis test by adding these substances to rheumatoid-factor-negative sera (i.e., RA-latex negative) that had been previously classified either as immune complex negative or positive at one of three arbitrary levels (i.e., high, medium, or low immune complex content). Five sera were included in each group. These test sera were analyzed in the presence and absence of each of the following substances at the final concentrations indicated: DNA, 2 g/L (type I, Sigma); lipopolysaccharide, 5 g/L (from Serratia marcescens or Escherichia coli 026:B6; Sigma); and heat-aggregated human γ-globulin, 5 g/L (immune serum globulin, Squibb, New Brunswick, NJ 08903), prepared by diluting the globulin preparation to 100 g/L with isotonic saline (9 g/L) and heating for 10 min in glass tubes in a water bath set at 65 °C, which produced an opalescent solution with little visible precipitate that remained stable at 4 °C for several weeks. The aggregated γ-globulin stock solution was mixed 1:20 with each of the 20 test sera and, in addition, was examined for the presence of γ-region artifacts by directly applying the stock solution to the gel. We determined the effect of polyclonal rheumatoid factor by mixing equal parts of a Waaler-Rose positive serum (end-point titer: 1/640) that lacked circulating immune complexes with each of the 20 test sera. All mixed sera were checked for alteration of the test results. Aliquots of lipemic, icteric, turbid, or hemolysed sera that showed no abnormalities ascribable to immune complexes were mixed in equal proportion with either normal or immune-complex-positive sera containing low, medium, or high concentrations of circulating complexes, to determine whether these abnormalities influenced the outcome of the test result. Effects of storage conditions—i.e., time and temperature—were assessed by repeated analyses of both positive and negative sera stored at 4 or -20 °C.

Comparison of Zone Electrophoresis with Other Methods for Immune Complex Detection

Raji cell assay: Both assays were performed on sera of 46 patients clinically suspected of having circulating immune complexes. These sera were drawn and tested at the Scripps Clinic Immunology Reference Laboratory (La Jolla, CA) by a standard procedure for the Raji assay (17) and by the procedure described here for electrophoresis.

Cryoglobulin analysis: Sera submitted for cryoglobulin analysis were first examined for monoclonal immunoglobulins and/or circulating immune complexes by zone electrophoresis in agarose gel and, according to the results, were classified as lacking these abnormalities or as possible type I, type II, or type III (18) cryoglobulin-containing specimens. All sera were centrifuged after refrigeration at 4 °C for 72 h, and any precipitate was washed three times with cold isotonic saline. We redissolved the precipitates by warming and repeated the zone electrophoresis, to study the appearance of washed cryoglobulins. We identified the immunoglobulin classes present by immunofixation (19).

Reproducibility

Six representative immune complex-containing sera were selected to provide a range of high, medium, and low concentrations of abnormal complexes in each of two categories: (a) type A, sera with low concentrations of homogeneous immunoglobulins within the complex, and (b) type B, sera that lacked homogeneous immunoglobulins within the complex. Coded immune complex-containing and normal sera were randomly assigned a plate position within a run (limited to 12 sera because of equipment design) by use of a random-number table. Sufficient plates were poured from a single batch of agarose to permit each sample to be analyzed 20 times. The necessary number of runs was completed on two successive days. The resulting zone electrophoresis patterns were classified according to type and concentration of immune complex, and then the code was broken to determine the percentage of correct identifications.

Between-day variation was studied by including a high, medium, low, and negative immune-complex-containing serum in the first run each day for 20 consecutive days. The samples were coded and randomly assigned a position within the run by use of a random-number table. At the end of the 20-day interval the code was broken and the number of correct identifications calculated.
Results

Electrophoretic Mobility of Immune Complexes

Figure 1 shows the appearance of stained agarose gels after electrophoresis of (A) free tetanus toxoid, (B) affinity-column-purified anti-tetanus antibodies, (C) tetanus/anti-tetanus immune complexes prepared in three-fold antigen excess, and (D) tetanus/anti-tetanus immune complexes prepared in three-fold antibody excess. Free antigen migrated in the α2 region, but staining for the purified antibody was confined to the γ-region. Formation of immune complexes, with a three-fold molar excess of either reactant, reduced the staining attributable to free toxoid in the α2 region, and introduced a new pattern in the β-γ region. This was a broad zone of heavier staining, with well-defined anodic and/or cathodic edges that differed markedly from the normal staining pattern of background polyclonal immunoglobulin. Electrophoretic mobility of the complex pattern was influenced by the antigen/antibody ratio. Dilution studies showed that the method could detect immune complexes, prepared with a three-fold excess of either reactant, in concentrations exceeding 200 ng per sample application.

