EVALUATION OF A NEW RAPID IMMUNOCHROMATOGRAPHIC ASSAY FOR SERODIAGNOSIS OF ACUTE HEPATITIS E INFECTION

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Abstract. A rapid and reliable diagnostic assay for acute hepatitis E virus (HEV) infection is needed. We evaluated a rapid, immunochromatographic assay for IgM antibodies to HEV (ASSURE™ HEV IgM Rapid Test) using acute-phase HEV samples (n = 200) from Indonesia and Nepal and convalescent-phase HEV samples (n = 70) from Nepal. Blood donors in Thailand (n = 100), individuals with hepatitis A (n = 80), hepatitis B (n = 45), and hepatitis C (n = 50) in Thailand and Nepal, acute-phase sera of individuals with Epstein-Barr virus infection (n = 20), and rheumatoid factor–positive blood (n = 26) served as negative controls. The assay had a sensitivity of 93% (95% confidence interval [CI] = 88.5–96.1%) and a specificity of 99.7% (95% CI = 98.3–100%). The positive and negative predictive values were 99.5% (95% CI = 97.1–100%) and 95.8% (95% CI = 93.1–97.7%), respectively. These results suggest that this assay is a sensitive and specific tool for the rapid diagnosis of acute HEV infection.

INTRODUCTION

Hepatitis E virus (HEV) is a common cause of epidemic and sporadic viral hepatitis with more than 50 outbreaks reported throughout southeast and central Asia, the northern and western parts of Africa, and Mexico. Recent outbreaks of HEV have been reported in refugee and internally displaced persons camps in Sudan, Chad, and Iraq. Serologic studies suggest that HEV may be endemic, albeit at low levels, in industrialized countries such as United States, Europe, and Japan. Symptomatic hepatitis E is normally observed in young adults with the highest case fatality rate (15–20%) among pregnant women. To date, HEV is known to exist as a single serotype, although at least four major genotypes have been identified.

Most HEV cases are diagnosed serologically using enzyme immunoassays (EIAs) for IgM and IgG antibodies to HEV or a reverse transcriptase–polymerase chain reaction (RT-PCR). IgM antibody to HEV appears early during acute clinical illness but wanes over a few months. IgG antibody to HEV appears a few days later but persists for at least a few years. The detection of HEV-specific IgM or an increasing titer of IgG antibodies to HEV is diagnostic of acute infection. Two commercial kits are currently marketed for detecting IgM antibodies to HEV in blood. Different methods to detect viral-specific nucleic acid by RT-PCR have been developed and evaluated. A rapid RT-PCR procedure using universal oligonucleotide primers was also developed for detection of HEV-RNA in serum. However, the EIA can be performed only by relatively skilled personnel in well-equipped laboratories and the RT-PCR is time-consuming, expensive, and difficult to conduct. The tests routinely use for the laboratory diagnosis of hepatitis E are too elaborate for widespread application under field or outbreak conditions.

There is an urgent need for a rapid, reliable, and user-friendly assay to diagnose acute HEV infection in disease-endemic areas. A kit based on immunochromatography should be a reliable and rapid method for clinical diagnosis, even in the hands of inexperienced personnel, and easy to perform under harsh field conditions. The aim of this study was to conduct an evaluation of the performance of the rapid immunochromatographic assay based on the antibody capture format for IgM antibodies to HEV with laboratory-confirmed acute-phase sera from patients with hepatitis E, as well as with control sera from hepatitis E–free individuals.

MATERIALS AND METHODS

Acute HEV infection. Acute HEV infection was defined based on symptomatic hepatitis with identification of a HEV genome by RT-PCR from plasma and IgM antibodies to HEV > 100 U/mL by the Walter Reed Army Institute of Research (WRAIR) EIA from acute-phase specimens.

Serum samples. The following panel selected from existing collections was used: 1) sera (n = 200) obtained from patients with acute HEV infection in Indonesia and Nepal; 2) sera from patients with other confirmed types of hepatitis: acute hepatitis A (n = 80), acute hepatitis B (n = 45), and acute hepatitis C (n = 50) from Thailand, Nepal, and Indonesia; 3) healthy blood donors from Thailand (n = 100); 4) sera from patients with acute Epstein-Barr virus (EBV) infections (n = 20); 5) rheumatoid factor (RF)–positive blood (n = 26); and 6) convalescent-phase HEV sera collected 10 days or more after acute onset sera were negative for HEV by an RT-PCR (n = 70). All HEV specimens were collected under previous protocols. Written, informed consent was obtained from study participants. These studies were reviewed and approved by the U.S. Army Medical Research and Material Command and its associated institutional review board(s), as well as the institutional review boards of the host countries. Some specimens were submitted from outbreak investigations or routine patient care. These specimens were devoid of any patient identifiers. All control sera including EBV- and RF-positive sera did not contain IgM antibodies to HEV. All samples were stored at −20°C, except for sera from healthy blood donors and RF- and EBV–positive blood, which were stored at −70°C. The assay was not evaluated with whole blood specimens in this study.

