The Radioimmune Assay Inhibition test for the Detection of Antibody to Hepatitis B Antigen

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OBJECTIVE: To develop methods of detecting, quantifying and identifying the specificity of antibody to hepatitis B antigen (HBsAg).

BACKGROUND: The detection of antibody to hepatitis B surface component (anti—HBs) has been a problem in Thailand as well as elsewhere because of inability of standard serological tests, e.g., complement fixation (CF), to detect small amounts of antibody. The application of the immunoelectroosmophoresis (IEOP) test allowed for the detection of antibody in approximately 10% of urban Thais and was used to determine the development of antibody in recipients of blood containing HBsAg; however, the patterns of antibody acquisition revealed by this detection method pointed to secondary rather than primary antibody responses. Individuals suffering from acute hepatitis B were not found to develop anti—HBs detectable by IEOP following infection. The development of a passive hemagglutination test (PHA) allowed a check on the validity of the IEOP antibody test.

Table 1 demonstrates the increased sensitivity of PHA over IEOP. The lesser sensitivity of the IEOP method and the difficulty of manufacturing and transporting sensitized red blood cells for the PHA method led us to investigate other sensitive methods of antibody determination.

DESCRIPTION: Utilizing a radioimmune assay (RIA) developed and sold by Abbott Laboratories (Ausria kit), an additional step was added to allow for the identification of anti—HBs. This procedure was called

Table 1. Comparison of IEOP and PHA for Detecting Anti—HBs in Sera Collected in Huay Kwang 1972.

PHA Reciprocal Titer	Sera Tested	IEOP No.	Positive %
128	495	0	0
256	32	1	3.1
512	46	3	6.5
1024	30	9	30.0
2048	40	30	75.0

* PHA tests were performed by the American National Red Cross Blood Research Laboratory, Bethesda, Md.

radioimmune assay inhibition (RIAI). For each antigen subtype to be used a serum was selected and a RIA extinction curve was performed using normal human serum as a diluent. A dilution of serum containing HBsAg was selected for use in the RIAI. Two–tenths (0.2) ml of the appropriate antigen dilution was incubated for 2 hours at 37°C with 0.2 ml of the serum sample to be tested for antibody activity. One – tenth ml of the mixture was then placed in the bottom of each of two plastic tubes coated with guinea pig anti–HBs. The tubes were stoppered and allowed to incubate at room temaperature for 16–18 hours overnight. The samples were then removed and the tube rinsed 5 times with 1 ml each of 0.01M Tris–HCL, pH 7.4. One–tenth ml of the ^{125}I–labeled guinea pig anti–HBs solution (approximately 0.1 μCi) was then delivered to the bottom of each tube. The tube was capped and allowed to incubate at room temperature for 90 minutes, then aspirated and rinsed 5 times as above.

Seven negative control tubes were prepared using a standard HBsAg negative control provided by Abbott Laboratories. Seven positive control tubes were run for the dilution of HBsAg positive serum used in the RIAI. Each tube was counted in a gamma ray spectrophotometer and the mean counts per minute (CPM) and standard deviation of the positive and negative control tubes were calculated. The percent inhibition of the RIA (% RIAI) of sample sera was computed using the formula:

$$\frac{(MPD - MNC) - ([PD + Sample] - MNC)}{MPD - MNC} \times 100 = \% \text{ RIAI}$$

Where M indicates the mean counts per minute, NC indicates the CPM for the negative control and PD indicates the CPM for the dilution of HBsAg positive serum used in the RIAI.

PROGRESS: Rodioimmune assays were run on dilutions of sera containing the three major subtypes of HBsAg; adr, adw and ayw. Figure 1 illustrates dilution extinction curves on three such sera. Dilutions containing approximately 50% of the CPM of the highest counting dilution were used as the antigen for the RIAI, as small changes in antigen concentration should lead to large changes in the amount of ^{125}I–labeled antibody complexed to the antigen. This should be reflected in large changes in the number of counts per minute.

Initial testing of the RIAI method was performed using dilutions of sera known to contain high titers of anti–HBs. These sera were obtained from multiply transfused individuals with complement fixation titers for anti–HBs. Figure 2 shows the percent inhibition of the radioimmune assay of one such serum with a CF titer of 1:8. RIAI was run on 57 sera obtained from 11 Thai individuals recovering from HBsAg positive hepatitis using an adr antigen, the predominant subtype in Thailand. Of these 11 individuals, antibody capable of inhibiting the radioimmune assay by greater than 50% developed in 10. Thus the RIAI was capable of detecting antibody following acute hepatitis B infection.