C3 Concentrations and Proteinuria during Serum Sickness

Figure 2A shows serum complement component C3, expressed as a relative percentage of the pre-immunization control values, of two rabbits during the onset of serum sickness. The response was characterized by a series of fluctuations in C3 values, with the major phase of consumption occurring between days 9 and 12. Proteinuria was greatest in urine samples collected on the 11th day after immunization, although individual variation was considerable (Figure 2C). As expected, albumin constituted most of the protein lost in the urine.
Appearance of γ-Region Abnormalities

We first noted alterations in serum electrophoretic patterns (i.e., the appearance, in the γ-region, of broad zones of increased staining with well-delineated edges superimposed upon the more diffuse polyclonal Ig background) three to four days after inoculation with bovine serum albumin. The staining of these patterns increased in intensity for a day or two, then decreased, coincident with the rapid fall in serum C₃ concentrations (Figure 3A–C). As the proteinuria resolved, the staining intensity of the pattern once again began to increase, but a change in its appearance was noted. The zone of increased staining was now enclosed by low-concentration homogeneous proteins (Figure 3D), which persisted throughout the course of the experiments (i.e., >61 days), although the staining intensity of the zone between them diminished with time so that the final pattern resembled, in essential details, a bi-clonal gammapathy (Figure 3E and F). Figure 2B shows the relative amounts of protein in these γ-region patterns, as estimated by visually inspecting the stained gels and arbitrarily grading 0 to 5, for a single representative animal during the course of a serum sickness experiment. γ-Region abnormalities were greatest after the acute episode.

Presence of Antigen in the γ-Globulin Fractions

We examined γ-globulin fractions by counterimmunoelectrophoresis for the presence of the antigen, bovine serum albumin; the results were negative for all sera that lacked γ-region abnormalities and weakly positive (before immune complex dissociation) for all sera that contained the broad zone of increased staining superimposed upon the polyclonal background. The weakly positive reaction is attributable to antigen released during equilibration of the complex in the buffered saline. Decreasing the pH of globulin fractions to 3.0 dissociates immune complexes, and this was found to strengthen all the positive test results (Figure 4). On repeat zone electrophoresis of the acid-treated γ-globulins the broad zone of increased staining was no longer present and in some cases was replaced by two low-level homogeneous proteins (Figure 3F).

Control Studies and Interfering Substances

Control studies by the zone electrophoretic method revealed a 15% incidence of banding abnormalities in the γ-region, similar in appearance to those seen in the rabbit sera (see above), in human sera submitted for pre-marital and hospital job applicant serological testing. The hospitalized control population had a 16% incidence of these abnormalities. Nei-

| Table 1. Zone Electrophoresis and Raji Cell Assay Compared for Detecting Circulating Immune Complexes in Human Serum |
|---|---|---|---|
| Zone-electrophoresis method | (+) | (−) |
| Raji assay | | |
| (+) | 22 | 8 | 30 |
| (−) | 3 | 13 | 16 |
| | 25 | 21 | 46 |

χ² = 10.427, p <0.01. In this table only sera containing immune complexes at levels >2 units, as assessed by visual examination, were considered (+) in the zone-electrophoresis method.

Correlation of Electrophoretic Procedure with Raji Assay and Cryoglobulin Analysis for Immune Complex Detection

Forty-six sera of patients clinically suspected of having circulating immune complex (es) were first evaluated by the Raji assay, then coded and screened by the electrophoretic method. These sera were sent from hospitals throughout the country to the Scripps Clinic and Research Foundation for detection of immune complexes by the Raji cell technique. The staining intensity (i.e., the concentration) of the γ-region patterns detected by electrophoresis was graded from 0 to 5 by visual inspection, with 0 indicating none detected. The results are shown in Tables 1 and 2. In Table 1 both the 0 and

| Table 2. Zone Electrophoresis and Raji Cell Assay Compared for Detecting Circulating Immune Complexes in Human Serum |
|---|---|---|---|
| Zone-electrophoresis method | (+) | (−) |
| Raji assay | | |
| (+) | 27 | 3 | 30 |
| (−) | 11 | 5 | 16 |
| | 38 | 8 | 46 |

