Microtiter Solid-Phase Radioimmunoassay for Hepatitis B Antigen

ROBERT H. PURCELL, DORIS C. WONG, HARVEY J. ALTER, AND PAUL V. HOLLAND

Laboratory of Infectious Diseases, National Institute of Allergy and Infectious Diseases, and the Clinical Center
Blood Bank of the National Institutes of Health, Bethesda, Maryland 20014

Received for publication 18 May 1973

A micro-solid-phase radioimmunoassay (micro-SPRIA) for hepatitis B antigen (HB Ag) was developed for use with microtiter serological equipment. Radiolabeled immunoglobulin G was prepared from human and animal sera containing hepatitis B antibody (HB Ab); it was not necessary to isolate specific HB Ab by immunochemical means. A micro-SPRIA prepared with guinea pig reagents was approximately as sensitive as the AusRIA radioimmunoassay, but, like the AusRIA test, yielded false positive results. A micro-SPRIA prepared with human reagents was slightly less sensitive but did not yield false positive results. These micro-SPRIA tests offer several advantages, including conservation of reagents, adaptability to other antigen-antibody systems, ease of performance (especially when testing large numbers of specimens), and economy.

Recent progress in hepatitis research has been, in part, dependent upon the development of tests and assays for hepatitis B antigen (HB Ag) and antibody (HB Ab). The need for simple, easily performed techniques for large-scale screening of blood donors on the one hand, and the need for highly sensitive and specific research tools on the other, has led to the development of a bewildering array of techniques that differ markedly in sensitivity and specificity. Among the most sensitive and specific of these techniques are the radioimmunoassays (1, 4, 6, 8, 10, 16, 17). Results obtained with these radioimmunoassays for HB Ag and HB Ab are markedly changing our concepts of the epidemiology of hepatitis B. One such test, a solid-phase radioimmunoassay for HB Ag (AusRIA; 10) that utilizes a polypropylene tube for the solid phase (3), has been licensed and is being used with increasing frequency for the detection of antigen-positive blood donors. This technique has also found wide application as a research tool for the study of hepatitis B infection of man and nonhuman primates. We have found the AusRIA test indispensable for the detection of HB Ag in experimentally infected rhesus monkeys (11). However, during the course of our studies, we found a need for a radioimmunoassay that could be performed with less serum than the AusRIA system and one with which large numbers of sera could be tested simultaneously. In addition, we wished to have a confirmatory test that utilized an entirely different set of reagents from those used in the AusRIA kit. For these reasons, we developed a solid-phase radioimmunoassay for HB Ag that could be performed in microtiter plates. We describe herein the micro-solid-phase radioimmunoassay (micro-SPRIA) for HB Ag.

MATERIALS AND METHODS

Reagents. Hyperimmune serum to HB Ag was prepared by inoculating guinea pigs with highly purified HB Ag as described previously (5, 13). The complement fixation (CF) titer of guinea pig hyperimmune HB Ab was 1:256. Human sera with CF titers of HB Ab of, respectively, 1:256 and 1:64 were obtained from patients who developed a secondary-type immune response to HB Ag after transfusion. Sera containing a low titer of HB Ag were obtained from patients convalescent from hepatitis B. These sera did not have detectable CF-reactive HB Ab but were positive for HB Ab when tested by a sensitive radioimmunoprecipitation technique (8). Immunoglobulin G (IgG) was purified from 1-ml samples of serum by precipitation with an equal volume of saturated ammonium sulfate. After centrifugation, the precipitate was resuspended in ammonium sulfate (one-third-saturated for human sera; one-half-saturated for animal sera). After recentrifugation, the precipitate was dissolved in 0.003 molar phosphate buffer, pH 8, and dialyzed 2 to 5 days against 0.005 molar phosphate buffer, pH 8, with 0.1% sodium azide. The dialyzed protein was chromatographed on a diethylaminoethyl-cellulose (Bio-rad) column (0.9 by 15 cm) equilibrated with 0.005 molar phosphate buffer, pH 8, with 0.1% sodium azide. The first protein peak
(IgG) was concentrated to original volume in an Amicon filter unit with an XM50 membrane. The IgG preparations were stored at 4 or −70°C.