A panel of sera, pretested by passive hemagglutination by the American National Red Cross Blood Research Laboratory (Bethesda, Maryland, USA) were selected for analysis by RIAI. Since the PHA was run using adw antigen coated cells, adw antigen was also used in the experiment pictured (See Figure 3). A scatter diagram of the PHA titers versus the RIAI demonstrates a clear correlation between these two techniques. All of the sera with titers of antibody by PHA of 1:64 or more inhibited the RIAI by greater than 50%. Those with titers of less than 1:64 by PHA showed considerable scatter, with the majority of sera inhibiting the RIAI by less than 50%. Sera considered to have no antibody by PHA also showed considerable scatter, with RIAI ranging from 80% to 0%. The majority (9/12) of these clustered below 30% RIAI but two were greater than 50%. Repetition of this test using the same antigen and panel of sera showed some lack of reproducibility with those sera having PHA antibody titers less than or equal to 1:64. Similar experiments using other sera tested by PHA also showed variability of RIAI inhibition if the PHA titer was less than 1:64.

With sera showing a high % RIAI it was sometimes possible to demonstrate a difference in the antibody titer to different subtypes. Figure 4 shows the RIAI results of two such sera, run simultaneously with each

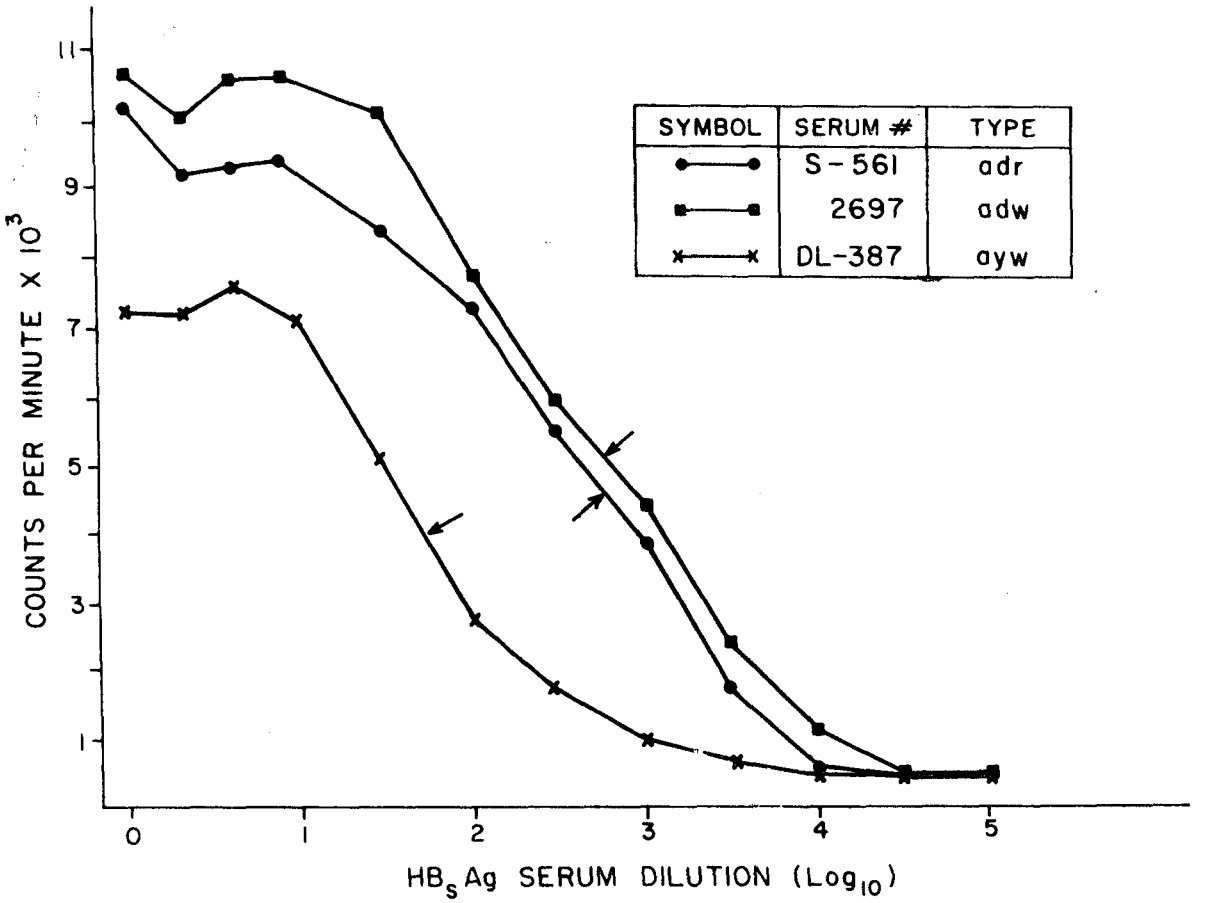

Figure I. Dilution extinction curves of three sera containing HB_sAg of different subtypes tested by radioimmune assay. Each point represents the mean value of duplicate determinations. Arrows indicate the antigen dilutions used in RIAI tested for detecting HB_sAg; adr (1:512), adw (1:512) and ayw (1:64).

