

## Are Specimens with "at Risk" Biochemical Profiles More Likely to Be Infectious for Hepatitis B Virus?

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The potential infectivity of 640 plasma specimens with various biochemical profiles was directly assessed by hepatitis B virus (HBV) DNA dot-hybridization. We found that specimens with "at risk" biochemical profiles typical of various forms of liver disease were not significantly more likely to carry HBV than the general patient population. Specimens containing HBV cannot be distinguished from non-infectious specimens by any simple biochemical tests, including aminotransferases and bilirubin. The only predictive feature of HBV-positive samples was that they were more likely to be labeled as "biohazardous" by the medical staff, but even this was not always the case.

It is commonly taught that infection with hepatitis B virus (HBV) produces an acute or chronic disease with a pathognomonic pattern of biochemical abnormalities (1-3).<sup>2</sup>

Previous studies of the prevalence of HBV in clinical specimens have made use of markers such as the surface antigen (HBsAg, Australia antigen) to infer the presence of this virus. However, only a small proportion of all HBsAg-positive specimens contain intact viral Dane particles and are potentially infectious (4). Recent developments in molecular biology now permit direct detection of the HBV viral genome in serum or plasma, by use of DNA hybridization with a radiolabeled specific HBV probe. The presence of HBV-DNA correlates closely with other markers of infectivity, such as endogenous viral DNA polymerase (5), and the e-antigen (4, 6-7). Thus, assay for HBV-DNA should identify those specimens that are potentially infectious for hepatitis B.

We recently determined (8) the presence or absence of HBV-DNA in a large number of randomly selected clinical specimens and compared these results with those for a number of commonly performed biochemical analytes. We found that specimens containing HBV (and which were potentially infectious for hepatitis B) could not be identified on the basis of any combination of simple biochemical tests.

An unexpected finding from this earlier study was that there appeared to be little or no increase in risk of carrying HBV in specimens that had grossly abnormal "at risk" profiles than in those with "normal" profiles in our study group. Unfortunately, the low prevalence of HBV-DNA-positive samples in that study population prevented our

exploring this interesting observation in more detail. Here, we have used a selection algorithm that would enrich our study population for various abnormal biochemical profiles usually associated with various forms of liver disease. The duration of the sampling period was also extended to decrease the likelihood of multiple sampling from the same patients.

### Materials and Methods

**Patients' specimens.** We examined 2012 plasma samples in this study: unselected, consecutive specimens received in the laboratory of a large metropolitan tertiary referral hospital on six randomly selected days covering a period of approximately seven weeks. All specimens were coded, then analyzed in a Technicon SMAC analyzer. We used a computer program to select for further study some 640 specimens on the basis of their biochemical profiles (*vide infra*). These specimens were recoded, stored at -20 °C, and later analyzed for HBV-DNA in batches.

**Biochemical analyses.** The following analytes were measured in the specimens: sodium, potassium, chloride, total CO<sub>2</sub>, glucose, creatinine, urea, calcium, phosphate, uric acid, total protein, albumin, total bilirubin (TBIL), alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyltransferase (GGT), iron, cholesterol, and triglyceride.

**Hepatitis B virus DNA dot hybridization.** The DNA diagnostic probe used was pHBV.CB, which consists of the entire genome of the hepatitis B virus (subtype adwy), cloned in the bacterial plasmid pBR322 (9). This probe hybridizes to all the known serotypes of HBV. The HBV insert, excised from the plasmid vector before use, was radiolabeled with <sup>32</sup>P to high specific activity.

Details of the hybridization protocol were as described earlier (8).

**Statistical analysis.** The SPSS program (10) was used for analysis of results.

Because of the skewed distributions of the results for the HBV-negative specimens and uncertainty about the shape of the distributions for the HBV-positive specimens, we compared groups with a nonparametric test (the Mann-Whitney U test), as before (8). For tests for significant differences in the frequencies of HBV-positive specimens among the various groups we used the Chi-square test.

### Results

We postulated some criteria that might define high- and low-risk biochemical profiles, as follows:

(1) "Classical hepatitis"—any sample with either ALT >250 U/L or with AST <250 U/L [the values 250 U/L were chosen to be four times the upper reference limit (URL) of our laboratory].

(1a) "Classical hepatitis, without increased ALP"—as for profile 1, above, but also requiring ALP <150 U/L (which is 1.5 times URL).

