Evaluation of Diagnostic Assays for Hepatitis E Virus in Outbreak Settings

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Hepatitis E virus (HEV) is a major cause of hepatitis. We evaluated five HEV antibody diagnostic assays by using outbreak specimens. The Abbott immunoglobulin G (IgG), Genelabs IgG, and Walter Reed Army Institute of Research (WRAIR) IgM assays were about 90% sensitive; the Abbott IgG and WRAIR total Ig and IgM assays were more than 90% specific.

Hepatitis E virus (HEV) is the principal cause of acute hepatitis on the Indian subcontinent, in southeastern and central Asia, in the Middle East, in Mexico, and in parts of Africa. It is associated with the consumption of fecally contaminated drinking water (7, 8, 12, 15). Recent outbreaks have occurred in Iraq, Chad, Sudan, and India (2, 9). Although HEV is associated with a low case fatality rate in the general population, pregnant women in the second and third trimesters are at greater risk (case fatality rates of 10 to 24%) for fulminant hepatitis and fetal loss (14, 18).

HEV has not been cultured in vitro, and most enzyme immunoassays (EIAs) for HEV infection are based on either recombinant HEV proteins or synthetic peptides. These assays have varied significantly (16), and assays based on open reading frame 2 (ORF2) were shown to be more sensitive in detecting anti-HEV than those based on ORF3 (1, 11, 17). These recombinant-protein-based tests have detected anti-HEV in 90 to 95% of symptomatic HEV cases (3, 10).

From March to May 1998, an HEV outbreak occurred in the Bondowoso District, East Java Province, Indonesia. Investigations included a retrospective review of hospital records, a cross-sectional study, and case detection with household contact follow-up (19). Sera from 82 symptomatic and 174 asymptomatic individuals from the four affected villages and the local health centers and from 496 subjects from an unaffected village were used for this study. Five different serological assays for the detection of anti-HEV were evaluated by using HEV RNA detection by reverse transcriptase PCR (RT-PCR) as a reference. The mean time from onset of jaundice to time of sample collection was 23 days (range, 4 to 70 days). Informed consent was obtained from all individuals. All assays were performed blinded.

WRAIR EIA. Immunoglobulin M (IgM) and total Ig anti-HEV EIAs developed by the Walter Reed Army Institute of Research (WRAIR) were performed at the Armed Forces Research Institute of Medical Science (AFRIMS) according to previously published methods (13, 20). In these assays, titers of IgM and total Ig to recombinant HEV were measured by quantitative sandwich EIA with a recombinant protein encoded by ORF2 of the Pakistani HEV strain expressed in the baculovirus system. The cutoffs for the IgM anti-HEV EIA and the IgG anti-HEV EIA are ≥100 U/ml and ≥50 U/ml, respectively.

GLD EIA. Testing by IgM (batch BH3023) and IgG anti-HEV EIA (batch BF3021) kits from Genelabs Diagnostics (GLD) Pty. Ltd., Singapore, was performed at AFRIMS according to the manufacturer’s instructions. These are commercially available assays based on the ORF2 and ORF3 recombinant proteins of the Burmese and Mexican strains of HEV that use ortho-phenylenediamine as a substrate to detect IgM and IgG anti-HEV (4).

Abbott EIA. Testing with IgG anti-HEV EIA kits from Abbott (Abbott Diagnostika, Wiesbaden-Delkenheim, Germany) was performed at U.S. Naval Medical Research Unit No. 2 according to the manufacturer’s instructions. This assay uses recombinant proteins encoded by ORF2 and ORF3 from the Burmese strain of HEV expressed in Escherichia coli that are used to coat polystyrene beads (4).

RT-PCR. HEV PCR was performed at AFRIMS by the method of Tsarev et al. (22, 23). HEV RNA was extracted from 100 μl of human serum with TRIzol reagent (Gibco/BRL) by following the manufacturer’s instructions. Viral cDNA was produced with ORF3-specific primer 2783 (5'-GGT TGG TTG GAT GAA TAT AGG-3') and 6 U of avian myeloblastosis virus RT at 42°C for 2 h. The first round of PCR was accomplished with 35 cycles consisting of 1 min each at 94°C, 55°C, and 72°C with HEV primer 2782 (5'-GGD CTB GTT CAT AAC CTG AT-3') and the primer used for priming cDNA synthesis. Following the first amplification round, the resulting DNA products were further amplified with 5 μl of the first-round product with a pair of internal nested primers, HEV primers 2781 (5'-GTT CAT AAC CTG ATW GGY ATG CT-3') and 2784 (5'-GGA TTG CGA AGG GCT GAG AAT CA-3'). The GenBank accession number of Burmese strain Bur-121, which was used to design the primers, is 73218.
The amplification reaction protocol consisted of 35 cycles of 1 min each at 94°C, 55°C, and 72°C. The nested-PCR products were then analyzed on an electrophoresis gel. The presence of a 310-bp DNA band indicated detection of HEV RNA.

