

## CLINICAL VIROLOGY

# Assessment of hepatitis B “e” antigen and “e” antibody status—tests of status or status of tests?

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**Summary.** Forty-seven sera that gave positive results in tests for hepatitis B surface antigen and core antibody were examined for the presence of “e” antigen and “e” antibody by various commercially available assays. Considerable discordance was observed between results of tests performed on the same sample in different assays. Examination of the sera for the presence of hepatitis B DNA failed to resolve the discrepancies. Increasingly, “e” antigen and its antibody are used as measures of infectivity in carriers of hepatitis B. The absence of reliable tests has implications for patients, for infection control within hospitals and for the implementation of Department of Health guidelines on safe working practices for hepatitis B-infected health care workers.

## Introduction

It has been recognised for many years that the amount of virus circulating in the blood of persons infected with hepatitis B virus is reflected by the presence or absence of “e” antigen.<sup>1–4</sup> This antigen, thought to be a subunit of hepatitis B core antigen,<sup>5</sup> is present in sera that contain large amounts of whole virus. As hepatitis B infection is controlled by the patient’s immune system, circulating virus decreases and “e” antigen becomes undetectable. There follows a period when both “e” antigen and “e” antibody are present at low or undetectable levels, and then antibody to “e” becomes apparent.<sup>6</sup> These changes are common to recovery from acute infection and after chronic carriage and are eventually followed by loss of circulating hepatitis B surface antigen (HBsAg) and the development of antibody to it.

Because the presence of circulating “e” antigen correlates with high levels of circulating virus, blood and other body fluids which are positive for HBsAg and “e” antigen are much more likely to transmit infection than those positive for “e” antibody.<sup>1</sup> Individual hepatitis B carriers who have developed “e” antibody have a low risk of transmitting infection whether to sexual partners, to infants during parturition or to “parenteral” contacts through sharps injuries.<sup>1, 7, 8, 9</sup>

This difference in infectivity between “e” antigen positive and “e” antibody positive carriers of hepatitis

B has led to the development of different patient management strategies in surgery and obstetrics and, most recently, has been recognised by the Department of Health in the UK as of crucial importance in determining safe practice by health care workers.<sup>10</sup>

The risk of transmission of hepatitis B from health care worker to patient during exposure-prone procedures has been deemed unacceptably high if the health care worker is positive for HBsAg and also “e” antigen. Health care workers who are HBsAg positive but negative for “e” antigen are considered to be of acceptably low infectivity to perform exposure-prone procedures safely on patients.<sup>10</sup> Thus the reliability of tests for “e” antigen and “anti e” antibody is of considerable importance.

We have assessed several commercially available enzyme-linked immunoassay (ELISA) kits for “e” antigen and “e” antibody. All the sera examined were known to be positive for HBsAg and hepatitis B core antibody. Some sera were also tested for the presence of hepatitis B virus DNA which is another indicator of circulating whole virus and a correlate of infectivity.<sup>11–14</sup>

## Subjects and methods

### *Serum samples*

Sera examined in this study were those submitted routinely to the virus laboratory for hepatitis B marker evaluation. All sera were confirmed HBsAg positive and positive for antibody to hepatitis B core antigen.

Of 47 sera examined, 25 were from asymptomatic carriers of hepatitis B who had been diagnosed following screening tests, e.g., prior to cardiac surgery or as part of ante-natal care (nine of these patients were from areas where hepatitis B prevalence is high). One patient was identified as a hepatitis B carrier after the development of acute hepatitis B in a sexual contact; the patient himself was well. Three sera from blood donors were referred by the Blood Transfusion Service for confirmation of hepatitis B status. Two sera were from well haemophilic patients and one was from a patient receiving chronic renal dialysis; these three patients had been known to be long-term carriers for many years. Twelve sera were from patients with evidence of chronic liver disease who were known to be long-term carriers of hepatitis B. Three sera were from patients who had recently recovered from acute hepatitis B with jaundice.

#### *ELISA tests for hepatitis B "e" antigen and "e" antibody*

A maximum of five assays was performed on each sample. Limitation of sample size was the main reason for testing a sample by three or four assays only. ELISA assays included in the evaluation were:

1. Enzygnost HBe; enzyme immunoassay for the detection of hepatitis B "e" antigen (Behring; Hoechst UK Ltd, 50, Salisbury Road, Hounslow, Middlesex).
2. Syva Microtrak HBeAg/Anti HBe EIA; enzyme immunoassay for the detection of hepatitis B "e" antigen and anti-HBe in human serum or plasma (Sorin Biomedica SpA, Saluggia, Italy; distributed by Syva Company Pao Alto, CA, USA).
3. Wellcozyme HBeAg/anti HBe; enzyme immunoassay for the detection of hepatitis B "e" antigen and antibody (Murex Diagnostics Ltd, Central Road, Temple Hill, Dartford, Kent).
4. Hepanostika HBeAg/anti HBe MicroElisa System (Organon Teknika Ltd, Science Park, Milton Road, Cambridge).
5. IMX HBe Hepatitis B antigen and IMX anti HBe (Abbott Laboratories, Diagnostic Division, Abbott Park, IL 60064, USA).