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<sup>2</sup> Nonstandard abbreviations: HBV, hepatitis B virus; TBIL, total bilirubin; ALP, alkaline phosphatase; AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, gamma-glutamyltransferase; URL, upper reference limit; LFT, liver function tests.

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(2) "Icteric"—any sample with TBIL >50  $\mu\text{mol/L}$  (2.5 times URL).

(2a) "Cholestatic"—any sample with TBIL >50  $\mu\text{mol/L}$ , and also either ALP >150 U/L or GGT >100 U/L (two times URL).

(3a) "Abnormal Liver Function Tests" [LFT]—Type 1"—any sample with ALT >150 U/L (2.5 times URL).

(3b) "Abnormal LFT—Type II"—any sample with ALT >150 U/L, and also with any one of the following: AST >150 U/L (2.5 times URL), TBIL >40  $\mu\text{mol/L}$  (two times URL), ALP >150 U/L, GGT >100 U/L, or albumin <36 g/L (our laboratory's lower reference limit).

(4) "Biochemically normal LFT"—all samples with all six of the following criteria satisfied: TBIL <20  $\mu\text{mol/L}$ , ALT <60 U/L, AST <60 U/L, ALP <100 U/L, albumin >36 g/L, and GGT <60 U/L.

From the original population of 2012 samples, these various biochemical profiles resulted in the selection of a sample population consisting of 640 specimens, derived from 561 different patients. This sample population was classified into the above groups, and the prevalence of HBV-DNA-positive specimens in each group was determined (Table 1).

There were no significant differences between the frequency of HBV-DNA-positive samples in any of the groups and the frequency in the sample population as a whole. In particular, the frequency of HBV-DNA-positive samples in the "hepatitis" profiles (groups 1 and 1a) was not significantly higher than in the general population, and the frequency of HBV-DNA-positive samples in the two groups with increased total bilirubin (groups 2 and 2a) was (to our surprise) lower than that for the sample population as a whole, but this difference was not statistically significant.

We also studied the entire sample population and compared the biochemical analyses of the HBV-DNA-positive and -negative subpopulations. As before (8), we could find no useful significant differences in the values of the analytes measured in the two populations (data not shown).

The significance of a specimen carrying a "universal biohazard" warning was also assessed. We noted that 26 specimens (derived from 23 patients) in the sample population had been labeled by the medical staff as being "biohazardous." Presence of this warning on a specimen significantly increased the likelihood of a specimen carrying HBV-DNA, although absence of this warning did not preclude infectivity (Table 1).

## Discussion

We found that specimens with biochemical profiles usually considered pathognomonic for various forms of liver

disease, including "hepatitis," seem to carry no greater risk of containing HBV-DNA than does the general specimen population. None of the "at risk" biochemical profiles tested were significantly different from the general population in their likelihood to be potentially infectious for hepatitis B.

Our earlier study suggested that mildly increased ALT and AST, and slightly lower values for ALB, TBIL, and iron may have been distinguishing characteristics of HBV-DNA-positive specimens, although we surmised that none of these properties was likely to be useful in practice. Comparison of the larger number of HBV-DNA-positive samples in this present study with the HBV-DNA-negative samples failed to reveal any statistically significant differences in analyte values between the two groups and confirms that even these five analytes cannot be used as a discriminant tool in looking for the presence of hepatitis B virus. The finding that three of 17 HBV-DNA-positive samples were in the "normal" control group (Table 1, group 4) supports this conclusion.

Indeed, the only useful discriminating feature possessed by HBV-DNA-positive samples was that they were more likely to carry a biohazard warning. In our hospital, this warning is used to tag specimens from patients known or suspected to have any one of a variety of infectious conditions, of which hepatitis B would be the most common. Although a third of all specimens carrying this warning were demonstrated to carry HBV, an equal number of HBV-DNA-positive samples lacked this warning; in most cases this was because the detection of HBV in the patients' blood was an incidental finding in the course of this study, and had not been suspected by the medical attendants.

We can draw several conclusions from this study relating to the infectious risks of specimens received in the clinical biochemistry service. Firstly, there appear to be no useful distinguishing biochemical features at all that can be used to characterize those specimens that are potentially infectious for hepatitis B. Secondly, while some patients with "textbook" biochemical profiles typical of hepatitis will be infectious for hepatitis B, most of these patients are either not in the viremic stage of their illness, or else have diseases unrelated to HBV. Thirdly, specimens marked as being biohazardous do, indeed, carry an appreciably higher risk of carrying HBV than do unmarked specimens; however, specimens not so marked can still be infectious, albeit less frequently. And finally, specimens with "normal" biochemical profiles pose no less a degree of potential infectivity for hepatitis B than those with "at risk" biochemical profiles; as in our original study, those with normal bilirubin values should be treated with as much care and respect as those with "icteric transaminitis."

