Multicentric Evaluation of New Commercial Enzyme Immunoassays for the Detection of Immunoglobulin M and Total Antibodies against Hepatitis A Virus

M. C. Arcangeletti, 1* E. Dussaix, 2 F. Ferraglia, 1 A. M. Roque-Afonso, 2 A. Graube, 2 and C. Chezzi 1

Department of Pathology and Laboratory Medicine, University of Parma, Parma, Italy, 1 and National Reference Center for Hepatitis A, Paul Brousse Hospital, Villejuif, France 2

Received 21 September 2010/Returned for modification 11 November 2010/Accepted 27 May 2011

A multicentric clinical study was conducted on representative sera from 1,738 European and U.S. subjects for the evaluation of new anti-hepatitis A virus enzyme immunoassays from Bio-Rad Laboratories. Comparison with reference DiaSorin S.p.A. tests confirmed the good performance of Bio-Rad assays (99.85% and 99.47% overall agreement in detecting total antibodies and IgM, respectively).

The etiological agent of hepatitis A is a nonenveloped, positive-stranded RNA virus that is typically transmitted via the fecal-oral route (4).

Hepatitis A virus (HAV) infection is still the most common cause of acute viral hepatitis worldwide and remains a serious health problem not only in the developing world, but also in industrialized countries (6, 10). It can lead to a multiplicity of clinical features, ranging from asymptomatic infection to fulminant fatal disease (16).

Over the last decades, improvement in the living conditions of several world populations has prevented young people from acquiring asymptomatic infection (which provides lifelong protection), leaving them susceptible to hepatitis A infection (2, 4). With regard to adults, certain categories of people, such as men who have sexual intercourse with men, both injection and noninjection drug users, and travelers returning from developing countries, are exposed to a higher risk of infection and therefore are subjected to cyclic outbreaks of hepatitis A (1, 3, 8, 11, 12, 15, 17, 18). It is also worth mentioning that HAV-infected persons with chronic liver disease are likely to develop fulminant hepatitis A (14, 16).

This global scenario of hepatitis A epidemiology calls for an increase in research to be carried out in the coming years and for measures to be taken to control and prevent the spread of this infectious disease. In particular, vaccination against hepatitis A is recommended for all the aforementioned high-risk categories of people and represents an important tool in the prevention of virus spread and epidemic outbreaks (5).

Laboratory diagnosis of hepatitis A is based mainly on the detection of antibodies associated with acute and past infection (IgM and IgG, respectively); complementary tests can be used in some cases for the diagnosis of recent infection (9, 13). In prevaccination programs, however, the detection of total anti-HAV antibodies is also critical for establishing whether individuals have acquired immunity and for identifying those susceptible to HAV infection.

This study aimed to evaluate the performance of two novel anti-HAV enzyme immunoassays (EIA) developed by Bio-Rad Laboratories (the Monolisa anti-HAV IgM EIA and the Monolisa anti-HAV EIA) and to compare their results to those obtained using the FDA-approved ETI-AB-HAV IgM Plus and ETI-AB-HAVK Plus assays from DiaSorin S.p.A.

The tests were performed and the results interpreted according to the manufacturers’ instructions. Relative sensitivity and specificity, as well as agreement between the Bio-Rad and DiaSorin assay results, were calculated, with unresolved equivocal (concordant and discordant borderline) data excluded; 95% binomial confidence intervals (CI) were applied to the results.

The study was conducted on representative serum samples from 1,738 European and U.S. subjects from Parma University Medical School in Italy (n = 586), Paul Brousse Hospital in France (n = 366), and the United States (n = 786).

A retrospective study (to detect total antibodies and IgM) was performed on 235 sera (stored at −20°C) that had been collected from a population with a known hepatitis A status: 84 acutely infected, HAV IgM-positive patients and 151 formerly infected, HAV IgG-positive patients who had recovered.

A prospective study (to detect total antibodies and IgM) was conducted on 1,097 sera from subjects with an unknown hepatitis A status. This population consisted of the following categories: general hospitalized patients (345 Europeans), patients with symptoms of hepatitis (n = 426; 252 Europeans and 174 U.S. individuals), subjects at high risk for hepatitis A (n = 292; 62 Europeans and 230 U.S. individuals), and health care workers (34 Europeans).

A prevalence study (for total antibodies) was performed on 755 subjects (the above-mentioned European groups of 345 general hospitalized patients and 34 health care workers and a further 376 general hospitalized U.S. individuals) whose conditions were not related to hepatitis infections and who were representative of the healthy population.

Total HAV antibody response was also evaluated in 30 subjects who had received one of the three vaccines licensed in the

---

* Corresponding author. Mailing address: Microbiology Section, Department of Pathology and Laboratory Medicine, University of Parma, Viale Antonio Gramsci 14, 43126 Parma, Italy. Phone: 39 0521 033499. Fax: 39 0521 993620. E-mail: mariacristina.arcangeletti@unipr.it.