Quantitative EIA for IgM to recombinant HEV (rHEV) capsid. Serum analysis for the presence of IgM antibodies to rHEV antigen was performed using a WRAIR EIA, which

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can also be used to quantitate antibody to HEV. In this assay, IgM antibody to rHEV was detected by a sandwich EIA in 96-well plates. The antigen is the putative capsid protein of HEV, Sargodha 1987 strain, expressed in \textit{Spodoptera frugiperda} (SF9) cells by a recombinant baculovirus. More than 100 U/mL of WRAIR IgM to HEV (with an acute hepatitis illness) was considered evidence of an acute HEV infection.

**Detection of HEV by RT-PCR.** An RT-PCR was used to detect the presence of HEV virus RNA.\(^{16,19}\)

**ASSURE™ HEV IgM rapid test.** The immunochromatographic test device intended for the rapid detection of IgM antibodies to HEV in human serum, plasma, or whole blood was provided by Genelabs Diagnostics (Singapore). The test is an IgM capture solid-phase immunochromatographic assay. IgM, when present in the test sample, can be captured by anti-human IgM monoclonal antibodies immobilized on the membrane. A colloidal gold-labeled antibody to HEV is pre-incubated with HEV antigen prior to being embedded on the membrane. In addition, immobilized rabbit IgG antibodies, which can be recognized by colloidal gold-labeled anti-rabbit IgG antibodies, were used as a procedural control. When present, these rabbit IgG antibodies confirm the validity of the assay by formation of a visible red line. In the test procedure, 25 μL of serum (as used in this study) or plasma or 35 μL of whole blood was placed on the sample application well, and the result was read in 15 minutes. The test was performed according to the manufacturer’s instructions and was read blinded to prevent reader bias. The test result was considered positive if both the internal control and the test band were stained, irrespective of the intensity of the staining (Figure 1). The test result was considered negative if only the internal control was visible. The test result was considered invalid if the internal control line was not visible.

**Data analysis.** The sensitivity of this new rapid test was assessed with sera from patients with confirmed HEV (n = 200). Convalescent-phase HEV samples (n = 70) were evaluated separately from the confirmed positive samples because the test is intended only for acute phase specimens. Sera from healthy controls (n = 100), patients with other acute non-HEV virus infections (n = 195), and from RF-positive subjects (n = 26) were used to determine the specificity of the test. For the performance characteristics, the following values were used: true positive (TP), false positive (FP), true negative (TN), and false negative (FN). Sensitivity was calculated as TP/(TP + FN), specificity as TN/(FP + TN), positive predictive value as TP/(TP + FP), and negative predictive value as TN/(FN + TN). Exact confidence intervals (CIs) were calculated by the method of Clopper and Pearson.\(^{20}\)

**RESULTS**

A total of 301 samples (100 healthy donors, 80 acute-phase HAV samples, 45 acute-phase HBV samples, and 50 acute-phase HCV samples, 20 acute-phase EBV samples, and 26 RF-positive samples) were confirmed as negative controls (RT-PCR negative and an HEV IgM titer \(< 100 \text{ U/mL}\)) and 200 acute-phase sera were confirmed as true positive samples (RT-PCR positive and an HEV IgM titer > 100 U/mL). Additionally 70 follow-up samples from convalescent patients following an HEV infection, with negative RT-PCR result for HEV but with IgM titers between 11.3 and 11,885.1 U/mL (mean ± SD = 1,617.5 ± 2,453.1), were analyzed.

In samples collected from acutely ill HEV patients, 186 of 200 tested positive by the rapid test. Table 1 shows the sensitivity and specificity of the rapid test using the HEV IgM seropositive and seronegative samples. The test had a sensitivity of 93% (95% CI = 88.5–96.1%). The specificity was 99.7% (95% CI = 98.3–100%) with HEV-negative samples from healthy donors, as well as other patient samples, including those infected with EBV or hepatitis A, B, or C.

In convalescent-phase specimens obtained an average of 37 days (range = 10–119) after the acute-phase samples, 72.9%

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**FIGURE 1.** a, Schematic diagram of the hepatitis E virus (HEV) rapid test. A = blue line; B = control; C = test line. The blue line is for test operation and will later fade. b, Interpretation of test line: − = nonreactive; +/− = faintly positive; 1+ = clearly positive; 2+ = distinctly positive; 3+ = moderately positive; 4+ = strongly positive. This figure appears in color at www.ajtmh.org.
(95% CI = 60.9–82.8%) still tested positive in the rapid test. In convalescent-phase samples, the percentage of positive results, as well as the intensity of the test lines, significantly decreased with time after acute infection (R = 0.554, P < 0.001). However, even after more than two months, 5 (45.5%) of 11 samples (95% CI = 16.7–76.6%) still tested positive. Of the 26 RF- and 20 EBV-positive sera, all except one EBV serum (97.8%, 95% CI = 88.5–99.9%) tested negative in the rapid test. The positive predictive value for the rapid test was 99.5% (95% CI = 97.1–100%) and the negative predictive value was 95.8% (95% CI = 93.1–97.7%).