IgG was labeled with $^{125}$I by a modification of the method of Hunter and Greenwood (7). The following reagents were added, in order, to a small conical vessel: 20 μl of 0.25 M phosphate buffer, pH 7.4; 200 μCi of high-specific-activity $^{125}$I (in 1 to 2 μl); 10 μg of the protein to be labeled (in 1 to 10 μl); 15 μl of a solution of chloramine T (3.5 μg/μl); 20 μl of a solution of sodium metabisulfite (4.8 μg/μl); and 20 μl of a solution of sucrose (22.5%), potassium iodide (2 mg/ml), and aqueous phenol red (0.025%). After the addition of chloramine T, the reaction was allowed to proceed for 15 s before being terminated by the addition of sodium metabisulfite. The mixture was applied to the top of a column (0.9 by 15 cm) packed with Sephadex G-200 and equilibrated with phosphate-buffered saline, pH 7.4, with 0.1% sodium azide. The protein was eluted from the same buffer; fractions containing the first peak of radioactivity were pooled and diluted with an equal volume of calf serum. This stock mixture was stored at 4°C and diluted 1:2 with calf serum just before use. The chromatographic column, collection vessel, and transfer pipette and storage vial were all preserved with 1% bovine serum albumin in saline to diminish nonspecific adsorption of the globulin.

Method. Polyvinyl V-bottom microtiter plates (Cooke Engineering) served as the solid phase for the radioimmunoassay and were used as received. Seventy-five microliters of an appropriate dilution of whole guinea pig or human antiserum was added to the bottom of the microtiter well with an Eppendorf pipette. The plate was incubated at 4°C for 4 h, and the residual antiserum was removed by aspiration. The wells were washed twice by immersion in saline. After the two wash cycles, the wells received 0.2 ml of 1% bovine serum albumin in saline from a microtiter dropper. The plate was incubated overnight at 4°C and washed twice as described above. Residual fluid was aspirated from the wells, and 25 μl of the sample to be tested for HB Ag was added to the bottom of the well with an Eppendorf pipette. The plate was incubated at 4°C (16 to 20 h when guinea pig reagents were used; 40 to 44 h for reagents of human origin) and, after aspiration of the residual serum, washed five times by alternately filling with saline from a reservoir dropper (Cooke Engineering) and aspirating with a Pasteur pipette connected to a vacuum or with a special vacuum manifold that aspirated all 96 wells simultaneously (Fig. 1). Fifty microliters of antibody labeled with $^{125}$I were added with a microtiter dropper or Eppendorf pipette, and the plate was covered and incubated on a rocker platform for 4 h at 37°C. After aspiration of residual serum, the wells were washed five times. After the final wash, the microtiter plate was transferred to a holder (Cooke Engineering), and the wells were cut apart with a hot dissecting needle or spatula as described previously (8). The separate wells were transferred to gamma counting tubes and counted in a Nuclear Chicago gamma spectrometer for 4 to 20 min. Results were expressed as the ratio of residual counts in the sample well to the mean residual count of wells that received a serum sample known to be free of HB Ag (positive/negative, or P/N).

Twofold dilutions of a “standard” HB Ag-positive serum served as a positive control in each test. Normal serum controls were included on each microtiter plate because slight variations were found to exist from plate to plate. Mean residual counts for normal serum controls were calculated for each individual plate, and these results were used for determining the positivity of other samples on that plate.

**Specificity testing of AusRIA and micro-SPRIA.** The specificity of AusRIA-positive but counterelectrophoresis (CEP)-negative sera was tested as described elsewhere (H. J. Alter, P. V. Holland, R. H. Purcell, and J. L. Gerin, Blood J. Hematol., in press). Briefly, 0.1 ml of the sample to be tested was incubated overnight in each of four AusRIA tubes. After washing, 100 μl of the following was added, respectively, to three tubes: human serum containing CEP-reactive HB Ab, normal guinea pig serum, and normal human serum. Nothing was added to the fourth tube; this tube served as the positive control. The tubes were incubated for 90 min, washed, and inoculated with 100 μl of $^{125}$I-labeled guinea pig HB Ab (as provided in the AusRIA test). The test then proceeded as for the standard AusRIA test. A test was considered specific for HB Ag if the prior addition of unlabeled human HB Ab reduced the radioactive counts by at least 50% (or to the level of normal serum controls if the P/N value of the positive control was less than 2.1) and suppression of counts by normal guinea pig or human serum was less than one-half that seen with HB Ab. A test was considered nonspecific (false positive) if the counts were not suppressed by HB Ab or were suppressed by normal guinea pig serum or human serum, or both, as well as HB Ab. This specificity test was adapted to the micro-SPRIA by adding 25 μl of saline to the positive control and decreasing the volume of blocking reagents added to the other wells to 25 μl.