χ² = 1.967, p >0.1. In this table all sera containing immune complexes at >1 unit, as assessed by visual examination, were considered (+) in the zone-electrophoresis method.
1 gradations of γ-region patterns for the electrophoretic procedure are considered negative. The chi square test is significant at \( p < 0.01 \), indicating a positive association between the two tests. In Table 2 all electrophoretic results of 1 or greater are considered positive, and it is apparent that the electrophoretic procedure (83% positive) detects low concentrations of immune complexes in patients' sera when the Raji test (65% positive) does not. Figure 5B–E shows representative γ-region patterns encountered in human sera.

Type II and type III (18) cryoglobulins are generally conceded to be immune complexes. Fourteen sera submitted during the course of this study were found to have type II or type III cryoglobulins. In each case a γ-region pattern, with or without homogeneous components, was noted after zone electrophoresis in agarose gel (Figure 5E). Analysis of the washed cryoglobulins before immunofixation confirmed that the isolated materials gave rise to patterns analogous to those encountered previously in rabbit and human sera (Figure 5F).

Thus, all of 14 isolated washed immune complexes of human origin produced patterns on zone electrophoresis in agarose gel that resembled in all essential features the γ-region abnormalities that appeared in sera of rabbits subjected to experimental serum sickness. In two cases a monoclonal immunoglobulin, IgM/kappa in both instances, was evident in the center of the γ-region pattern (i.e., the immune complex pattern) of the washed cryoglobulin (i.e., type II cryoglobulin) when it was not detected by zone-electrophoretic analysis of the whole serum. This is consistent with a shift in the antigen/antibody ratio of the washed cryoglobulin toward antibody excess, thus allowing the monoclonal nature of the free antibody (i.e., monoclonal IgM/kappa with rheumatoid factor activity) to be identified. In two other sera—both from patients with chronic active hepatitis—a similar phenomenon was observed, but the monoclonal protein was IgG/kappa and was detected outside the immune complex pattern. These monoclonal immunoglobulins could not be absorbed with the usual reagents for rheumatoid factor (i.e., human γ-globulin-coated latex particles or sheep erythrocytes sensitized with rabbit antibody), but they could be shown to react with immunoglobulin present in the autologous immune complex by counterimmunoelectrophoresis (manuscript in preparation).

Thus they fit the specificity requirements for anti-idiotypic antibodies, which are known to exist in these patients in the form of anti-HBs (20). Of the 121 sera included in this study, 14 (12%) were found to contain immune complexes by analysis of cryoprecipitates, but 71 (59%) showed immune complex patterns by zone electrophoresis. Evidently zone electrophoresis is a more sensitive method for immune complex detection than is cryoprecipitation. Immunofixation of isolated washed cryoglobulin is shown in Figure 5F and G.

We have recently tested (manuscript in preparation) sera from 108 patients with progressive systemic sclerosis for circulating immune complexes by three different procedures: the Raji assay, the C₁₉₄ binding assay, and the electrophoretic method. This study showed good correlation \(( p < 0.01, \text{chi square analysis})\) of the zone electrophoresis results with results of both the Raji and C₁₉₄₄ assay.

**Reproducibility**

We studied within-run reproducibility by using two types of immune complex, A (homogeneous Ig within complex) and B (no homogeneous Ig within complex) at three different concentrations in each category and showed that these sera were always (in the 120 such tests) distinguished from negative controls. Type A immune-complex-containing sera invariably \(( n = 60) \) were correctly identified as to type, although the arbitrary concentration gradings were sometimes confused, i.e., a medium concentration type A immune complex serum was once classified as a type A high concentration and once as a type A low concentration. The type B high-concentration immune complex sera were always \(( n = 20) \) correctly identified as to type and concentration. The medium- and low-concentration type B sera \(( n = 40) \) were invariably correctly identified by type, but were incorrectly classified by concentration—i.e., medium vs. low—about one time in five. The samples included in the between-run variation study were correctly identified as negative or positive at the appropriate concentration 98.3% \(( 59/60) \) of the time. On one occasion a low-concentration positive was read as negative.

