

Comparison of Two Methods of Immunelectroosmophoresis for the Detection of
Hepatitis B Antigen and Antibody

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OBJECTIVE: To compare the performance of the existing technique for immunelectroosmophoresis (IEOP) to that used at Walter Reed Army Institute of Research (WRAIR).

BACKGROUND: IEOP is the basic screening test for hepatitis B antigen and antibody at SMRL. Previous studies have compared the sensitivity of IEOP to that of complement fixation (CF) and solid phase radioimmunoassay (RIA) (1). Although basically the procedures are the same, the results of IEOP tests at SMRL have always revealed more antigen and antibody positives than found at WRAIR. Most of the differences in results between SMRL and WRAIR are probably related to the greater prevalence of hepatitis B antigen (HBsAg) and antibody (anti-HBsAg) in Thailand. Nevertheless the two laboratories use somewhat different procedures which might affect the relative sensitivity of the tests.

DESCRIPTION: The differences in the two IEOP procedures are listed in Table 1. In addition, human antiserum is used at SMRL as opposed to high titered rabbit antiserum at WRAIR. Because of insufficient rabbit antiserum, only a human antiserum, designated P.T., was used in this study.

All tests were conducted in standard electrophoresis cells (Buchler and/or Shandon VoKam) with medium grade filter paper wicks (Schleicher & Schuell grade 407W) at room temperature on 7 x 5 cm glass slides overlaid with 10 ml of 1% agarose. Following electrophoresis, the slides were washed for 72 hours in normal saline and stained with 0.025% benzalkonium chloride for 30 minutes. All slides were read with an indirect fluorescent light against a black background.

Test specimens included serial dilutions of serum containing HBsAg/adr (designated 48/0) and anti-HBsAg/adw (P.T.) and a panel of 58 selected clinical sera (25 with HBsAg, 22 with anti-HBsAg and 11 negative)

All preparation of reagents, testing and slide interpretation was done by a person thoroughly experienced in both the SMRL and WRAIR procedures.

Table 1. Differences in IEOP procedures used at SMRL and WRAIR.

Characteristic	SMRL	WRAIR
Chamber buffer	Veronal 0.05M pH 8.6	Veronal 0.05M pH 8.6
Slide buffer	TRIS-EDTA-NaCl 0.01M pH 9.6	Same as chamber
Electrophoresis	Constant current 30 ma/sl for 90 min.	Constant voltage 12 v/sl for 120 min.
Well diameter	5 mm pair	2 vs 3 mm (Ab vs Ag)
Staining	Yes	No
Reference	Alter et al 1971	Prince and Burke 1970

Table 2. Effect of Benzalkonium Chloride Staining on the Titer of anti-HBsAg

HBsAg Dilution	Unstained Reaction			Stained Reaction		
	Intensity	Ab Titer		Intensity	Ab Titer	
1 : 8	4+	at	1 : 64	4+	at	1 : 64
1 : 16	3+	at	1 : 64	3+	at	1 : 64
1 : 32	2+	at	1 : 8	2+	at	1 : 64
1 : 64	0	at	1 : 4	1+	at	1 : 64
1 : 128	0	at	1 : 4	0	at	1 : 4

PROGRESS: When the two methods were performed as described in Table 1, the results were identical in nearly all respects. This was also true when the SMRL slides were run at constant voltage and the WRAIR slides at constant current. This indicates that the buffer and pH of the slide and the mode of electrophoresis are not significant factors.

The factor that most influenced the performance was well size. The SMRL technique, using pairs of wells 5 mm in diameter, detected antigen and antibody two dilutions higher than the WRAIR procedure with 2 vs 3 mm wells. Yet, when the panel of clinical specimens was tested, the results were the same regardless of well size; only the intensity of the reactions was different.

Staining of the slides with 0.025% benzalkonium chloride increased the number of positive reactions (Table 2). With the clinical sera, however, only the detection of antibody was enhanced. No new antigen reactions were revealed by staining. Unfortunately, staining also accentuated all proteins remaining in the agar, giving rise to artifacts.

DISCUSSION: The SMRL procedure is more sensitive than that of WRAIR when the two are used to screen for both hepatitis B antigen and antibody. When screening sera for anti-HBsAg and when using human antibody to detect HBsAg, the 5 mm wells and subsequent staining lend greater sensitivity to the test. The other differences listed in Table 1 do not seem to affect the sensitivity in any significant way. Similar results were reported by Kissling and Barker⁽⁴⁾. They, too, found that variations in buffers, pH, volume of sera employed or amperage applied, did not seem to influence results. They did observe, however, that the selection of antiserum can greatly affect the sensitivity of IEOP procedures.

It can be concluded, then, that the higher rate of IEOP positive reactions obtained at SMRL is influenced by both the prevalence of hepatitis B antigen and antibody within the population and the sensitivity of the test methods. Future IEOP tests at SMRL will continue to use the existing procedure.

REFERENCES:

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