Indirect Immunofluorescence Assay for the Detection of Hepatitis A Virus-Specific Serum Immunoglobulins

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Hepatitis A virus-infected BSC-1 cells were used for the detection of serum immunoglobulins to hepatitis A virus by indirect immunofluorescence. Of 150 serum samples tested, specific immunoglobulin M was detected only in patients with serologically confirmed acute hepatitis A, while specific immunoglobulin G was detected in patients with acute or past clinical hepatitis A as well as many patients with no known history of hepatitis.

Hepatitis A is a common infection of humans caused by hepatitis A virus (HAV), an Enterovirus within the family Picornaviridae (3). Rapid diagnosis of acute hepatitis A is important for the management of patients and their contacts, in the investigation of outbreaks, and in assisting in the diagnosis of non-A, non-B hepatitis. The detection of rising titers of HAV-specific serum immunoglobulin G (IgG) or the presence of IgM by radioimmunoassay (1, 10) or enzyme immunoassay (6, 8) are the most common methods used for the diagnosis of hepatitis A. The production of sufficient reagent HAV for these assays requires large-scale growth and purification of HAV, although the availability of monoclonal antibodies to HAV (1, 7) has reduced the level of purity required.

HAV generally produces a persistent infection of susceptible cells in vitro (11), with continual production of virus and minimal destruction of cells (2, 11). Most of the virus present in infected cells remains tightly cell associated (9, 11), leading to difficulties in the extraction and purification of HAV from contaminating material. Despite the low yield of HAV in cell cultures, hepatitis A antigen (HAAg) can readily be detected by indirect immunofluorescence in BSC-1 cells acutely or persistently infected with HAV strain HM175 (4). This system was examined as an alternative method for the detection of HAV-specific serum IgG and IgM.

BSC-1 cells were cultivated in minimal essential medium containing 5% fetal calf serum. All cultures were incubated at 34°C. Cultures persistently infected with HAV, designated BSC-1-HM175, were obtained from S. M. Feinestone, National Institutes of Health, Bethesda, Md., and were subcultured for 14 to 28 days. Culture supernatant fluid from BSC-1-HM175 cells in subculture 12 contained 10,000 infectious units per ml, as determined by radioimmunofocus assay (5) on BSC-1 cells under minimal essential medium containing 2% fetal calf serum and 0.5% Noble agar (low ash) (results not shown), and was used as the infectious stock for these studies.

Confluent monolayers of BSC-1 cells in eight-chamber tissue culture chamber slides (Miles Scientific, Div. Miles Laboratories, Inc., Naperville, Ill.) were washed with phosphate-buffered saline (PBS) (pH 7.4), infected with 0.3 ml of BSC-1 or BSC-1-HM175 culture supernatant for 4 h at 34°C, and then washed and refed with 0.3 ml of minimal essential medium containing 2% fetal calf serum for a further 3 days.

Monolayers were then washed with PBS and fixed in acetone for 2 min at 4°C. After being air dried for 3 h, the fixed slides were stored in an airtight container at 4°C until use. For indirect immunofluorescence, the slides were reacted with 50 μl per well of either monoclonal antibody K3-4C8 (7) at a concentration of 25 μg/ml or dilutions of human sera in PBS for 30 min at 34°C. The slides were washed in two changes of PBS for a total of 30 min, stained with a 1:40 dilution of fluorescein-labeled antibodies to mouse immunoglobulins (Dako) or fluorescein-labeled, affinity-purified antibodies to human IgG or IgM (Kirkegaard and Perry) for 30 min at 34°C, and then washed twice in PBS as described above. The slides were then mounted in PBS (pH 8.2) containing 80% (vol/vol) glycerol and examined at a magnification of 320× with a Zeiss standard microscope with an incident-light condenser and an HBO 50 light source.

When fixed at 3 days after infection, 40 to 80% of the cells in HAV-infected wells showed specific immunofluorescence when reacted with K3-4C8 (results not shown) or human convalescent-phase hepatitis A serum at a 1:40 dilution in PBS and fluorescein-labeled antibody to human IgG (Fig. 1B). No specific fluorescence was detectable in uninfected BSC-1 cells under these conditions (Fig. 1A). When incubation of the cells was continued for longer than 3 days, a gradual reduction in the intensity of HAAg staining was observed, although a greater proportion of the monolayer was infected. Fixed slides have been stored at 4°C for 6 months without any appreciable change in reactivity.

