Two RIA Tests for the Diagnosis of Hepatitis A

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Case history
A 27-year-old man was diagnosed by his private physician as having acute hepatitis A one week after being hired as a food handler at a large hotel. During that week he had been ill with "flu-like" symptoms and had only assisted in preparing salads for a large convention dinner. This dinner had been attended by 163 people, all of whom were subsequently contacted by the Department of Public Health. They were instructed that they had potentially been exposed to hepatitis A and should receive prophylactic immune serum globulin (ISG).

Of these 163, 47 refused ISG prophylaxis and three people in this group subsequently developed symptomatic hepatitis A. Family contacts of these three individuals received ISG and no subsequent cases were identified. No symptomatic cases of hepatitis A were observed in the group that received prophylactic ISG.

Background
Hepatitis A is caused by a non-enveloped, 27-nm, RNA virus with biophysical characteristics that place it in the enterovirus group of picornaviruses. Although the hepatitis A virus (HAV) is usually acquired by the fecal-oral route, several recent reports have documented cases secondary to blood transfusion. The incubation period ranges from 15 to 45 days with a mean of 30 days and the icteric phase lasts from one to three weeks. During the late incubation and the acute preicteric phases, the virus can be recovered from blood, bile, stools, and other body secretions. In general, complete recovery is the rule and the mortality rate is less than 1 percent. There is no apparent carrier state or association with chronic liver disease. Infection confers lifelong immunity to reinfection.

Hepatitis A has a worldwide distribution and 20,000 to 30,000 cases are reported in the United States each year. Seroprevalence studies reveal 40 to 50 percent antibody positivity in the adult populations of Europe and the United States, however, higher rates are seen in third world countries. The majority (>80%) of antibody positive individuals do not recall having had hepatitis and the ratio of inapparent to apparent cases is believed to be about 8:1.

Viral replication occurs primarily in hepatic parenchymal cells, which accounts for the clinical manifestations of the disease. A graphic representation of the course of hepatitis A is shown in Fig. 1. Several features of this figure should be noted: (1) viral excretion in stool occurs prior to the onset of symptoms and peaks prior to or in conjunction with any demonstrable laboratory abnormalities; (2) viral excretion decreases fairly rapidly after the appearance of IgM (and IgA) antibodies; (3) alanine aminotransferase (ALT) levels peak after the period of maximal viral excretion and parallel the IgM response; and (4) serum IgG antibodies appear relatively late and remain at detectable levels for years after infection. (In some persons infected at an early age, IgG levels may decline to undetectable levels using available assays.)

The clinical manifestations of hepatitis A differ depending on the age of the patient. In adults with symptomatic disease the symptoms/signs most often encountered are: jaundice (88 percent), dark urine (68 percent), malaise (63 percent), and light-colored stools (58 percent). In children the symptoms are similar, but jaundice is seen with slightly less frequency (65 percent) and nausea/vomiting is seen more often (65 percent). The symptoms in neonates are extremely nonspecific and consist of diarrhea, vomiting, and failure to thrive. Jaundice in the neonatal group is seldom noted in connection with hepatitis, and when present the jaundice is usually attributed to other causes.

Testing
Before the advent of serologic testing for hepatitis A, the diagnosis was
generally made on the basis of clinical findings, history (i.e. no recent transfusions), and serum transaminase levels. Occasionally, in research or epidemiologic settings, immuno-electron microscopy to detect viral particles in stool may be of use. Yet this technique is extremely time-consuming and too expensive for use in clinical diagnosis. Serum aminotransferase levels, although perhaps useful in monitoring patients with hepatitis, are nonspecific indicators and may be elevated in a variety of both hepatic and non-hepatic diseases.

Early serologic tests for antibodies to HAV utilized complement fixation and immune adherence hemagglutination (IAHA) techniques. While these tests displayed high sensitivity and specificity, both were unable to discriminate between acute infection and immunity related to past infection. Several methods for the separation of IgG from IgM antibodies were developed using IgM blocking (anti-IgM), bacterial absorption, or gradient centrifugation techniques. These latter methods were able to diagnose acute disease, but they were too cumbersome for routine laboratory use.

HAVAB test

The HAVAB test (Abbott Diagnostics) is a competitive solid phase radioimmunoassay (RIA) which detects both IgG and IgM antibodies to HAV. In the test procedure approximately 10 μL of patient serum is added to a microtiter well along with 200 μL of a suspension of radiolabeled anti-HAV antibody. A polystyrene bead coated with viral antigen is then added to the well and allowed to react for approximately 20 ± 2 hours at room temperature. (An optional four-hour incubation at 45 C may also be used with equivalent sensitivity.) The beads are then washed and counted in a gamma counter. Using the counts per minute of the positive and negative control wells, a "cutoff" value is obtained using the following formula:

\[
\text{Positive cpm} \div 10 + \text{Negative cpm}
\]

A specimen is "positive" if the cpm obtained is less than this cutoff value. (In a competitive RIA test, the lower the cpm the higher the titer of antibody in the patient specimen.) Specimens with counts within a 10 percent range of the cutoff value should be retested to confirm the initial result.

In comparison studies this assay was shown to be more sensitive and specific than electron microscopy or IAHA. Furthermore, this HAVAB test could detect anti-HAV antibodies several weeks before the IAHA assays became positive. In a study in which volunteers were inoculated with HAV, anti-HAV was detectable by RIA five days after the onset of symptoms (35 days after exposure). The IAHA tests in this study did not become positive until 30 days later. This difference is explained by the fact that the HAVAB test detects both early and late antibodies (IgG and IgM), while the IAHA tests detect only the late (IgG) antibody response.