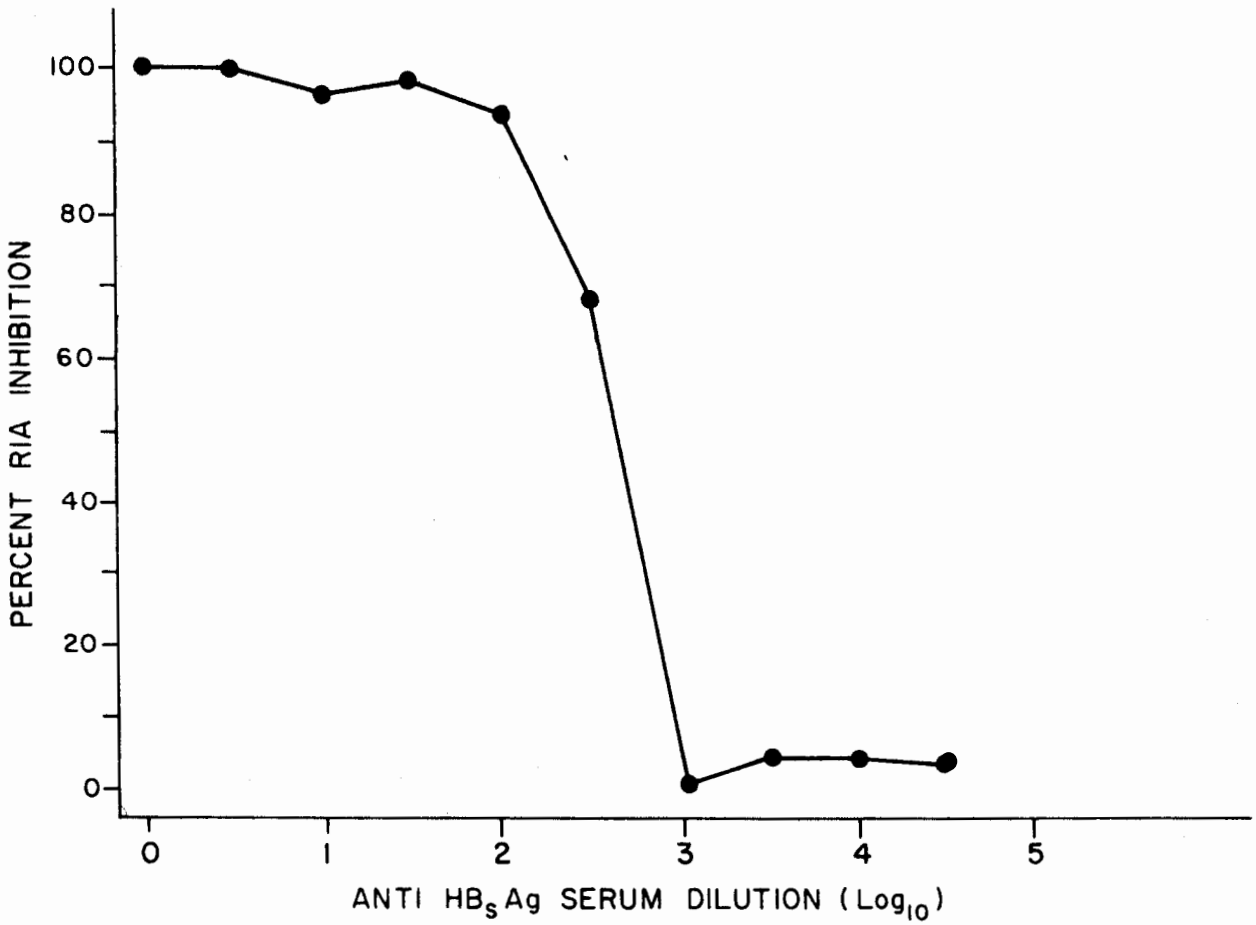

Figure 2. Radioimmune assay inhibition of a constant amount of HB_sAg/adr (S-361) by serial dilutions of a human serum containing anti-HB_s. The serum (P. T.) had antibody titers of 1:8 by CF and 1:300 ($10^{-2.5}$) by RIAI.