† Published ahead of print on 8 June 2011.
The relative sensitivities of the Monolisa anti-HAV EIA (versus ETI-AB-HAVK Plus) and the Monolisa anti-HAV IgM EIA (versus ETI-HA-IgMK Plus), determined with the samples included in the retrospective and prospective studies, were 100% in both cases (reactive for total antibodies, 933/933 samples [CI, 99.61% to 100%]; reactive for IgM, 87/87 samples [CI, 95.85% to 100%]). The relative specificities were 100% in both cases (reactive for total antibodies, 933/933 samples [CI, 99.49% to 99.94%]; reactive for IgM, 87/87 samples [CI, 98.16% to 99.94%]). The overall agreement was 99.85% (1,235/1,242 samples [CI, 99.47% to 99.98%]) for total antibodies and 99.44% (1,323 samples [CI, 99.61% to 100%]) for IgM antibodies. The relative specificities were 99.49% (388/390 samples [CI, 98.16% to 99.94%]) for total antibodies and 99.44% (1,325/1,329 samples [CI, 99.46% to 99.98%]) for total antibodies and 99.47% (1,322/1,329 samples [CI, 98.92% to 99.77%]) for IgM.

HAV antibody prevalence in subjects representative of the healthy population (health care workers and patients hospitalized with conditions unrelated to hepatitis virus infection) was estimated with the Monolisa anti-HAV EIA for Europe (n = 379) and for the United States (n = 376) (Fig. 1). The results obtained from the above-named populations show that prevalence is higher in Europe than in the United States and that, mostly in the former case, it is related to the subjects’ age range, with older individuals demonstrating progressively higher prevalences, in accordance with data obtained by other authors (7).

The HAV total antibody response using the Monolisa anti-HAV EIA and ETI-AB-HAVK Plus was also evaluated in subjects who had received one of the three vaccines licensed in the United States (Vaqta, Havrix, or Twinrix vaccine). It is important to note that only a limited number of vaccinated subjects develop a transient IgM anti-HAV response (13), while immunized people always produce IgG anti-HAV. This
is why only total antibodies were assayed in the evaluation of samples from vaccinated populations.

Postvaccination samples from 6 subjects who had received the Vaqta vaccine were assayed, and both tests found all samples to be reactive (data not shown). With regard to the Havrix vaccination, the HAV total antibody response was evaluated in 10 pre- and postvaccination samples (2-dose schedule). Once again, coincident results were found with both tests, showing a correct improvement of the HAV antibody response in postvaccination samples (not shown). For the evaluation of Twinrix (3 injections at 0, 1, and 6 months), 14 subjects were examined. A prevaccination sample was collected the day of the first vaccination dose, and a second sample was obtained before the second dose was injected (1 month after the first one), but the second and third vaccination dose samples were not available. With regard to the Monolisa anti-HAV EIA (Fig. 2), the results show a slight increase in the HAV antibody response after the first injection in the majority of the subjects enrolled in the study (subject 14 was already immunized against HAV before the first vaccination dose); there were 4 cases (subjects 5, 9, 10, and 13) that were found negative after the first dose. The ETI-AB-HAVK Plus assay gave identical results, except for the borderline values of postvaccination samples from subjects 10 and 13 (data not shown).

In summary, data derived from this multicentric study indicate that the Monolisa anti-HAV IgM and anti-HAV (total Ig) EIA (Bio-Rad Laboratories) are specific and sensitive assays which may be used effectively in the laboratory diagnosis of acute or past HAV infection and for the identification of HAV-susceptible individuals who should be enrolled in vaccination programs. Their performance is comparable with that

