

**The Prevalence of Hepatitis B with e-Antigen
and Antibody in Thai Blood Donors**

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OBJECTIVE :

1. To determine the prevalence of e-antigen and antibody in Thai blood donors.
2. To develop and test methods for detection of the e-antigen and antibody (anti-e).

BACKGROUND : Hepatitis B Virus (HBV) has now been associated with three apparently independent antigens; hepatitis B surface antigens, (HB_sAg), hepatitis B core antigen (HB_cAg) and e-antigen. HB_sAg is present on the surface of the putative virus; the 40 nm "Dane" particle. It also appears to be produced in over-abundance in the form of particles and rods, 28 nm in diameter. HB_cAg is associated only with the Dane particle and is found only in the nuclear capsid of the particle. Recently a third antigen, the e-antigen has been described. This antigen is associated with the intact Dane particle and appears to be a marker of infectivity (1). Antibody has been identified against each of these antigens. This reports a study of e-antigen and antibody (anti-e) in HB_sAg positive Thai blood donors and also describes the preliminary testing of a counterimmuno-electrophoresis test (CIEP) for the e-antigen and antibody.

DESCRIPTION : HB_sAg positive sera, collected from Thai blood donors were examined for the presence of e-antigen and antibody using a routine immunodiffusion test (ID). The ID test used a micro Ouchterlony technique with two seven well patterns punched in 0.8% agarose gel diluted in a buffer (pH 7.6) coated microscope slide. Reference human serum containing anti-e (EH-421) was placed in the central well of the left pattern and in the top well of the right pattern. Reference human serum containing e-antigen (Donor No. H. 50483) was placed in the central well of the right pattern and the top well of the left pattern. Sera to be tested were placed in coordinate wells of both patterns. The loaded slides were placed in a moist chamber, incubated at room temperature for 48 hours and observed for the presence of precipitin lines between test and reference sera. A counterimmuno-electrophoresis test (CIEP) for detection of e-antigen and anti-e was developed. The conditions of the test were similar to that used for detection of HB_sAg (2). A reference sera, donor-H-50483 was used for e-antigen, and EH-421 was used for anti-e. Appropriate controls were included in each test.

PROGRESS : Table 1 illustrates the prevalence of e-antigen and anti-e among HB_sAg positive blood donors. e-Antigen was found in 14% of 105 HB_sAg positive Thai blood donors. Of the 105 donors 87.6% were HB_sAg/adr and 12.4% were HB_sAg/adw. The prevalence of e-antigen was the same in each subtype. Anti-e was found only in HB_sAg/adr positive sera, however, the number of HB_sAg/adw positive individuals was small.

Comparisons were made of the ID and the CIEP test for the detection of e-antigen and anti-e. A 12% (3/25) increase in the detection of e-antigen and a 42% (3/7) increase in detection of anti-e were found using the CIEP test (Tables 2 and 3). The CIEP also detected anti-e in sera negative for HB_sAg.

DISCUSSION : The e-antigen has been detected in the sera of Thai blood donors using a reference serum (EH-421) containing anti-e. Anti-e has also been detected in Thai blood donors using sera containing e-antigen; detected in Thailand. A selection of those sera in which e-antigen and anti-e were detected has been sent to Dr. George L. Le Bouvier, Yale University for confirmation (Table 4). The e-antigen and anti-e were detected using both an ID and a CIEP technique. In preliminary testing the CIEP technique appeared to be more sensitive than the ID.

A similar prevalence of e-antigen was found among blood donors carrying either HB_sAg/adr or HB_sAg/adw. This suggested that e-antigen is not subtype specific. Since e-antigen has been suggested as a marker for hepatitis B infection, further studies of this antigen will be carried out.

SUMMARY : The e-antigen and anti-e has been detected in Thai blood donors. A CIEP test has been developed which appears to increase the sensitivity of detection. Further studies are planned to determine the usefulness of e-antigen as an indicator of infectivity of hepatitis B virus.

REFERENCES :

1. Okada, K., Kamiyama, I., Inomata, M., Imai, M., Miyakawa, Y., and Mayumi, M.: e-Antigen and anti-e in the Serum of Asymptomatic Carrier Mothers as Indicators of Positive and Negative Transmission of Hepatitis B Virus to their Infants. *New Eng. J. Med.* **294**: 746, 1976.
2. Snitbhan, R., and Top, F.H., Jr.: Comparison of the Sensitivity of Complement Fixation (CF) Test and Immunoelectroosmophoresis (IEOP) Test for Detection of Hepatitis B.

Table 1. Prevalence of e-Antigen and Antibody Among HB_sAg Carrier Blood Donors (Immunodiffusion Test)

HB _s Ag Subtype	e-Ag			Anti-e		
	No. tested	Positive		No. tested	Positive	
		No.	%		No.	%
adr	92	13	14	83	4	5
adw	13	2	15	10	0	0
Total	105	15	14	93	4	4

Table 2. The Comparison of e-Ag Tested by ID and CEP Methods

HB _s Ag (IEOP)	No. tested	Test for e-Antigen			
		ID		CJEP	
		No. positive	Percent	No. positive	Percent
Positive	51	22	43	25	49
Negative	25	0	0	0	0
Total	76	22	28	25	32

Table 3. The Comparison of Anti-e Tested by ID and CEP Methods

HB _s Ag	No. tested	Test for Anti-e			
		ID		CIEP	
		No. positive	Percent	No. positive	Percent
Positive	28	4	14	4	14
Negative	25	0	0	3	12
Total	53	4	14	7	13

Table 4. Red Cross Blood Donors Sera Containing e-Antigen and Anti-e

e-Antigen	H. 50475	*H. 50685	
	H. 50480	*H. 50720	
	H. 50482	*H. 50771	
	H. 50483	*H. 50772	
	H. 50485	*H. 50773	
	H. 50624	*H. 50778	
	*H. 50636	*H. 50836	
	*H. 50682		
	Anti-e	H. 47960	*H. 50655
		H. 47994	*H. 50664

* Sent to Dr. George L. Le Bouvier for confirmation.