#### *ELISA tests for hepatitis B surface antigen and core antibody*

All sera were HBsAg antigen positive by two test methods:

1. Bioelisa HBsAg ELISA test for the detection of HBsAg in human serum (Biokit SA, 08186 LLissa d'Amunt, Barcelona, Spain).
2. IMX HBsAg Microparticle Enzyme Immunoassay for the detection of HBsAg in human serum or plasma (Abbott Laboratories).

All sera were found to be anti-core IgG positive by IMX CORE for the detection of antibody to hepatitis B core antigen (Abbott Laboratories).

#### *Hepatitis B DNA testing*

Hepatitis B DNA was detected by the Digene Hybrid Capture System HBV DNA Assay, a chemiluminescent molecular hybridisation assay for the detection and quantitation of hepatitis B virus (HBV) DNA in serum (Murex Diagnostics).

#### *Quality control*

All assays were performed according to the manufacturer's instructions. Results included in the evaluation were from runs which met the manufacturer's criteria for acceptability.

#### **Results**

The sera showed considerable inconsistency with regard to "e" antigen status when tested by different kits. Testing for hepatitis B DNA did not resolve these inconsistencies. Sixteen sera were examined for the presence of "e" antigen and "e" antibody by all five ELISA assays. The results are summarised in table I. Only three sera gave concordant results in all five tests. Eleven of the 16 sera examined gave discordant results which would have led to different conclusions being drawn about the infectivity of the serum and hence of the patient from whom the sample was taken. The figure shows the scatter of results obtained in "e" antigen evaluations of the 16 sera examined by five ELISA kits.

Twenty further sera were examined by four ELISA assays (table I). Eleven sera gave concordant results; of the nine sera that gave discordant results, eight gave results which would lead to conflicting conclusions about the infectivity of the serum and the patient. Another 11 sera were examined by three assays (table I), six sera gave concordant results and five sera gave discordant test results. Table II summarises the results obtained from hepatitis B DNA assays performed on

**Table I.** Results obtained with sera examined for the presence of hepatitis B "e" markers by commercial assay kits

Assay results	Number of sera examined in		
	5 assays (16)	4 assays (20)	3 assays (11)
"e" antigen positive by all assays	1	3	1
"e" antibody positive by all assays	2	7	5
*eag/eab negative by all assays	0	1	0
"e" antigen positive or eag/eab negative/negative	2	1	1
"e" antibody positive or eag negative/eab negative	7	1	4
"e" antigen or "e" antibody detected by different assays	4	7	0

\* eag, "e" antigen; eab, "e" antibody.