**Serum test panels.** Five panels of sera were used for comparing the sensitivity and specificity of the micro-SPRIA with the AusRIA system. The first of these was the Bureau of Biologics test panel no. 2, a set of 60 human sera consisting of antigen-negative

*Fig. 1. Manifold for simultaneous aspiration of all 96 wells in the microtiter plate.*
sera and sera containing HB Ag in concentrations ranging from very high to very low, including antigen-positive sera diluted beyond the detectability of existing methods. Both ad and ay subtypes (9) were represented in the panel.

The second panel was prepared by the Blood Resources Branch of the National Heart and Lung Institute and consisted of various dilutions in normal serum of two HB Ag-positive sera containing, respectively, subtypes ad and ay, as well as negative control sera (J. K. Roche and J. M. Stengle, Transfusion, in press). Twenty-six coded specimens were represented in this panel.

The third panel consisted of serial serum samples obtained from rhesus monkeys experimentally infected with hepatitis B virus. These samples were obtained as part of another study (11). This panel consisted of 290 sera either negative for HB Ag or containing HB Ag at concentrations not detectable by CEP.

The fourth panel consisted of acute-phase serum specimens from 16 hepatitis patients. All were positive by AusRIA but negative by CEP. They had not been previously tested for specificity.

The fifth panel of sera contained 20 AusRIA-positive but CEP-negative samples identified by routine screening of blood donors. All sera had been tested for specificity in the AusRIA test as described above and were found to yield false positive results.

RESULTS

Optimal conditions for performance of micro-SPRIA: specific activity of radiolabeled IgG. Purified IgG was labeled with different quantities of $^{125}$I to yield protein with specific activities ranging from 5 to 94 $\mu$Ci/$\mu$g of protein. These radiolabeled antibodies were tested for their ability to detect HB Ag in serial twofold dilutions of a standard HB Ag-containing serum. Labeled antibody with different specific activities detected HB Ag with the same level of sensitivity. Therefore, all subsequent experiments were performed with antibodies with an average specific activity of approximately 7 $\mu$Ci/$\mu$g of protein (range of 3 to 17 $\mu$Ci/$\mu$g). Such specific activities were obtained by labeling with a ratio of $^{125}$I to protein of 20 $\mu$Ci/$\mu$g. Approximately 0.2 $\mu$Ci of such $^{125}$I antibody was used in each micro-SPRIA test; only 0.03% of this was retained in tests of normal serum (negative controls). Routinely, samples were counted for 4 min to yield negative control counts of 400 to 500.

Radiolabeled antibody could be used for 2 to 3 weeks after labeling, but it occasionally exhibited diminished sensitivity or increased nonspecific binding thereafter. Consequently, antibody was labeled at 2-week intervals.

Microtiter plates. Flat-bottom and V-bottom microtiter plates were compared for suitability as a solid phase for the micro-SPRIA. Both types of plates proved suitable, but a slightly more sensitive test was obtained with the V-bottom plate, which was also easier to wash.