**Discussion**

The advent of clinical laboratory procedures for detecting and measuring circulating immune complexes has stimulated interest in the application of these tests in three principal areas: (a) patient management (e.g., assessing disease activity and response to therapy, as prognostic indicators of risk, or as staging factors), (b) diagnostic aids, and (c) investigations into pathogenesis. Unfortunately, relatively little clinically useful information has been generated by these tests, and a review of the recent literature suggests poor correlation among results obtained by different methods (8). These inconsistencies can be understood when one considers the restricted molecular composition of immune complexes that are detected by the various tests. For example, the C₉₄₄ methods detect principally large complexes, >19 S, which must activate complement by the classical pathway (20); the Raji cell assay
detects complexes consisting of IgG, C₃b–C₃d, and a molar excess of antigen (10); and rheumatoid factor assays principally detect small IgG-containing complexes (21). Thus, it is apparent that a negative result from any single test cannot be taken as conclusive evidence that circulating immune complexes do not participate in the pathogenesis of the disease.

The method described in this paper overcomes many limitations of the earlier procedures (Table 3) and, because of its relative methodological simplicity, appears to be suitable for use in screening in most clinical laboratories. The principal attribute of the method is that it exploits a consequence of the binding of antibody to antigen as a means of detection (i.e., the electrophoretic mobility of the complex differs from that of either the free antigen or the free antibody), and thus eliminates the need for troublesome measurements of other phenomena such as interaction with complement components, rheumatoid factors, cellular receptors, or cryoprecipitation. By zone electrophoresis, immune complexes appear as a broad area of uniformly increased staining with well-demarcated edges. Progression of the immune response is characterized by cyclic periods of increased antibody synthesis (22) and by the emergence of dominant antibody-producing clones of plasma cells (23, 24). After the acute nephritic episode in the rabbit model, circulating immune complexes are present in antibody excess (10), and on zone electrophoresis these complexes are found to be associated with low-level, flanking homogeneous immunoglobulins, presumably the products of dominant antibody clones. Similar clonal restriction has been observed in rabbits inoculated with antigens other than bovine serum albumin (25). Furthermore, in a single patient with recurrent blymphohistiocytosis followed by one of us (R.H.K.), the morphology of the immune complex pattern found was seen to progress from a broad zone of heavier staining at the time of flareup to one marked by flanking homogeneous immunoglobulins after successful therapy with an antibiotic. Table 4 shows attending physicians' diagnoses for an additional 181 hospitalized patients found to have circulating immune complexes by zone electrophoresis. The electrophoretic method also lends itself to several follow-up procedures that are useful for further characterization of the immune complex.

A decreased concentration of native C₅ in immune complex-containing sera is detected by reduced staining intensity of the β₂ band after zone electrophoresis. Immunofixation (19) can be readily applied to determine the Ig class of the complex. The complex may be dissociated by acid or 3 mol/L KCNS to confirm its lability in the unlikely event that its appearance is confused with that of a monoclonal Ig, or to facilitate analysis of individual components.

In terms of sensitivity for immune complex detection, the electrophoretic method compares favorably with the Raji assay, both in experimental (10) and clinical studies, as shown here. The seemingly high incidence of circulating immune complex (es) in normal (15%) and hospitalized control populations (16%) suggests that the formation of immune complexes is a physiological process (5), with food and normal gut and skin microbial flora serving as likely sources of antigens, that proceeds without pathological sequelae in most instances. Furthermore, patients with immune complex disease could be distinguished from their normal counterparts by examining the status of serum acute-phase reactants (11), which are altered in the former but normal in the latter. The reproducibility of this method, including both between-run and within-run sources of variation, for detection of circulating immune complexes was found to exceed 99% (179/180).