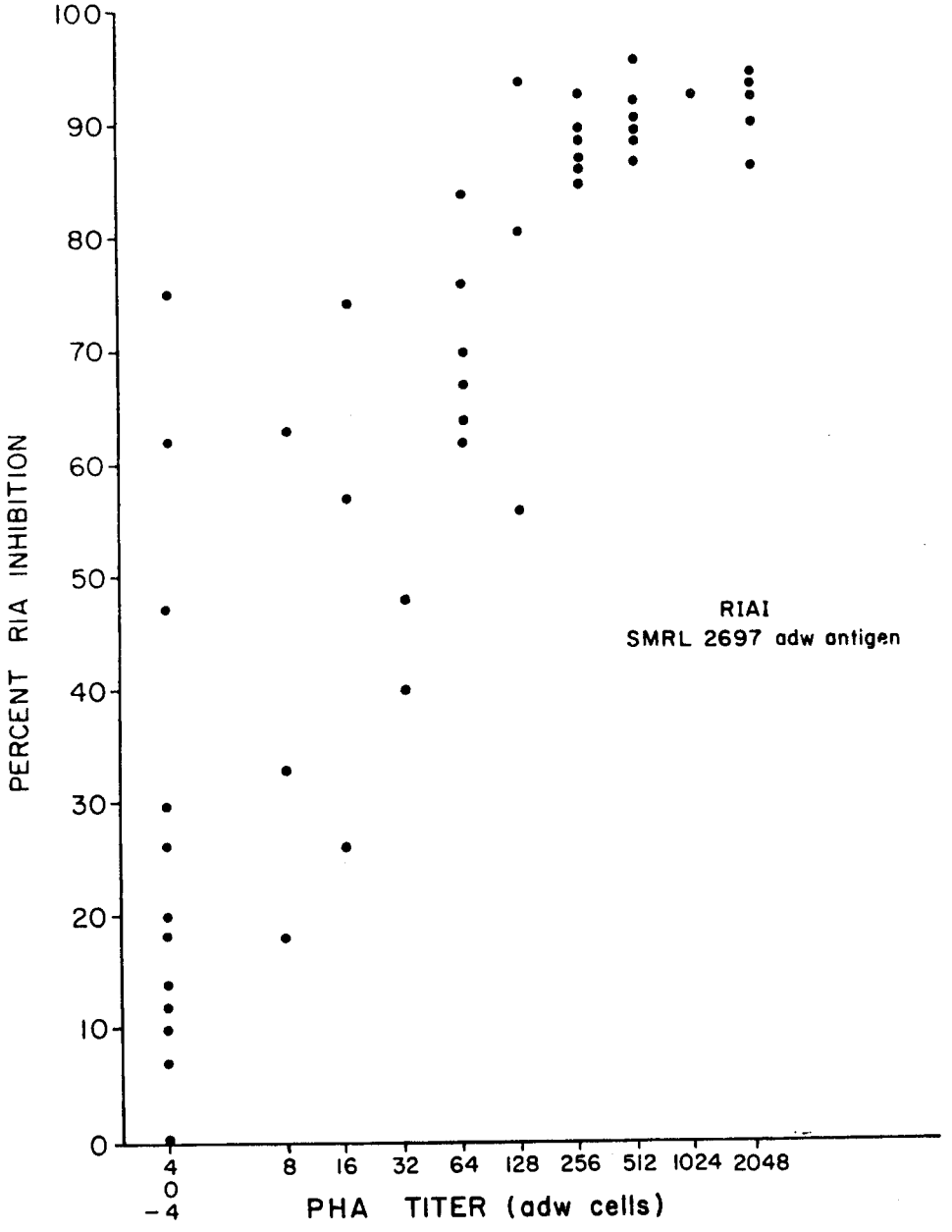

FIGURE 3. PASSIVE HEMAGGLUTINATION TITERS PLOTTED AGAINST PERCENT RADIOIMMUNE ASSAY INHIBITION.