Volumes and incubation times for the reagents used in the micro-SPRIA. The absolute and relative volumes, as well as the incubation times yielding maximal sensitivity and reproducibility, were determined for each of the reagents used in the micro-SPRIA. Those conditions found most suitable are listed under Materials and Methods and in Table 1. Hyperimmune guinea pig HB Ab with a CF titer of 1:256 could be diluted at least 1:400,000 for coating the microtiter wells. A dilution of 1:4,000 was used routinely; such antibody was used once and discarded. One milliliter of

| Parameter                  | Measurement | Variables tested |
|----------------------------|-------------|------------------|
| Coating with antibody      |             |                  |
| Volume                     | 50 $\mu$liters |                  |
|                            | 75 $\mu$liters* |                  |
|                            | 100 $\mu$liters  |                  |
|                            | 150 $\mu$liters  |                  |
| Duration                   | 1 h         |                  |
|                            | 2 h         |                  |
|                            | 4 h*        |                  |
| Secondary coating          |             |                  |
| Type                       | Guinea pig serum |                  |
|                            | Human serum  |                  |
|                            | Human albumin|                  |
|                            | Bovine albumin| (1%)*            |
|                            | Bovine albumin| (10%)            |
| Volume                     | 200 $\mu$liters* |                  |
| Duration                   | 1 h         |                  |
|                            | 2 h         |                  |
|                            | 4 h         |                  |
| Incubation with sample     |             |                  |
| Volume                     | 25 $\mu$liters* |                  |
| Duration                   | Overnight*  |                  |
| Incubation with $^{131}$I antibody |     |
| Volume                     | 50 $\mu$liters* |                  |
| Duration                   | 1 h         |                  |
|                            | 2 h         |                  |
|                            | 4 h*        |                  |
|                            | Overnight*  |                  |

* Conditions found most suitable for micro-SPRIA (guinea pig and human HB Ab reagents).
* Superior when guinea pig HB Ab reagents were used.
* Superior when human HB Ab reagents were used.
guinea pig HB Ab was sufficient to coat over 13,000 microtiter wells. Human hyperimmune HB Ab of comparable titer could be diluted at least 1:10,000 but yielded a more sensitive test when used at a dilution of 1:10.

Human sera from patients convalescent from primary exposure to hepatitis B virus (and therefore positive for antibody only with the sensitive passive hemagglutination and radioimmunoprecipitation techniques) could also be used as reagents in the micro-SPRIA. However, the sensitivity of tests performed with these reagents was less than that obtained with hyperimmune animal or human sera.

**Washing of microtiter plates.** A 96-port manifold for the simultaneous aspiration of all the wells in the microtiter plate (Fig. 1) was designed and built by the National Institutes of Health Mechanical Fabrication Section. This device was useful for the rapid removal by vacuum of fluid from the plates, but the wells also could be evacuated moderately rapidly with a single Pasteur pipette attached to a vacuum system.

**Stability.** To test the storage stability of micro-SPRIA plates, microtiter plates coated with antibody and secondarily coated with bovine albumin were rinsed with distilled water, dried in vacuo overnight at 4°C, and stored at −80°C. Plates stored for 8 months were as sensitive as freshly prepared plates for detecting HB Ag.

**Comparative sensitivity of AusRIA and micro-SPRIA: titrations of a standard HB Ag-containing serum.** Serial twofold dilutions of human serum containing the ad subtype of HB Ag were prepared and tested under code with the AusRIA test and a micro-SPRIA technique utilizing guinea pig reagents. Comparable titers were obtained with the two methods in repeated tests. On the basis of these observations, the AusRIA criteria for differentiating between positive and negative sera (P/N < 1.5 = negative; P/N > 1.5 but <2.1 = borderline, P/N ≥ 2.1 = positive) were used for evaluation of subsequent micro-SPRIA test results. Tests in which the titration of standard HB-Ag-positive serum indicated a slightly greater or lesser sensitivity than usual were either discarded or the criteria for positivity adjusted accordingly.

In other experiments, the relative sensitivity of tests performed with reagents derived from guinea pig hyperimmune HB Ab (GP-micro-SPRIA) and human secondary-exposure HB Ab (H-micro-SPRIA) was studied by using serial twofold dilutions of a standard HB Ag. Wells were coated with either guinea pig HB Ab or human HB Ab, and 125I-labeled guinea pig HB Ab or one of the two 125I-labeled human HB Ab globulins was used as the final reagent. Tests utilizing 125I-labeled guinea pig HB Ab were slightly more sensitive than those utilizing 125I-labeled human reagents (Table 2). Coating of wells with human HB Ab instead of guinea pig HB Ab also resulted in a reduction in the sensitivity of the micro-SPRIA (Table 2). For this reason, in subsequent experiments with the H-micro-SPRIA a “hybrid” assay was used that utilized guinea pig HB Ab-coated wells plus 125I-labeled human HB Ab.

