

Radioimmune Assay Inhibition Tests for the Detection
of Antibody to Hepatitis B Surface Antigen

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OBJECTIVE: To compare the radioimmune assay inhibition (RIAI) test for antibody to hepatitis B surface antigen (anti-HB_s) using two radioimmune assay (RIA) techniques.

BACKGROUND: The RIAI for anti-HB_s was developed in this laboratory using the Ausria I RIA kit manufactured and sold by Abbott Laboratories (SEATO Medical Research Laboratory Annual Report 1973-1974). To the procedure for the Ausria I, an initial absorption step was added. The sera, whose antibody content was to be determined, was used to absorb a standard amount of antigen. A change in the technical aspect of this test was brought about by the discontinuation of production of the Ausria I kit and its replacement by the manufacturer with a newly developed Ausria II kit. This change in material necessitated a series of comparative tests to insure that the results of the RIAI based upon the Ausria II (RIAI_{II}) were comparable to those based upon the Ausria I (RIAI_I).

DESCRIPTION: In February 1975 the Ausria I kit on which the RIAI_I was based was withdrawn from the market. A final order of Ausria I kits and an equal number of the replacement Ausria II kits were provided by the manufacturer for cross testing and standardization. These kits were used to compare both the RIA and the RIAI tests in our laboratory.

The major technical difference between the Ausria I and the Ausria II was a change in the antibody carrier from a polystyrene tube to a polystyrene bead. The technique for the RIAI_I was presented in last year's annual report. The initial absorption technique for the Ausria II is briefly reported: the RIAI was performed using a standard dilution of serum containing a known amount of antigen. Exactly 0.1 ml of this antigen dilution was incubated with 0.1 ml of each serum to be tested. After 1½ hours incubation a polystyrene bead was introduced into the well and submerged in the mixture. From this point the test was performed using the directions for the Ausria II kit provided by the manufacturer and is essentially the same as with the Ausria I.

Included in each test run were seven negative controls, testing a pool of human serum shown to have neither HB_sAg or anti-HB_s activity. The standard antigen dilution which was used as a maximum for the RIAI_I was also tested in seven replicates. The number of counts per minute (CPM) in each test was determined using a gamma ray spectrometer.

The percent radioimmune assay inhibition (% RIAI) was calculated using the following formula:

$$\frac{D - X}{D} \times 100 = \% \text{ RIAI}$$

D is the mean of the CPM of the standard antigen dilution from which the mean of the negative controls had been subtracted and X is the CMP of the serum-antigen mixture following a similar manipulation.

Data from the RIAI_I and RIAI_{II} were compared on the basis of the percent RIA inhibition. Also, a positive or negative score was assigned to each test using 50% inhibition as a cut-off point for differentiating positives from negatives.

PROGRESS: In order to establish the appropriate dilution of antigen to use in the RIAI tests, antigen extinction curves using a sera containing HB_sAg/adw were run using both RIA tests. The results were found to be almost identical (Figure 1). Dilutions of 1:400 and 1:800 were selected as candidates for the standardized antigen for use in the RIAI tests. These dilutions were selected because they showed 50% or less of the CPM of the highest counting antigen dilution and were located on the steepest part of the antigen dilution curve. In this part of the curve, small changes in the concentration of antigen in the substance tested should lead to large changes in the amount of ¹²⁵I-labelled antibody complexed to the antigen.

Dilutions of serum known to contain a high titer of anti-HB_s (Serum PT) were tested by both tests using dilutions of HB_sAg/adw of 1:400 and 1:800. Figure 2 illustrates the percent RIAI of the dilutions of antibody using a 1:800 dilution of the HB_sAg/adw antigen. Use of the 1:400 dilution of the HB_sAg/adw antigen produced similar curves with both tests but the RIA was inhibited by lower antibody dilutions and therefore was less sensitive. For this reason a 1:800 dilution of HB_sAg/adw was chosen as the standard antigen dilution for the RIAI with both the Ausria I and the Ausria II kits.

A panel of 100 sera from a Thai population known to have a high prevalence of anti-HB_s was tested by RIAI using both techniques and the standard 1:800 dilution of HB_sAg/adw. Figure 3 illustrates the relationship between the percent RIAI on these 100 sera using the RIAI, versus the RIAI₁₁. The correlation coefficient (r) of results obtained from these two techniques was 0.96. This very high correlation indicates that the two tests are measuring the same variable. The proportion of common variance (r²) was 0.93, indicating that 93% of the variation in one test is accounted for by the variation of the other test.

When the scores assigned to each test on the basis of the 50% cut-off point were examined, 45 of the 100 sera were positive by the RIAI₁₁ and 38 of the 100 were positive by the RIAI (Table 1). The correlation coefficient (r_{phj}) of the tests scored in this way, was only slightly lower than that derived from the numerical data, again indicating the similarity of the two tests (Table 2).