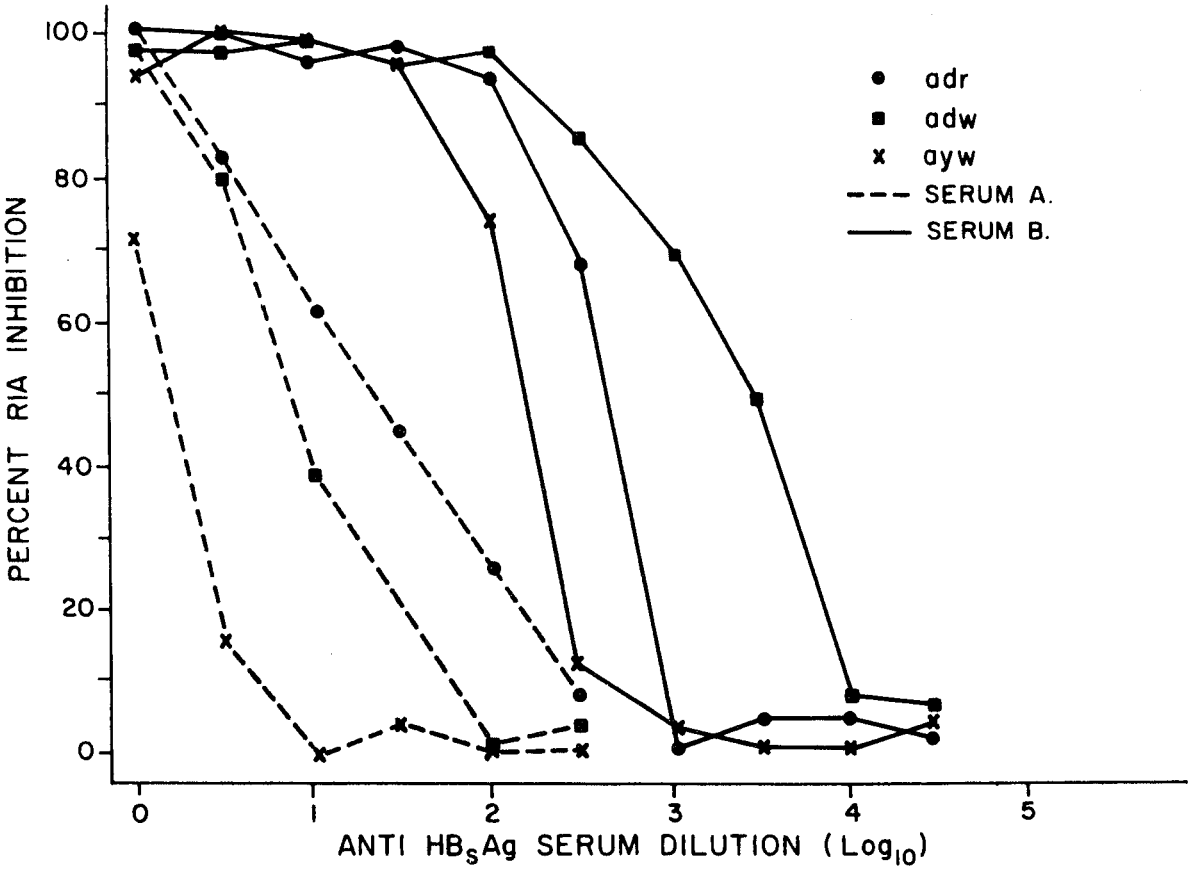

Figure 4. Radioimmune assay inhibition dilution curves on two sera showing differences in titer to three different subtypes of HB_sAg (adr, adw, ayw). Serum A was obtained from a patient approximately 18 months after a documented clinical infection with HB_sAg/adr. Serum B was obtained from a blood recipient following transfusion with at least one unit of HB_sAg/adw positive blood.

of the three antigens. Serum (A) was obtained from an individual from whom HBsAg/adr was isolated during an episode of hepatitis approximately one year prior to the date the serum was obtained. Serum (B) was obtained from a hospitalized patient who received multiple transfusions. At least one transfusion prior to the time blood was obtained was shown to contain HBsAg/adw. Serum (A) inhibited 50% of the adr antigen activity as indicated by the CPM at a dilution three times that of adw. Serum (B) appeared to contain a higher titer of antibody against adw than to adr, showing a ten-fold difference in titer at the 50% level.

DISCUSSION: In our hands RIAI appears to be sensitive, specific and reproducible in cases where large amounts of anti-HBs are present. In cases of multiply transfused individuals with CF titers or in cases with recent hepatitis B, RIAI is capable of detecting antibody. Furthermore, RIAI is from 40-50 times more sensitive than IEOP.

Unfortunately, RIAI was unable to demonstrate antibody in some sera that titered less than or equal to 1 : 64 by PHA. The RIAI, however, was often repeatable, suggesting the possibility of false negatives and positives by the PHA method.

RIAI, when run using different antigen positive sera, appears to be capable of identifying the subtype of the antigen with which an individual was infected.

Methods of improving the RIAI sensitivity are being tried. Other procedures for the detection of anti-HBs are being sought.