HAVAB-M test

The HAVAB-M test (Abbott Diagnostics) is a solid phase RIA specific for anti-HAV of the IgM class. In this assay, 10 μL of a 1:200 dilution of patient serum in normal saline is placed in a microtiter well along with 200 μL of diluent. A polystyrene bead coated with goat anti-human IgM is then placed in the well and incubated at room temperature for 2 hours ± 5 minutes. The bead is washed and 200 μL of a suspension of HAV antigen is added and allowed to incubate for 18 to 22 hours at room temperature. The bead is again washed and 200 μL of a suspension of radiolabeled anti-HAV is added and incubated at 45 C for four hours. The beads are then washed and counted in a gamma counter.

Using the counts per minute of the positive and negative controls, a "cutoff" value is obtained using the following formula:

\[
\text{Positive cpm} \div 10 + \text{Negative cpm}
\]

Specimens with counts greater than or equal to the cutoff value are positive and counts less than this value are negative. Counts within 10 percent of the cutoff value should be repeated to confirm the initial result.

Studies with volunteers have shown that the HAVAB-M test becomes positive within a week after symptoms develop in patients with normal immune function. The test is specific for hepatitis A and sera from patients with other viral infections such as rubella, EBV, and HBV, and alcoholic hepatitis are nonreactive. Serum from patients with rheumatoid factors are generally nonreactive, but one recent
study described false-positive results in patients with a non-IgM, rheumatoid factor-like substance, although this has not been confirmed.

**Test utilization**

The HAVAB test, which detects both IgG and IgM antibodies to HAV, is generally useful only for determining the immune status of an individual with respect to HAV. Given the high incidence of IgG antibody positivity in the adult population, this test should not be used by itself in the diagnosis of acute hepatitis. In the proper clinical setting with supportive laboratory abnormalities and in a young patient, a positive test would be very suspicious for acute disease. Nevertheless, even in this instance the diagnosis should be confirmed with the HAVAB-M assay. A positive test would be considered diagnostic only for a patient with a recent HAVAB test that had been negative.

The HAVAB test may be of use in evaluating the immune status of persons at risk of exposure to hepatitis A and thereby determining the need for ISG prophylaxis. Yet since the cost and risk of ISG prophylaxis are relatively low, such testing should be reserved for specific individuals and would not be appropriate for routine screening of large numbers of possible contacts.

The sensitivity and specificity of the HAVAB-M assay makes it an excellent test to confirm the diagnosis of acute or subacute hepatitis A. As a confirmatory test, however, its use should be restricted to those patients who have symptoms and laboratory abnormalities compatible with the diagnosis of hepatitis A (i.e. elevated aspartate aminotransferase [AST], ALT and/or bilirubin levels). In addition, testing for other diseases such as hepatitis B should be performed along with the HAVAB-M test.

**Prospective screening**

In the setting of a nosocomial or focal outbreak of hepatitis A, the major goal is to limit the spread of disease as rapidly and completely as possible. Due to the estimated inapparent to apparent case ratio of 10:1, there is concern in such outbreaks that laboratory surveillance of the exposed population is needed to ensure that all cases are identified. The use of the HAVAB-M assay in such a prospective testing protocol may therefore appear worthwhile. Epidemiologic studies of such outbreaks, however, demonstrate that the most important factors in limiting such an epidemic are: (1) identification and isolation of the source or index case, (2) institution of enteric precautions, and (3) prophylactic administration of ISG to exposed individuals. Once these measures are undertaken the incidence of new cases decreases dramatically (usually to less than 3 percent of the individuals remaining at risk develop acute disease). This would indicate that, even though a potentially large number of inapparent cases may occur, failure to identify them does not contribute significantly to the overall spread of the disease. This conclusion is supported by a study of an outbreak of hepatitis A in a Navy training facility. In this study, examination of 43 stool specimens from 19 anicteric patients failed to reveal any virus particles, whereas HAV was found in 29 percent of stool samples taken from icteric patients exposed at the same time.

Similar concerns have been expressed over the use of prophylactic ISG. It has been shown that ISG administration during the incubation period may attenuate the symptoms of hepatitis A, while not actually preventing infection. In this way ISG provides "passive-active" immunization against HAV while increasing the number of inapparent cases of disease. Even though the individuals in such cases are infected, it is likely—as in other inapparent infection—that they excrete virus in low titers and therefore have a low potential for infectivity. The paucity of secondary cases of hepatitis attributable to individuals who have received ISG supports this assumption.

Finally, the potential cost of such a screening protocol must be considered. A recent study at the University of California, Davis Medical Center examined the costs of such prospective testing protocols. The potential cost per positive diagnosis ranged from $2,100 to $21,000 depending on the nature of the strategy chosen. Therefore, use of the HAVAB-M assay to identify cases of inapparent disease would probably have little impact on the extent of an epidemic and would be prohibitively expensive.

In conclusion, the HAVAB test is useful to the clinician who wants to establish the immune status of a patient, but generally has little other utility. The HAVAB-M assay is useful for confirming or excluding the diagnosis of acute hepatitis A in a patient with compatible clinical symptoms or laboratory abnormalities, but as with other confirmatory tests it is not suitable for prospective or routine screening of exposed populations.

**References**

12. Dumuwrey W, Wieland F, van der Veen J: A new principle for the detection of spe...