Tests were read blinded to eliminate reader bias and the staining intensity of the color band was graded from − to +++ (− = nonreactive; ± = faintly positive; + = clearly positive; ++ = distinctly positive; +++ = moderately positive; ++++ = strongly positive) (Figure 1). The faintly positive bands were read and confirmed by an independent observer. The test was generally simple to interpret with the majority (65.4%) of positive test results (n = 237) showing line intensities between 2+ (distinct) and 4+ (strong). The line intensity of the positive rapid test results corresponded well with the IgM levels measured by an enzyme-linked immunosorbent assay (ELISA) (R = 0.347, P < 0.001). The median IgM level was 656.7 IU/mL in faintly positive test results, 1,616.6 IU/mL in clearly positive test results, 1,641.1 IU/mL in distinctly positive test results, and 2,417.5 IU/mL in strongly positive test results (Figure 2).

**DISCUSSION**

The accurate and efficient diagnosis of acute HEV infection in support of surveillance activities and outbreak investigations in disease-endemic areas remains a great challenge. Health care institutions typically lack HEV diagnostic capabilities. The problem is compounded in rural areas, where electricity and refrigeration for storage of diagnostic test kits, reagents, and specimens may be interrupted or are unavailable.

To simplify the diagnosis of acute HEV infection, a new rapid test for the detection of IgM antibodies to HEV based on immunochromatography has been developed and evaluated with well-characterized sera from HEV-infected patients. Our evaluation showed that the rapid assay has excellent specificity and sensitivity (>90%) compared with our in-house reference assays to diagnose acute HEV infection (Table 1). The sensitivity was related to individual IgM levels because most of the false-negative samples (85.7%, 95% CI = 57.2–98.2%) had IgM levels consistently below the average value (mean for false-negative samples = 679.1 IU/mL compared with the overall mean of 1,617.5 IU/mL). The practicability and reliability of this rapid detection test was investigated with sera collected from three countries in southeast Asia. The rapid assay has a high sensitivity and specificity to detect IgM antibodies to HEV because no cross-reactivity was observed with other hepatitis viruses. Also, most of the acute-phase samples showed a line intensity reading ≥2+ that was easily interpretable.

The performance of the new rapid test was not validated against the commercial kits currently available for the diagnosis of acute HEV infection. Additional evaluations in other HEV-endemic regions are necessary to confirm that this rapid

**FIGURE 2.** Box and whisker plot of hepatitis E virus (HEV) IgM levels (IU/mL) for different rapid test line intensities for all samples that tested positive with the rapid test. Boxplots show the medians and error bars show the interquartile ranges. The color intensity of the positive test line was graded 0.5 (± faintly positive); 1+ (clearly positive); 2+ (distinctly positive); 3+ (moderately positive); 4+ (strongly positive).
test is appropriate for universal use with all strains and does not have regional limitations. However such limitations appear unlikely because the test is based on a recombinant antigen and monoclonal antibody shown to be widely reactive with different HEV strains.2,12 Because of the promising results obtained in the present study, a large field evaluation of the rapid test in a disease-endemic area might be undertaken.

Although not the intended use of the test, according to the manufacturer’s instructions, the sensitivity of the rapid test was also evaluated with convalescent-phase serum samples from patients with hepatitis E (n = 70). Due to an obvious decrease in IgM levels, the sensitivity of the rapid test in detecting convalescent-phase cases was lower (72.9%, 95% CI = 60.9–82.8%) than for acute-phase samples. However, all of these cases tested negative in the RT-PCR and 8 (11.4%, 95% CI = 5.1–21.3%) cases also had IgM levels not exceeding 100 U/mL and would therefore also test negative in an IgM ELISA. More importantly, the rapid test showed no cross-reactivity with RF serum, which commonly causes interference in serologic tests. Only 1 of 20 EBV sera showed a false-positive result. The rapid test was not evaluated with acute-phase secondary cases of hepatitis E with a predominant IgG antibody response. Also, the sensitivity of the rapid test could not be evaluated with onset of illness because this information was missing in most cases.

The rapid test is also ideal for use in developing countries and rural settings because this requires no laboratory infrastructure or highly skilled personnel, and can also be used under field or outbreak conditions and in situations where appropriate diagnostic facilities are lacking. Also, it does not require extensive training of the operator or cold storage and can be conducted with whole blood samples. We conclude that this new chromatographic test will be a reliable and rapid assay for the clinician or public health worker in developing countries, as well as in outbreak investigations.

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