A Comparison of Complement Fixation, Immunoelectroosmophoresis and Solid Phase Radioimmunoassay for the Detection of Hepatitis B Antigen in Thai and American Populations

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OBJECTIVE: To develop the radioimmunoassay (RIA) for detection of Hepatitis B Antigen (HB—Ag) and to compare the sensitivity of this assay with that of other tests for antigen used previously in this laboratory.

BACKGROUND: In previous years HB—Ag has been detected in sera submitted to this laboratory by three methods: Agar gel diffusion (AGD), Immunoelectroosmophoresis (IEOP) and Complement Fixation (CF). Over the past several years the prevalence of Hepatitis B Antigen has been determined by these methods in several different Thai and American populations. These populations include groups of United States military personnel entering the Republic of Vietnam, US military personnel suffering from acute hepatitis in the RVN, Thais living in a low income urban subdivision of Bangkok, paid Thai blood donors at Phra Mongkut Klao Hospital and Thai children with acute hepatitis seen at the outpatient clinic at Bangkok Children's Hospital. Because of the increased sensitivity attributed to the solid phase radioimmunoassay, the sera collected from these populations were submitted for HB—Ag testing by this method.

METHOD: The sera tested were taken from the six groups described above. The method for AGD, CF and IEOP have been presented before (1971—1972 Annual Report, SEATO Medical Research Laboratory). Reagents for the solid phase radioimmunoassay were obtained from Abbott Laboratories, Chicago, Ill. as Ausria Kits. The tests were performed according to the instructions provided. Briefly 1 ml of a sample to be tested was placed on the bottom of a plastic tube coated with guinea pig HB—Ag antibody. The tube was stoppered and allowed to incubate at room temperature for 16—18 hrs (overnight). The sample was then removed and the tube rinsed 5 times with 2 ml each of 0.01 M Tris—HCl, pH 7.1. One tenth milliliter of 125 I—labeled HB—Ag antibody solution (0.1 μCi) was then delivered to the bottom of the tube. After the tube was capped and allowed to incubate at room temperature for 90 minutes, it was aspirated and rinsed five times as above. Seven negative control tubes were prepared in the same manner with a standard normal human plasma provided by Abbott Laboratories. Each of the tubes was counted in a gamma ray spectrometer and the mean and standard deviation of the serum negative control tubes were computed. The coefficient of variance of these negative control tubes was between 10 and 20%. Samples were considered positive if they were greater than 5 standard deviations above the mean of the negative control value. Samples equal to or less than five standard deviations above the mean were considered negative.

PROGRESS: Tables 1 and 2 illustrate the increased sensitivity of the RIA over the CF and IEOP. Of a total of 1638 samples tested 145 or 8.8% were positive by CF, 197 or 12.0% were positive by IEOP and

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233 or 14.2% were positive by RIA. The increased sensitivity of the RIA over the CF in the total population was 88/233 or 38% while the increased sensitivity of the RIA over the IEOP was 40/233 or 17%.

That the RIA is capable of detecting HB—Ag later in the course of antigen positive hepatitis than other methods was demonstrated in a longitudinal study of 5 Thai children with HB—antigen positive disease. In one child followed for one year, the CF, IEOP & RIA remained positive, and this child was considered a carrier. In two cases followed for periods of time up to 8 months, the RIA remained positive beyond the IEOP or the CF for 1—3 months. Follow—up sera collected on both American and adult Thai hepatitis patients are at present under investigation.

A breakdown of the total population tested by RIA into component groups is shown in Table 3. It will be noticed that the RIA increases the detection of antigen in all of the groups tested; however, when used to detect HB—Ag on samples obtained from American personnel the RIA detected 3 to 4 times as many additional positive samples as when it was used to study Thai populations. Further analysis of histograms formed by plotting the counts/minute or standard deviations above the control mean on the abscissa and the number of persons studied on the ordinate, showed different curves for American and Thai HB—Ag positive samples. The Thai populations showed a unimodal curve with its mode greater than 100 standard deviations from the mean. The American military hepatitis patients showed a bimodal curve with peaks at about 50 and 100 standard deviatons from the standard control mean.