### TABLE 2. Analysis of discrepant IgM samples from subjects with a known and an unknown hepatitis A status

<table>
<thead>
<tr>
<th>Specimen category</th>
<th>Demographics (gender, age [yr])</th>
<th>Monolisa anti-HAV IgM EIA</th>
<th>ETI-AB-HAV-IgMK Plus</th>
<th>Complementary information</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Ratio&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Interpretation&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Sample OD</td>
</tr>
<tr>
<td>Formerly infected/recovered population</td>
<td>F, 59</td>
<td>1.13</td>
<td>R</td>
<td>0.06</td>
</tr>
<tr>
<td>General hospitalized population</td>
<td>F, 76</td>
<td>1.2</td>
<td>R</td>
<td>0.087</td>
</tr>
<tr>
<td>Symptoms of hepatitis population</td>
<td>M, 42</td>
<td>1.45</td>
<td>R</td>
<td>0.122</td>
</tr>
<tr>
<td></td>
<td>M, 42</td>
<td>1.18</td>
<td>R</td>
<td>0.114</td>
</tr>
<tr>
<td></td>
<td>M, 42</td>
<td>1.14</td>
<td>R</td>
<td>0.149</td>
</tr>
<tr>
<td></td>
<td>F, 18</td>
<td>4.08</td>
<td>R</td>
<td>0.037</td>
</tr>
<tr>
<td></td>
<td>F, 18</td>
<td>2.97</td>
<td>R</td>
<td>0.046</td>
</tr>
<tr>
<td></td>
<td>F, 18</td>
<td>1.22</td>
<td>R</td>
<td>0.18</td>
</tr>
<tr>
<td></td>
<td>F, 18</td>
<td>1.29</td>
<td>R</td>
<td>0.144</td>
</tr>
<tr>
<td></td>
<td>F, 18</td>
<td>3.28</td>
<td>R</td>
<td>0.025</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NE</td>
<td></td>
<td>NE</td>
</tr>
</tbody>
</table>

<sup>a</sup> F, female; M, male; R, reactive; NR, nonreactive; BRD, borderline; NE, not evaluated; OD, optical density; COD, cutoff OD.

<sup>b</sup> Results are expressed as the ratio of the sample OD to the COD.

<sup>c</sup> The presence or absence of anti-HAV IgM antibodies (Monolisa anti-HAV IgM EIA noncompetitive test) was determined as follows: specimens with ratio values greater than or equal to 1.1 were considered reactive (antibodies present) and those with ratios less than 0.9 as nonreactive (antibodies absent). Specimens with ratios greater than or equal to 0.9 times the COD value and less than 1.1 times the COD value (0.9 ≤ ratio < 1.1) were considered to be equivocal (borderline). For each run, the COD was calculated for each run as the mean absorbance of three calibration values (which must be greater than the positive-control OD and less than 0.6 of the COD value) and less than 60% of the COD value.

<sup>d</sup> For each run, the COD was determined by adding 0.250 to the mean of the calibrator absorbance values (which must be greater than 0.6 and less than 2.9) of the positive cutoff calibrator divided by 4.

<sup>e</sup> The presence or absence of anti-HAV IgM antibodies (ETI-AB-HAV-IgMK Plus noncompetitive test) was determined by comparing the OD value of the patient serum with the COD value. Patient specimens with absorbance values less than the COD value (and not within 20% of the COD) were considered nonreactive and those with values greater than the COD as reactive. Absorbance values ranging between 0 and 20% above the COD were considered equivocal (borderline).

<sup>f</sup> The presence of HAV RNA was evaluated using a one-step reverse transcription (RT)-PCR kit (Qiagen) amplifying a 512-bp fragment that encompasses the VP1/2A junction of the HAV genome. The sensitivity of the RT-PCR assay was 43 IU/ml, as assessed with serial dilutions of the World Health Organization HAV RNA standard.

<sup>g</sup> The presence of total HAV antibodies was confirmed using the DiaSorin ETI-AB-HAVK Plus (competitive format) reference immunoassay. In this case, the COD was calculated for each run as the mean absorbance of three calibration values (which must be greater than the positive-control OD and less than 60% of the negative-control OD). The presence or absence of anti-HAV antibodies was determined by comparing the OD value of the patient serum with the COD value; absorbance values less than the COD were considered reactive, and those with values greater than the COD (and not from 0 to 20% above the COD) nonreactive. Absorbance values ranging between 0 and 20% above the COD were considered equivocal (borderline).

### FIG. 1. HAV antibody prevalence in Europe (n = 379) and in the United States (n = 376) using the Monolisa anti-HAV EIA. The study was performed on subjects representative of the healthy population (health care workers and patients hospitalized with conditions unrelated to hepatitis virus infection). For each age range category, the number of reactive subjects (with total anti-HAV antibodies present) over the total number of subjects is displayed on each histogram.
of the ETI-AB-HAVK and IgMK Plus tests from DiaSorin S.p.A. The Bio-Rad Monolisa anti-HAV IgM and anti-HAV (total Ig) EIA thus represent valid alternative choices, with the shorter time required to perform the Bio-Rad tests being an additional advantage. The Bio-Rad assays have also been cleared by the FDA.

The few discrepant results (positive with the Bio-Rad versus negative with the DiaSorin tests), mostly related to IgM detection, could be due to a slightly higher sensitivity of the Bio-Rad assays, permitting the detection of small amounts of IgM that in some cases could persist after the acute stage; however, the possibility of false-positive results cannot be ruled out.

This work was supported by Bio-Rad Laboratories, Marnes La Coquette, France.

The results and conclusions in this report are those of the authors and do not necessarily represent the views of the funding manufacturer.

REFERENCES