The 100 sera were also tested by the passive hemagglutination test (PHA, Electronucleonics Inc.) and the immunoelectroosmophoresis test (IEOP) (Table 1). The correlation coefficients (r_{phj}) were also calculated between these tests and both of the RIAI tests (Table 2). The low correlation coefficients between the IEOP and other tests is indicative of the lack of sensitivity of the former as has been shown previously. The PHA identified anti-HB_s in 34 of 100 sera tested. All of these 34 sera were also positive by RIAI₁₁.

Table 1. A Comparison of Four Tests for Anti-HB_s
Results of 100 Sera

Test	Pattern of Positive Results					Sera positive for each test
RIAI						
AUSRIA II	X	X	X	X	X	45
AUSRIA I	X	X	X			38
PHA	X	X		X		34
IEOP	X					11
Total sera positive by tests indicated	11	20	7	3	4	

Table 2. Correlation Coefficient r_{phi}

AUSRIA II	vs	AUSRIA I	0.87
AUSRIA II	vs	PHA	0.79
AUSRIA I	vs	PHA	0.78
PHA	vs	IEOP	0.49
AUSRIA I	vs	IEOP	0.46
AUSRIA II	vs	IEOP	0.41

$$\text{Where } r_{phi} = \frac{BC - AD}{\sqrt{(A + B)(C + D)(A + C)(B + D)}}$$

In order to increase the confidence in the RIAI₁₁, an additional 100 sera, taken from the same population, were added to the original panel (Table 3). In this experiment the RIAI₁₁ identified anti-HB_s in 83 (41%) of the 200 sera as compared to 69 (35%) identified by the PHA. The differences seen here were due to 15 sera that were positive only by RIAI₁₁, and one serum which was positive only by PHA. The correlation coefficient (r_{phi}) was larger than that found with the original panel of 100 sera indicating an increased between-test reliability.

Table 3. A Comparison of Two Tests for Anti-HB_s
Results of 200 Sera

Test	Pattern of Positive Results			Sera positive for each test
RIA I				
AUSRIA II	X	X		83
PHA	X		X	69
Total sera positive by tests indicated	68	15	1	

DISCUSSION AND SUMMARY: The RIA_{II} has been used by this laboratory for the past year to identify anti-HB_s in various populations. This test was shown to be slightly less sensitive than the PHA. The introduction of the Ausria II test with the withdrawal of the Ausria I test from the market required the series of comparative tests reported here. The RIA_{II} proved to have an increased sensitivity over the RIA_I. Furthermore, using this test instead of the RIA_I, there appeared to be fewer sera in which anti-HB_s was identified by PHA and not by RIA. Proving increased sensitivity of the RIA_{II} remains a problem, as many of these positives cannot be confirmed by PHA. Nonetheless, the RIA_{II} has now replaced the RIA_I in this laboratory for the initial identification of sera containing anti-HB_s.