### Table 3. Advantages and Disadvantages of Immune Complex Assays<sup>a</sup>

<table>
<thead>
<tr>
<th>Basis of test(s)</th>
<th>Human reagents required&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Radioisotopes required&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Detects</th>
<th>All reagents commercially available</th>
<th>Class and sub-class of Ig detected</th>
<th>Interfering substances</th>
<th>Results expressed as</th>
</tr>
</thead>
<tbody>
<tr>
<td>C₁q interaction</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
<td>(−)</td>
<td>IgM, IgG&lt;sup&gt;1+3&lt;/sup&gt;</td>
<td>DNA, LPS</td>
<td>1. &quot;binding activity&quot;</td>
</tr>
<tr>
<td>Rheumatoid factor interaction</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
<td>(−)</td>
<td>IgG, IgA</td>
<td>RF, LPS</td>
<td>2. &quot;ng anti-IgM/tube&quot;</td>
</tr>
<tr>
<td>Interaction with cell receptors</td>
<td>(+)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>(+)</td>
<td>(+)</td>
<td>(−)</td>
<td>Varies</td>
<td>RF, C₁q&lt;sub&gt;c&lt;/sub&gt;, anti-lymphocytic antibody</td>
<td>3. &quot;μg equiv. ΔHGG/ml&lt;sup&gt;−1&lt;/sup&gt;</td>
</tr>
<tr>
<td>Electrophoresis</td>
<td>(−)</td>
<td>(−)</td>
<td>(−)</td>
<td>(+)</td>
<td>All</td>
<td>None of above</td>
<td>0 → +5 by visual assessment</td>
</tr>
</tbody>
</table>

<sup>a</sup> Table based on data compiled during own collaborative study for the evaluation of 18 methods for detecting immune complexes in serum (8). Methods are grouped by functional basis of the test procedure.

<sup>b</sup> A positive requirement indicates that in some of the methods in a category, usually the most sensitive, radioisotopes are used.

<sup>c</sup> Some tests may require reagents from other species, such as guinea-pig macrophages.

Abbreviations: ΔHGG, heat-aggregated human γ-globulin; LPS, lipopolysaccharide; RF, rheumatoid factor.

### Table 4. Disease Associations of 181 Patients Found to Have Circulating Immune Complexes by Zone Electrophoresis<sup>a</sup>

<table>
<thead>
<tr>
<th>Diagnostic category</th>
<th>Percentage</th>
</tr>
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<tbody>
<tr>
<td>Acute or chronic infections</td>
<td>29</td>
</tr>
<tr>
<td>Malignancies</td>
<td>20</td>
</tr>
<tr>
<td>Connective tissue disease</td>
<td>14</td>
</tr>
<tr>
<td>Liver disease (including all hepatitis)</td>
<td>10</td>
</tr>
<tr>
<td>Cardiac disease</td>
<td>2</td>
</tr>
<tr>
<td>Miscellaneous&lt;sup&gt;b&lt;/sup&gt;</td>
<td>23</td>
</tr>
<tr>
<td>No diagnosis</td>
<td>2</td>
</tr>
</tbody>
</table>

<sup>a</sup> Percentage of consecutive sera submitted for routine zone electrophoresis during a three-month period in which immune complexes were detected.

<sup>b</sup> Including six cases of acute glomerulonephritis, two cases of Henoch-Schönlein purpura; one to two cases each of pernicious anemia, trauma, iron deficiency anemia, diabetes mellitus, stroke, chronic gastritis, and myelofibrosis; and a miscellany of single cases.
Materials that give rise to false-positive results in other tests—such as DNA, bacterial lipopolysaccharides, and heat-aggregated Ig—are not a problem with the electrophoretic method: they do not produce patterns that can be confused with the appearance of an immune complex.

The finding that not all immune complexes give rise to identical patterns by zone electrophoresis is best explained by unique physical/chemical properties of different antigens involved, genetic variation of immune responsiveness, emergence of dominant antibody-producing clones with time after immunization (23, 24), and variations in the antigen/antibody ratio.

We conclude that zone electrophoresis in agarose gel seems to be suitable as a rapid, sensitive, and inexpensive method for immune complex screening in most clinical laboratories. Information gained from this analysis would aid in identifying specimens for which a follow-up procedure giving a titer, units of activity, or equivalents of heat-aggregated human γ-globulin might be indicated (Table 3).

We are grateful to Mr. N. Ratheev for performing the Raji assay, to Dr. B. S. Rabin for evaluating the zone electrophoresis patterns of human sera selected for comparison by these two methods, to Drs. S. L. Katyal and G. Singh for preparing the tetanus toxoid affinity column, to Ms. L. Shab for her photographic expertise, and to Ms. S. McNair for assistance in preparing the manuscript.


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