

Radioimmune Assays for Antibody to Hepatitis B Surface Antigen

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OBJECTIVE : To compare three assays for antibody to Hepatitis B surface antigen (Anti-HB_s): A direct radioimmuno assay, a passive hemagglutination test and a radioimmuno assay inhibition test. To develop a systematic method for screening sera for hepatitis B surface antigen (HB_sAg) and anti-HB_s.

BACKGROUND : The radioimmune assay inhibition test (RIAI, based on the AUSRIA II, Abbott Laboratories) and the passive hemagglutination test (PHA, Electronucleonics, Inc.) for anti-HB_s were shown to have similar sensitivity for the detection of antibody to hepatitis B surface antigen. (SEATO Medical Research Laboratory Annual Report Report 1974-1975). These tests, as well as a counterimmunoelectrophoresis (CIEP) technique have been used routinely at the SEATO Medical Research Laboratory for the past three years. In 1976 a new direct radioimmune assay for anti-HB_s (AUSAB, Abbott Laboratories), became commercially available. This necessitated an evaluation of the new test and the development of a new system for screening serum.

DESCRIPTION : Comparative tests of the CIEP, RIAI, PHA, and AUSAB were carried out. The PHA and AUSAB were performed using the instructions of the manufacturer and the CIEP and RIAI followed the method described previously (SEATO Medical Research Laboratory Annual Report 1971-1972 and 1974-1975). Appropriate controls were included for each test. In cases where one test was not confirmed by another, the presence of anti-HB_s was substantiated by neutralization with HB_sAg.

PROGRESS : A panel of 100 sera from a population of Thai women collected in 1973 and known to have a high prevalence of anti-HB_s were tested by the four methods. Of the 100 sera tested, antibody was detected in 66 by AUSAB, 37 by RIAI and 34 by PHA. Twenty-six sera were detected by AUSAB only, almost twice as many as were detected by the RIAI (Table 1).

The marked increase in sensitivity without loss of specificity or reproducibility, shown by the AUSAB over previously performed antibody detection methods led to the abandonment of the RIAI and the PHA for routine antibody screening. Because of this a new protocol for HBV testing had to be designed.

The laboratory now submits all sera to a testing sequence designed to identify HB_sAg and anti-HB_s by the most efficient but inexpensive method possible (Figure 1). Sera is initially screened for HB_sAg and anti-HB_s by CIEP. The CIEP was shown in previous studies to identify over 80% of HB_sAg containing sera and up to 10% of anti-HB_s containing sera (SEATO Medical Research Laboratory Annual Report 1972-1973 and 1974-1975). Sera that are negative for HB_sAg and anti-HB_s by IEOP are tested by AUSAB. AUSAB positives are confirmed and titrated by PHA or, if negative by PHA, they are substantiated by neutralization with HB_sAg. A serum is considered neutralized if the counts per minute are less than or equal to 50% of the counts per minute of

non-neutralized serum. Those rare sera in which neutralization does occur are called false positives, and are considered negative. The sera which are negative by AUSAB are tested by RIA (AUSRIA II) for HB_sAg. Sera found to be positive for HB_sAg by this technique are neutralized by absorption with anti-HB_s and are considered negative for HB_sAg if no neutralization takes place.

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

Test	Pattern of Positive Results						Sera positive for each test
AUSAB	X	X	X	X		X	66
RIA-I	X	X	X			X	36
PHA	X	X			X		34
IEOP	X						11
Total sera positive by tests indicated	11	19	6	4	1*	26*	

*Neutralized with HB_sAg

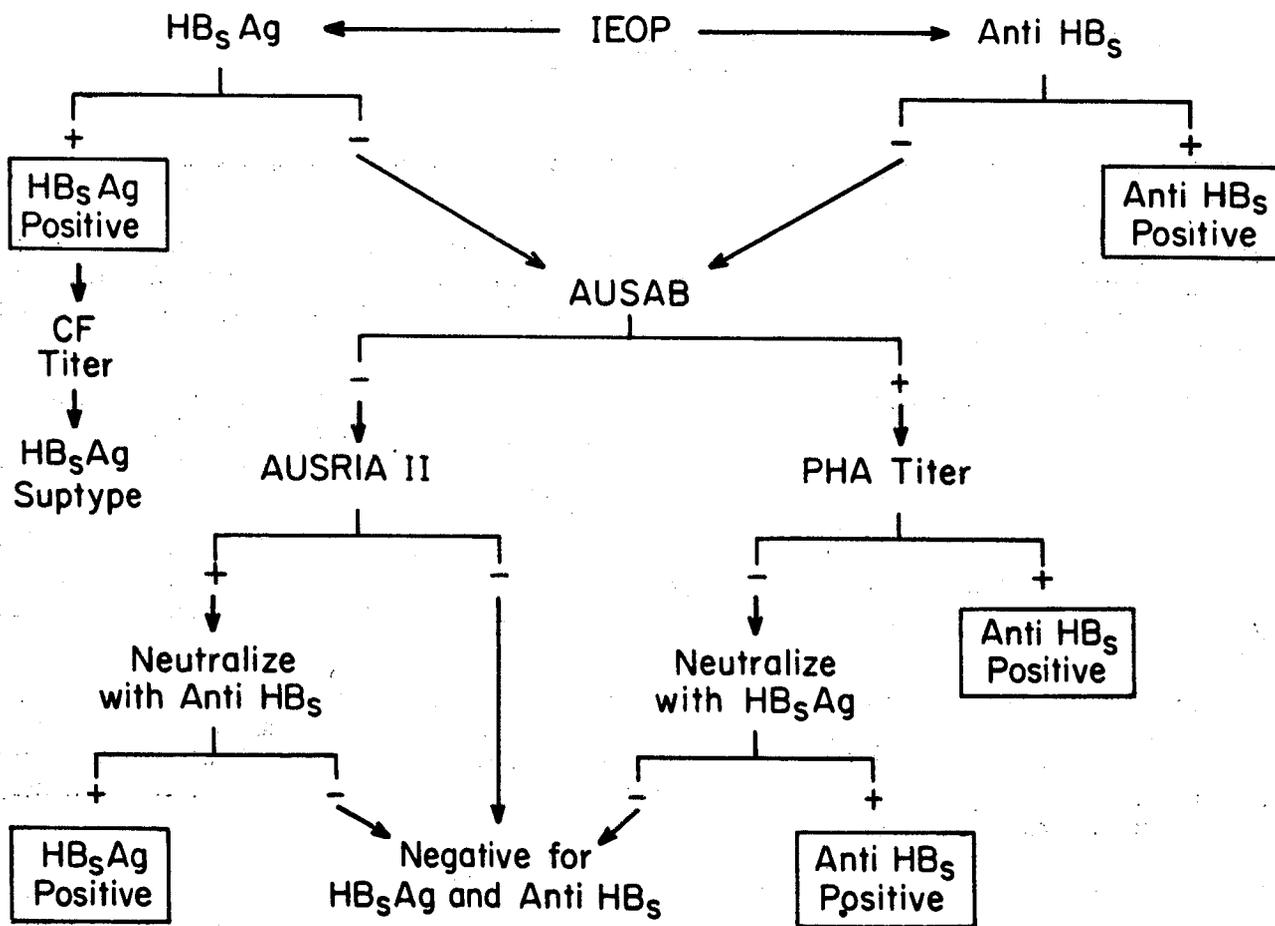


FIGURE I TEST SEQUENCE FOR HEPATITIS SERUM