Table 1. Frequency of HBV-DNA in Specimens with Various Biochemical Profiles

Group*	No. specimens	No. HBV-DNA positive (and %)
All specimens	640	17 (2.7)
1	48	3 (6.3)
1a	21	2 (9.5)
2	38	0 (0.0)
2a	33	0 (0.0)
3a	77	4 (4.9)
3b	67	2 (2.9)
4	138	3 (2.1)
Biohazard warning present	26	9 (34.6) <sup>b</sup>
Biohazard warning not present	614	8 (1.3)

\* See text. <sup>b</sup>  $p < 0.001$ . Frequency of HBV-DNA not statistically significant in the other groups (1 to 4) tested.

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## A Multilayer Element for Determining Hemoglobin in Whole Blood: Principles and Analytical Performance

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We describe the structure and analytical performance of a thin-film element for determining total hemoglobin in whole blood. The element can be used with the Kodak Ektachem DT60 analyzer. The method, analogous to the standard Drabkin's method, provides accurate and precise results on 10- $\mu$ L samples of undiluted whole blood. Dynamic range is linear from 0 to 200 g/L, and the method is relatively free from interference.

**Additional Keyphrases:** *multilayer film analysis · thiocyanomethemoglobin*

We report the construction and performance of a multilayer element for determining hemoglobin in undiluted whole blood. Whole blood (10  $\mu$ L) is applied to the element, where lysis occurs; the released hemoglobin is oxidized to methemoglobin and forms a complex. The assay is analogous to the cyanomethemoglobin procedure accepted as the international standard (1), except that in our method thiocyanate replaces the toxic cyanide. Various hemoglobin derivatives in blood are converted to thiocyanomethemoglobin, which is quantified at  $\sim$ 540 nm in the Kodak Ektachem DT60 analyzer.

### Materials and Methods

#### Multilayer Element

The multilayer element for determining hemoglobin in whole blood consists of a transparent support, a white reflective cellulose acetate-pigment ( $\text{BaSO}_4$ ) layer, and a layer containing polymeric beads, 20 to 40  $\mu$ m in diameter, for spreading the whole-blood sample (Figure 1). The bead

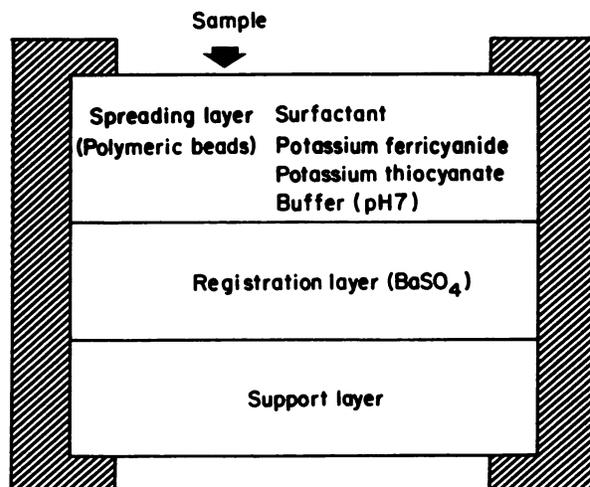
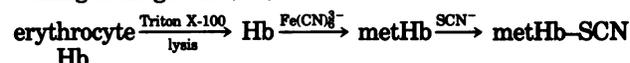


Fig. 1. Structure of the multilayer element for quantifying whole-blood hemoglobin

layer contains excess lysing surfactant (Triton X-100), potassium ferricyanide, potassium thiocyanate, and potassium phosphate buffer (pH 7). Whole blood applied to the slide is lysed and the liberated hemoglobin is oxidized to methemoglobin, which complexes with thiocyanate ion (2) to produce a species with an absorbance maximum at  $\sim$ 540 nm (Figure 2). Replacement of cyanide with thiocyanate in Drabkin's hemoglobinometry reagent (3) obviates toxicity concerns. The upper bead layer (Figure 1) serves as a site for blood spreading and lysis as well as a reagent reservoir; the white reflective cellulose acetate- $\text{BaSO}_4$  layer serves as the site of registration of the presence of hemoglobin.

The following conversions describe the process for determining hemoglobin (Hb):



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