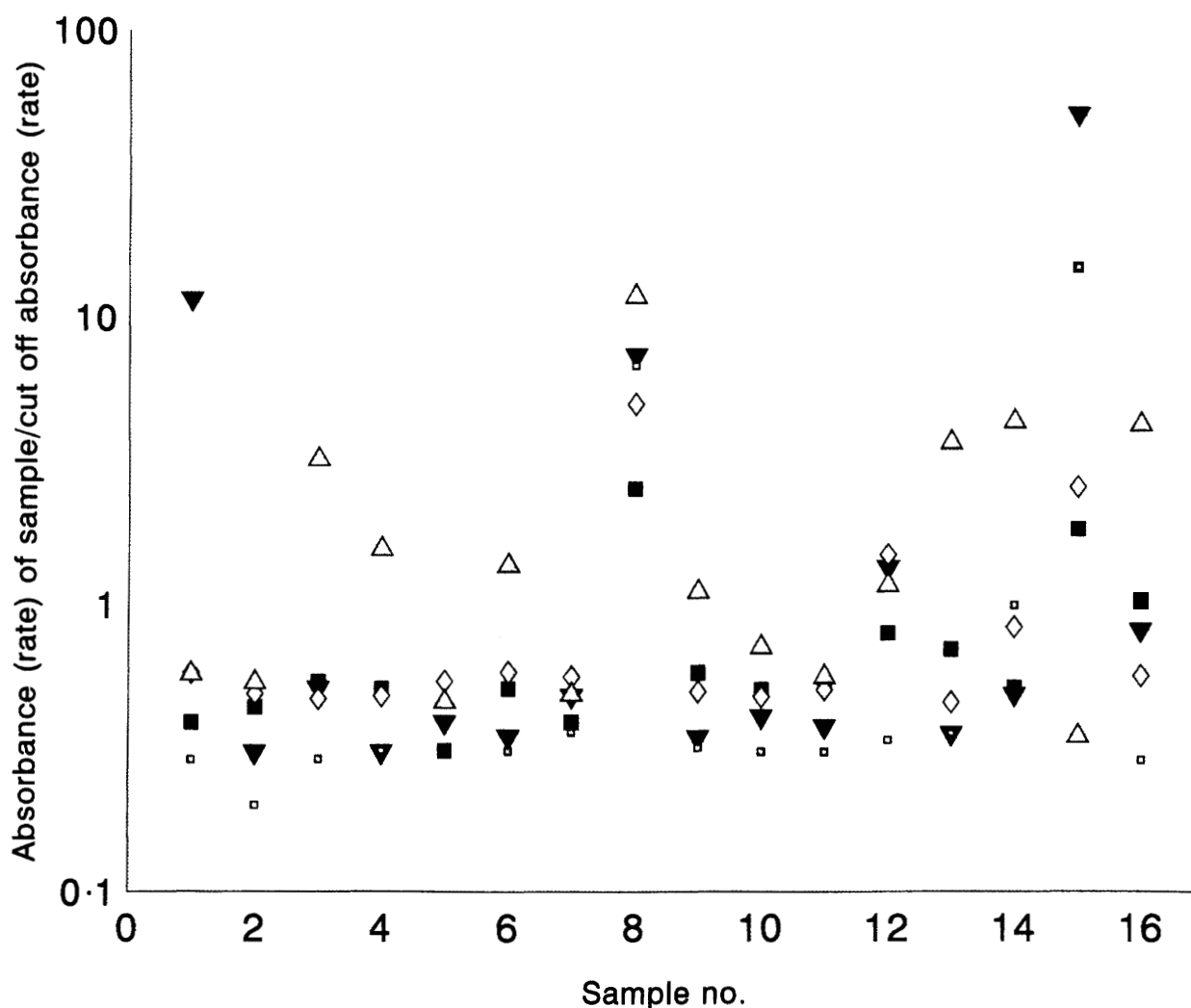


Figure. Results of "e" antigen tests on 16 sera examined by five ELISA methods (1,  $\Delta$ ; 2,  $\diamond$ ; 3,  $\square$ ; 4,  $\blacksquare$ ; 5,  $\blacktriangledown$ ) represented as sample: cut off ratio. Any specimen with a sample: cut off ratio  $> 1$  would be deemed "e" antigen positive; values  $< 1$  indicate "e" antigen negative results.

Table II. Results of hepatitis B DNA estimation on 26 sera

Result of "e" antigen and antibody assays	Number of sera		
	tested	HB DNA +ve	HB DNA -ve
Consistently "e" antigen positive	2	2	0
Consistently "e" antibody positive	10	2	8
Consistently eag negative/eab negative	1	1	0
Inconsistent results in "e" antigen	13	1*	12

eag, "e" antigen; eab, "e" antibody.

\*Known to be infectious; see *Results*.

26 of the sera in the evaluation panel. Two sera were "e" antigen positive by all ELISA methods and both gave positive results for hepatitis B DNA. Of 10 consistently "e" antigen positive sera, eight gave negative results for hepatitis B DNA but two gave positive results. One serum sample which was consistently "neg/neg" (no "e" antigen or "e" antibody)

by all five ELISA tests gave positive results for hepatitis B DNA. This serum was from a young woman in the recovery phase of acute hepatitis B. She had acquired her infection through heterosexual contact with a young man whose serum gave discordant "e" status results—one of five kits gave a result indicating "e" antigen negative and "e" antibody positive. Nonetheless his serum gave a positive result for hepatitis B DNA (table II) and he was recognised to be an infectious carrier of hepatitis B who had infected at least three sexual partners during the course of 1 year.

## Discussion

The results of this investigation of the "e" antigen and "e" antibody status of 47 HBsAg positive serum specimens showed a disquieting level of discrepancy. Inevitably, sera had been frozen and thawed several times during the course of the evaluation but no serum was  $> 1$  year old when examined. The patient group was representative of several types of hepatitis B

infection and the level of discrepancy between different kits was unexpected. We conclude that great caution must be exercised by laboratories in the selection of "e" marker kits. In the absence of external standards it was not possible to determine which kits gave correct results. The lack of standard sera of quantified "e" antigen and "e" antibody status undermines laboratory work on these markers of hepatitis B infection and the absence of official, definitive guidance regarding the sensitivity and specificity of "e" antigen and "e" antibody kits contributes further to the difficulties faced by laboratories undertaking these investigations.

These data demonstrate the urgent need for national standard sera of known "e" antigen and "e" antibody content. The attention of Infection Control Doctors and Occupational Health Departments, as well as

virology laboratories, should be drawn to the importance of circumspection and careful confirmation of hepatitis B "e" status in all cases, particularly in health care workers who undertake exposure-prone procedures. Recent Department of Health Guidelines in the UK make a clear distinction between the employment of "e" antigen positive hepatitis B carriers whose work should not include exposure-prone procedures and the employment of "e" antibody-positive carriers whose work is not so restricted. It is evident that the determination of "e" status of a hepatitis B carrier can vary depending on the type of assay used; such a bias is not in the interests of patients or health care workers, and recognising its existence is a first step towards improving the reliability and consistency of commercial assays for hepatitis B "e" antigen and antibody.

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