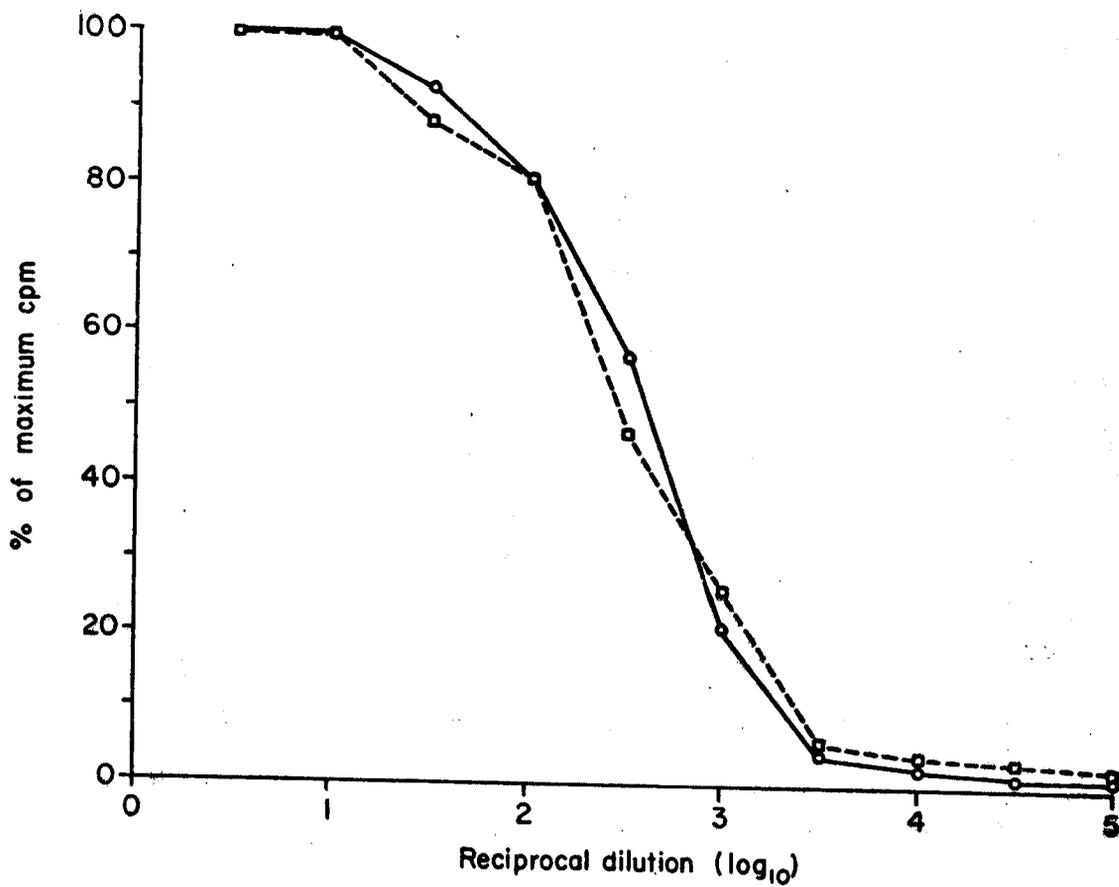


Figure 1. Antigen dilution extinction curves of sera containing HB_sAg/adw (CF titer 1:256) tested by radioimmune assays using both the Ausria I and the Ausria II tests provided by Abbott Laboratories.

□-----□ Ausria I, ○——○ Ausria II

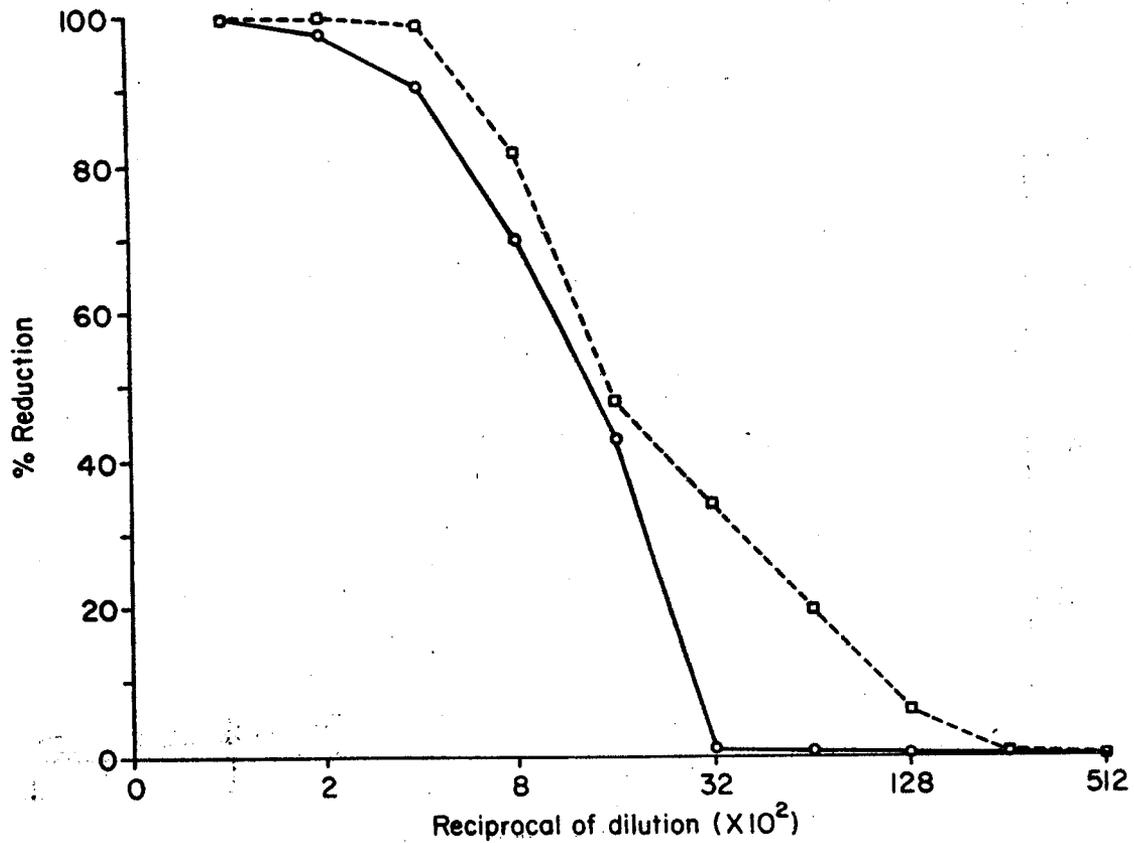


Figure 2. Radioimmune assay inhibition curves on dilutions of a serum containing a high titer of anti-HB_s (PT, CF titer = 1:64). The Ausria I and Ausria II kits were used with a standard antigen dilution of HB_sAg/adw of 1:800.