Statistical analysis. Statistical analysis of differences in the sensitivity and specificity of each anti-HEV assay compared to those of the WRAIR IgM and Ig anti-HEV assays was performed with a chi-square test.

Results. Table 1 illustrates results of the five HEV serologic assays evaluated. The reference for sensitivity was RT-PCR. All tests were significantly more sensitive for symptomatic assays evaluated. The reference for sensitivity was RT-PCR. A 310-bp DNA band indicated detection of HEV RNA. When HEV RT-PCR was selected as the reference assay for HEV detection in this study. However, this is open to criticism as viremia does not always coincide with the antibody response in the natural course of HEV infection. It was reported that detection of IgA alone or together with IgM provides better specificity and a longer duration of positivity for diagnosis of HEV infection (21). Inclusion of such assays for comparison would be helpful for evaluation.

Of the two IgM anti-HEV assays tested, the WRAIR assay performed better than the Genelabs IgM assay. However, the GLD kits used in this study were based on ortho-phenylenediamine; the current version based on tetramethylbenzidine was not tested and potentially performs differently. Among the three EIAs for detecting total Ig or IgG anti-HEV, the Abbott IgG anti-HEV EIA had the highest sensitivity, while the WRAIR total Ig anti-HEV EIA had the highest specificity. Overall, the WRAIR assays were the most specific, although the Abbott assay also had greater than 95% specificity. For sensitivity, there was no difference among the WRAIR IgM, the GLD IgG, and the Abbott IgG tests. Although it was reported that there was a significant lack of concordance among many serological assays available (10), we found a high degree of concordance among the assays that were evaluated in this study.

Serological assays for HEV infection, including the ones evaluated in this study, have not been extensively compared, especially under outbreak conditions. All HEV strains identified to date appear to belong to the same serotype, and recombiant HEV antigens react well with sera from all geographical origins.

A significant observation in this study was that the sensitivity of all serological assays was greater for symptomatic than for asymptomatic HEV infections. Cases of asymptomatic HEV infection are not well defined; however, subclinical forms of infection exist and they are thought to exceed icteric infections in HEV outbreaks (3, 6). More-sensitive assays to identify all infected persons rather than just those with clinical disease could be useful in investigations. Further evaluations of newer assays and kits (e.g., the new GLD EIA) are needed.

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We do not have a financial interest in any organizations that could be perceived as a real or apparent conflict of interest in the context of the material submitted here.

REFERENCES


### Table 1. Sensitivities and specificities of five HEV diagnostic assays evaluated with specimens collected from a hepatitis E outbreak in Bondowoso, Indonesia, March to May 1998

<table>
<thead>
<tr>
<th>EIA</th>
<th>% Sensitivity&lt;sup&gt;a&lt;/sup&gt; (P value) for:</th>
<th>% Specificity&lt;sup&gt;b&lt;/sup&gt; (P value) for healthy population</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall HEV cases</td>
<td></td>
<td></td>
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<tr>
<td>Asymptomatic HEV cases (no illness, PCR positive; n = 174)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Symptomatic HEV cases (acute hepatitis, PCR positive; n = 82)</td>
<td></td>
<td></td>
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<tr>
<td>WRAIR IgM anti-HEV</td>
<td>64&lt;sup&gt;b&lt;/sup&gt;) (&lt;0.01)</td>
<td>91&lt;sup&gt;a&lt;/sup&gt;) (&lt;0.01)</td>
</tr>
<tr>
<td>GLD IgM anti-HEV</td>
<td>52 (&lt;0.05)</td>
<td>72 (&lt;0.01)</td>
</tr>
<tr>
<td>WRAIR Ig anti-HEV</td>
<td>54 (&lt;0.05)</td>
<td>84 (&lt;0.05)</td>
</tr>
<tr>
<td>GLD IgG anti-HEV</td>
<td>63 (&lt;0.05)</td>
<td>89 (&lt;0.05)</td>
</tr>
<tr>
<td>Abbott IgG anti-HEV</td>
<td>60 (&lt;0.05)</td>
<td>91 (&lt;0.05)</td>
</tr>
</tbody>
</table>

<sup>a</sup> Compared with RT-PCR as the reference.

<sup>b</sup> The sensitivity and specificity of each anti-HEV assay were compared to those of the WRAIR anti-HEV assay with a chi-square test.