The rate of nonreproducible positive results obtained with the micro-SPRIA was approximately the same as that observed with the AusRIA system (approximately 1 to 2%). For this reason all positive results were confirmed by retest.

**Serum panels.** Bureau of Biologics Panel no. 2 was tested twice (each time in duplicate) by GP-micro-SPRIA, and the results were compared with the results of replicate tests by one of us (H. J. A.) with the AusRIA system. Comparable results were obtained with the two methods (Table 3). Results obtained with this panel by six reference laboratories, using several different radioimmunoassays, were almost identical with the results obtained with the micro-SPRIA. Although guinea pig HB Ab prepared against only the ad subtype of HB Ag was used in the micro-SPRIA, this method was as sensitive as the AusRIA method for both subtypes ad and ay.

The National Heart and Lung Institute panel was tested in duplicate with the micro-SPRIA, and the results were compared with the results obtained with the AusRIA system. Identical results were obtained for both ad and ay subtypes of HB Ag with the two techniques (Table 3).

### Table 2. Titer of an HB Ag standard when measured with human or guinea pig reagents in the micro-SPRIA

| Source of unlabelled coating antibody | Source of 125I-labeled antibody* |
|--------------------------------------|---------------------------------|
| Guinea pig                            | Guinea no. 1                     | Guinea no. 2                     |
| Human no. 1                           | 131,072*                         | 65,536                           | 32,768                           |
| Human no. 2                           | NT*                             | 16,384                           | NT                               |

* Complement fixation titer of whole serum: guinea pig = 1:256; human no. 1 = 1:256; human no. 2 = 1:64.

* Reciprocal.

* Not tested.
Serial serum samples from rhesus monkeys used in transmission studies of hepatitis virus were tested for HB Ag with the AusRIA system. Fifteen of 290 sera were found positive for HB Ag; antigen could not be detected in these sera by counterimmunoelectrophoresis. When retested by micro-SPRIA, 13 of the 15 positive sera were detected, and all of the AusRIA-negative sera were micro-SPRIA negative.

Comparative specificity of AusRIA and micro-SPRIA. The panel of acute-phase sera from hepatitis patients and the panel of false positive sera from blood donors were tested by GP-micro-SPRIA and H-micro-SPRIA. In addition, they were subjected to specificity-testing in the modified GP-micro-SPRIA as described in Materials and Methods. Results of H-micro-SPRIA tests were not tested for specificity.

The GP-micro-SPRIA detected HB Ag in all of the acute-phase sera from hepatitis patients but, as with the AusRIA test, also gave false positive results with the panel of donor sera (Table 4). However, these could be correctly identified as false positive by appropriate specificity testing. There was no agreement between the AusRIA test and the GP-micro-SPRIA as to whether false positive results were due to the presence of antibodies to guinea pig protein or to other unidentified causes: three of the 20 false positive sera appeared to be in the former group by AusRIA, but two different sera were inhibited by guinea pig serum in the GP-micro-SPRIA. In contrast, the H-micro-SPRIA successfully identified HB Ag-positive sera but did not react with the false positive sera. Thus, the specificity of the GP-micro-SPRIA was comparable to that of the AusRIA test and, as with AusRIA, required confirmation of results by appropriate blocking experiments; the H-micro-SPRIA was more specific than either of the tests using guinea pig reagents, and was almost as sensitive.

Modification of micro-SPRIA for detection of HB Ab. A solid-phase radioimmunoassay for the detection of HB Ab has been reported (10). In an attempt to see whether such an assay could be adapted to the microtiter system, we coated microtiter wells with human serum containing a high titer of HB Ag. HB Ag-positive serum was removed from the wells, the wells were thoroughly washed with saline, and samples to be tested for HB Ab were added and incubated overnight. After a thorough washing of the wells, HB Ag, purified and radiolabeled as described previously (8), was added to the wells and allowed to incubate overnight. After washing, the wells were cut apart and the residual radioactivity was measured in the gamma counter. HB Ab was detected in human sera by this method, but the technique was not as sensitive as passive hemagglutination or radioimmunoprecipitation, in part because optimal conditions for the micro-SPRIA for HB Ab were not sought. However, this preliminary study demonstrated the feasibility of adapting the SPRIA for the detection of HB Ab in microtiter plates.