Using cultures fixed at 3 days after infection, we examined serum samples collected at 1, 2, 4, 8, 12, and 16 weeks after the onset of dark urine from a patient with acute hepatitis A for the presence and titers of HAV-specific IgG and IgM (Fig. 2). HAV-specific IgG was detected in all samples, with a 32-fold increase in titer from weeks 1 to 4 after onset (Fig. 2A), establishing the infection as hepatitis A. HAV-specific IgM was detected at week 1, increased twofold in week 2, fell rapidly to a titer of 1:40 after 4 weeks, and was not detected after this time (Fig. 2B). However, a comparison of the relative titers of HAV-specific IgG and IgM after 4 weeks (10,240 and 40, respectively) suggested that this may have been due to saturation of the HAAg by the excess HAV-specific IgG. Accordingly, when sera were mixed with 3 volumes of a 10% suspension of Formalin-fixed Staphylococcus aureus cells (Immunoprecipitin G; Bethesda Research Laboratories, Inc., Gaithersburg, Md.) for 30 s at 24°C to remove IgG before dilution, IgM was detected at a

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distinguished from HAV-specific reactions solely by the distribution of fluorescence, which is strictly cytoplasmic and generally perinuclear for HAV-specific immunofluorescence. The results for the 150 serum samples examined are shown in Table 1.

Under the conditions used, the indirect immunofluorescence test detected HAV-specific IgM in 100% (24 of 24) of patients with acute hepatitis A and in none of the 126 others. HAV-specific IgG was detected in 100% (24 of 24) of patients with acute hepatitis A, 91% (21 of 23) of patients known to have recovered from hepatitis A, and 33% (10 of 30) of apparently healthy blood donors, who were also positive for HAV-specific IgG in the HAVAB radioimmunoassay for IgG.

The amount of virus-infected cell culture used in this indirect immunofluorescence assay is very much less than that required to produce sufficient reagent HAAg for radioimmunoassay or enzyme immunoassay, since intracellular HAAg is fixed in situ rather than being extracted from cells and purified. Culture supernatants and extracts from the small area of cells cultivated for this assay were generally below the detection limit for HAV in a sensitive radioimmunoassay with monoclonal antibodies (1; results not shown).

In summary, the indirect immunofluorescence assay described appears to be sufficiently sensitive and specific to be useful as a diagnostic test for the presence of serum immunoglobulins to HAV and requires less cell culture and virus purification to produce reagent HAAg than do other assays in common use.

![Image](http://jcm.asm.org/)

FIG. 1. Indirect immunofluorescence of BSC-1 cells mock infected (A) or infected with HAV strain HM175 (B). Cells were stained with serum collected 8 weeks after the onset of dark urine and with fluorescein-labeled antibody to human IgG. Note the specific, granular, cytoplasmic fluorescence in HM175-infected cells.

twofold higher titer at weeks 1, 2, and 4 and was detectable at 8 weeks after the onset of dark urine (Fig. 2B). Titration of these samples with commercial kits designed for the detection of HAV-specific IgG and IgM (HAVAB and HAVAB-M, respectively; Abbott Laboratories, North Chicago, Ill.) yielded similar results (results not shown).

A panel of 150 serum samples representing apparently healthy blood donors (including sera containing rheumatoid factor), patients with acute or convalescent hepatitis A or hepatitis B, and patients with other acute viral infections was screened under code for HAV-specific IgG and IgM. Aliquots (10 μl) of each serum sample were either mixed with 30 μl of Immunoprecipitin G for 30 s or untreated and were then diluted to 400 μl with PBS. Bacterial cells were removed by centrifugation for 2 min in an Eppendorf microcentrifuge, and 50-μl samples were then added to parallel wells containing uninfected or HM175-infected BSC-1 cells and stained for indirect immunofluorescence as described above. HM175-infected wells were then examined for immunofluorescence, and the specificity of any positive reactions was checked in the parallel control well. This was particularly important when preparations from patient serum samples containing rheumatoid factor were stained with fluoresceinlabeled antibody to human IgM, although most could be

![Image](http://jcm.asm.org/)

FIG. 2. HAV-specific serum immunoglobulins determined by indirect immunofluorescence. Serum samples collected from a patient at the indicated times after the onset of dark urine were titrated under code for the level of HAV-specific IgG (A) or IgM (B). IgM was determined in samples before (●) and after (○) the removal of IgG as described in the text. Note the increased sensitivity of the test for IgM after the removal of competing IgG.


<table>
<thead>
<tr>
<th>Patient group* (no. of sera tested)</th>
<th>No. (%) of sera positive by indirect immunofluorescence</th>
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<tbody>
<tr>
<td></td>
<td>IgG</td>
</tr>
<tr>
<td>Acute hepatitis A (24)</td>
<td>24 (100)</td>
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<tr>
<td>Convalescent hepatitis A (23)</td>
<td>21 (91)</td>
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<tr>
<td>Healthy blood donors</td>
<td></td>
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<tr>
<td>Positive by HAVAB (10)</td>
<td>10 (100)</td>
</tr>
<tr>
<td>Negative by HAVAB (20)</td>
<td>0</td>
</tr>
<tr>
<td>Acute hepatitis B (21)</td>
<td>NTb</td>
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<tr>
<td>Other viral infections (52)</td>
<td>NT</td>
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</table>

* Patient groups were defined according to HAVAB, HAVAB-M, and AUSRIA II (for hepatitis B; Abbott Laboratories) results and clinical features.

b NT, Not tested.

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LITERATURE CITED