□-----□ Ausria I, ○-----○ Ausria II

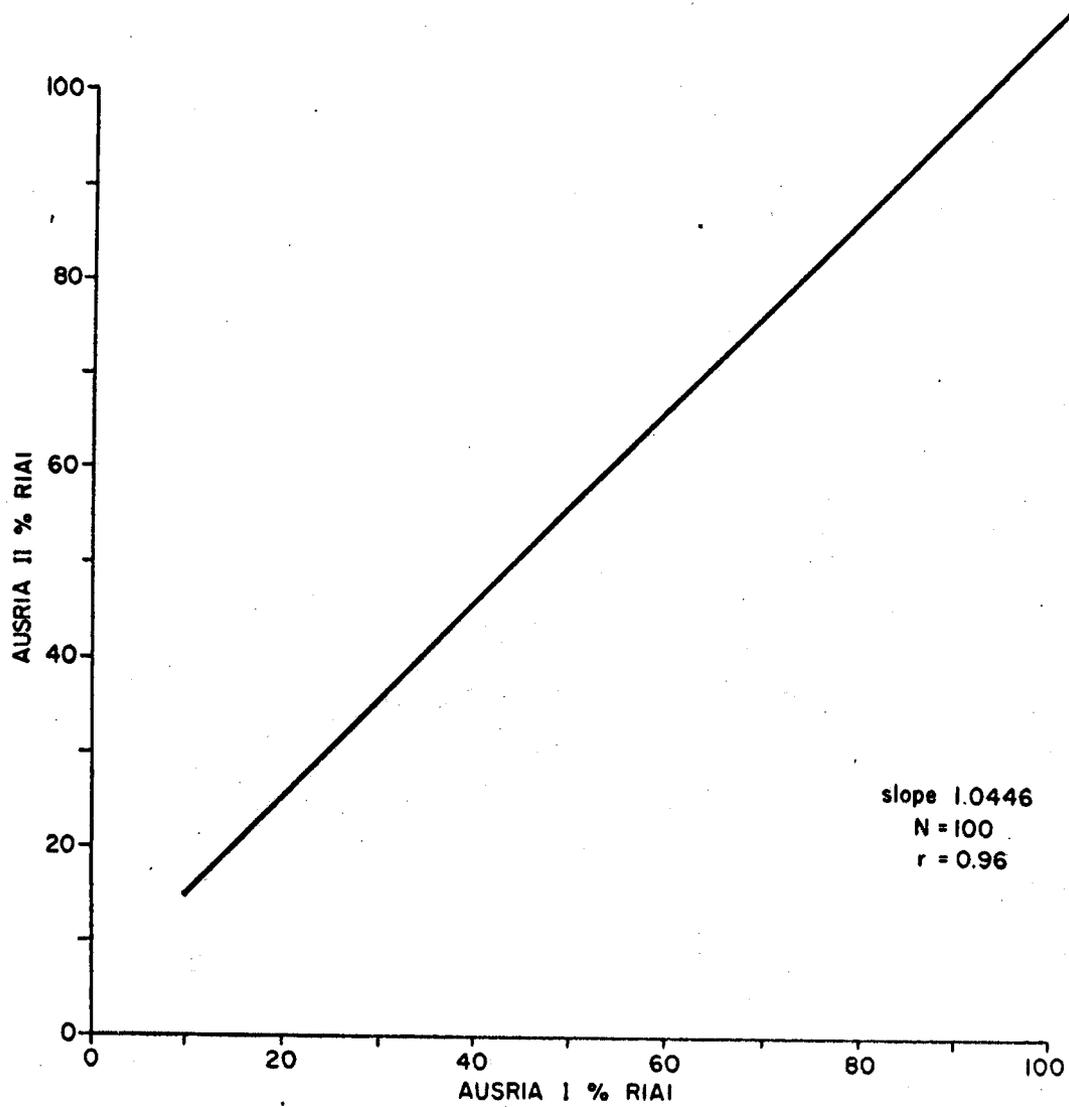


Figure 3. Relationship between the percent radioimmune assay inhibition as shown by the RIAI_I and the RIAI_{II}