TABLE 3. Comparison of HB Ag test results obtained with three serum panels by AusRIA and micro-SPRIA methods

| Panel*          | No. of sera in indicated category | No. of sera positive by indicated method |
|-----------------|----------------------------------|----------------------------------------|
|                 |                                   | AusRIA | Micro-SPRIA |
| Bureau of Biologics no. 2 | Positive* 40 | 34 (1)* | 32 (2) |
|                 | Negative 20 | 0 (1) | 0 (1) |
| National Heart and Lung Institute | Positive* 20 | 8 (1) | 8 (1) |
|                 | Negative 6 | 0 | 0 |
| Rhesus monkey sera | Positive* 15 | 15 | 13 |
|                 | Negative 275 | 0 | 0 |

* See Materials and Methods for description.
* Includes samples of HB Ag-positive serum diluted beyond the level of detectability of presently available techniques.
* As determined by AusRIA method.
* Number in parentheses denotes additional sera that yielded equivocal results.

DISCUSSION

Principal advantages of the micro-SPRIA tests described herein are the following: (i) ability to test sera for HB Ag with a sensitivity comparable to the AusRIA technique but with only one-fourth of the sample volume; (ii) ability to simultaneously test large numbers of sera with somewhat greater ease than with the AusRIA system; (iii) economy; (iv) ability to prepare custom reagents for specific purposes, i.e., the use of human HB Ab reagents for testing sera yielding false positive results in the AusRIA test.

Recent studies (12, 15; H. J. Alter et al., Blood J. Hematol., in press) have shown that many sera positive by AusRIA but negative by counterimmunoelectrophoresis are in fact false positive reactions caused by nonspecific binding of reagents or by the presence of antibodies in the test serum that are reactive with the radiolabeled...
guinea pig globulins. A high proportion of these false positive reactions has been found among blood donors, who have a relatively low frequency of HB Ag, than among hepatitis patients, who are more likely to be positive for HB Ag.

Tests of specificity have been developed to distinguish between true and false positive reactions. These consist of attempts to inhibit the positive AusRIA reaction by incubation of the test sample with appropriate reagents. When these specificity tests were adapted to the GP-micro-SPRIA, this test was found to yield false positive results with sera also giving false positive results with AusRIA. The cause of the false positivity could not be determined for most of the sera, but a few appeared to contain antibodies to guinea pig globulin. Nonspecific reactions were not detected with these sera when they were tested with the H-micro-SPRIA system; in addition, this test was almost as sensitive as tests using guinea pig-derived reagents for detecting HB Ag in the AusRIA-positive (CEP-negative) sera from hepatitis patients. Thus, the H-micro-SPRIA appeared to be useful for excluding false positive reactions regardless of their cause.

Another advantage of the technique described in this paper is the ability to use antibodies that are not immunochemically purified for reagents (the separation and purification of HB Ab from immune complexes of HB Ag and HB Ab has proven difficult for many laboratories). Furthermore, solid-phase radiolmmunoassays that utilize radiolabeled con-

valescent serum for reagents have, as their greatest potential, the fact that they do not require highly purified antigen or antibody. They therefore appear to provide a very sensitive means of detecting the presence of an antigen-antibody reaction when neither the antigen nor the antibody is available in purified form. In this respect they resemble the complement fixation test in its usefulness for a broad range of antigen-antibody reactions but markedly exceed it in sensitivity. Thus, in the present study, human secondary exposure antiserum was capable of demonstrating an antigen-antibody reaction between HB Ag and HB Ab with a sensitivity greater than that of complement fixation or counter electrophoresis. It is obvious that such techniques offer promise for developing assays for other antigen-antibody systems, and Rosenthal et al. recently developed several modifications of the micro-SPRIA for the detection of viruses and their antibodies (14). However, appropriate precautions must be taken to assure the specificity of such tests. We have applied the techniques described herein to the study of the internal component of "Dane" particles of hepatitis B antigen (2), an antigen for which simple assays are not yet available. Preliminary evidence indicates that the internal components of Dane particles can indeed be detected by micro-SPRIA and that this antigen-antibody reaction is distinguishable from that observed with HB Ag and HB Ab (R. H. Purcell, J. L. Gerin, J. D. Almeida and P. V. Holland, in preparation). Attempts to modify the micro-

SPRIA for the detection of a hypothetical hep-
titis A antigen are in progress.