DISCUSSION: The increased sensitivity of the radioimmunoassay over previous methods of detecting Hepatitis B Antigen is amply demonstrated by these data. The RIA not only detects more hepatitis B positive samples in point prevalence studies but also allows for the detection of HB antigen for longer periods in the convalescence from HB antigen positive disease.

A number of explanations may be offered for the differences in concordance of RIA with IEOP between Thai and American subjects. It might be that the RIA in our hands is less sensitive than it is elsewhere. This seems unlikely since results of the RIA done in this laboratory compare favorably with determinations performed on aliquots of the same Thai samples by the laboratory of Dr. Roger Dodd, Red Cross Research Laboratory, Bethesda, Md.

It might be that Thais carry antigen at a higher titer than do carriers in temperate zones. The majority (45 of 62 or 73%) of the Thai subjects in the lower socioeconomic subsection who manifested antigenemia by the radioimmunoassay had complement fixation titers of 1:64 or greater as did all of the HB--Ag positive blood donors tested. In the American soldier population only 1 of 5 (20%) had an antigenemia of this titer; however, despite the high CF titer in carriers, there were also relatively small differences between the number of IEOP and RIA positive Thais with hepatitis despite a large number (81%) of low or negative titer CF tests.

A third and most likely explanation is that the higher concordance between IEOP and RIA tests in Thais as opposed to Western populations is related to the homogeneity of HB—Ag in Thais and the heterogeneity in U.S. personnel. All antigens thus far identified in this laboratory from the Thai population have been subtyped ad and the majority of them have been adr (see elsewhere in this report). Subtyping of the sera from American hepatitis B carriers and hepatitis patients showed that 50% (18 of 36) were ayw.

Both the CF and the IEOP are performed in the laboratory using Thai human HB—Ag antibody which has been shown to have anti—ad activity but no anti—y activity. Because of the specificity of antibody for the Thai antigen tested, a high sensitivity of the IEOP and the CF test is possible. On the other hand, the HB—Ag subtypes of American populations are heterogeneous, therefore the specificity of the Thai ad antibody is less and the sensitivity of the test is reduced.

The RIA utilizes a guinea pig antibody against HB—Ag. This antibody pool has been found to have a much greater anti—ad activity than anti—y activity and for this reason should produce higher counts/

minute with equal amounts of ad versus ay antigen. The bimodal curve that was seen when the counts of sera from American hepatitis patients were plotted may indicate that the test is reading two subtype populations while the plot of the data from Thai subjects suggest that only a single antigen population ad is being detected.

Table 1. CF & RIA Results for Total Population

	CF Test		
RIA Test	NEG	POS	TOTAL
NEG	1405	0	1405
POS	88	145	233
TOTAL	1493	145	1638

Table 2. IEOP & RIA Results for Total Population

	IEOP Test		
RIA Test	NEG	POS	TOTAL
NEG	1405	0	1405
POS	40	193	233
TOTAL	1445	193	1638

Table 3. A Comparison of CF, IEOP, & RIA Results in the Detection of HB–Ag, in Several Thai and American Populations

	Total	CF	IEOP	RIA	Increased detection** of RIA over IEOP
U.S. Military Entering S.E.A.	500	2 (0.4)*	3 (0.4)	5 (1.0)	40%
U.S. Military Hepatitis	174	62 (36)	68 (39)	94 (54)	28%
Thai Lower Class Population	687	47 (7)	55 (8)	62 (9)	10%
Thai Blood Donors	100	8 (8)	9 (9)	9 (9)	0%
Thai Adult Hepatitis	113	21 (20)	51 (45)	54 (48)	10%
Thai Children Hepatitis	64	5 (8)	8 (13)	9 (14)	4%

* Number positive (% of total positive)
** Number of IEOP false negative/total RIA positive x 100