ACKNOWLEDGMENTS
We wish to acknowledge the excellent technical assistance of H. Smith. The Bureau of Biologics panel no. 2 and the National Heart and Lung Institute serum panel were supplied through the kindness, respectively, of L. Barker and J. Roche.

LITERATURE CITED
1. Aach, R. D., J. W. Grisham, and C. W. Parker. 1971. Detection of Australia antigen by radioimmunoassay. Proc. Nat. Acad. Sci. U.S.A. 68:1056-1060.
2. Almeida, J. D., D. Rubenstein, and E. J. Stott. 1971. New antigen-antibody system in Australia-antigen positive hepatitis. Lancet 2:1225-1227.
3. Catt, K., and G. W. Tregear. 1967. Solid-phase radioimmunoassay in antibody-coated tubes. Science 158:1570-1571.
4. Coller, J. A., I. Millman, T. C. Halbherr, and B. S. Blumberg. 1971. Radioimmunoprecipitation assay for Australia antigen, antibody and antigen-antibody complexes. Proc. Soc. Exp. Biol. Med. 138:249-257.
5. Gerin, J. L., R. H. Purcell, M. D. Hoggan, P. V. Holland, and R. M. Chanock. 1969. Biophysical properties of Australia antigen. J. Virol. 4:763-768.
6. Hollinger, F. B., V. Vorndam, and G. R. Dreesman. 1971. Assay of Australia antigen and antibody employing double-antibody and solid-phase radioimmunoassay techniques and comparison with the passive hemagglutination methods. J. Immunol. 107:1099-1111.
7. Hunter, W. M., and F. C. Greenwood. 1962. Preparation of iodine-131 labelled human growth hormone of high specific activity. Nature (London) 194:495-496.
8. Lander, J. J., H. J. Alter, and R. H. Purcell. 1971. Frequency of antibody to hepatitis-associated antigen as measured by a new radioimmunoassay technique. J. Immunol. 106:1166-1171.
9. LeBouvier, G. L. 1971. The heterogeneity of Australia antigen. J. Infect. Dis. 123:671-675.
10. Ling, C. M., and L. R. Overby. 1972. Prevalence of hepatitis B virus antigen as revealed by direct radioimmunoassay with 125I-antibody. J. Immunol. 109:834-841.
11. London, W. T., H. J. Alter, J. J. Lander, and R. H. Purcell. 1972. Serial transmission in rhesus monkeys of an agent related to hepatitis-associated antigen. J. Infect. Dis. 125:382-389.
12. Prince, A. M., B. Brotman, D. Jass, and H. Ikram. 1973. Specificity of the direct solid-phase radioimmunoassay for detection of hepatitis-B antigen. Lancet 1:1346-1350.
13. Purcell, R. H., J. L. Gerin, P. V. Holland, W. L. Cline, and R. M. Chanock. 1970. Preparation and characterization of complement-fixing hepatitis-associated antigen and antisera. J. Infect. Dis. 121:222-226.
14. Roseenthal, J. D., K. Hayashi, and A. L. Notkins. 1973. Comparison of direct and indirect solid-phase microradioimmunoassays for the detection of viral antigens and antiviral antibody. Appl. Microbiol. 25:567-573.
15. Sgouris, J. T. 1973. Limitations of the radioimmunoassay for hepatitis B antigen. N. Engl. J. Med. 288:160-161.
16. Tilden, R. L., and F. H. DeLand. 1972. Electrophoretic radioimmunoassay: application to hepatitis-associated antigen. J. Nucl. Med. 13:599-603.
17. Walsh, J. H., R. Yalow, and S. A. Benson. 1970. Detection of Australia antigen and antibody by means of radioimmunoassay techniques. J. Infect. Dis. 121:550-554.