

# LABORATORY DETECTION OF HEPATITIS A VIRUS IN STOOLS

Pages with reference to book, From 96 To 100

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## Abstract

The serum sample and serial stool specimens of an adult patient suffering from Hepatitis A were tested for serological markers of acute hepatitis by enzyme linked immunosorbent assay (ELISA) technique and presence of hepatitis A virus (HAV) by various methods namely, solid phase radioimmunoassay (SPRIA) and molecular hybridisation utilizing c DNA recombinant technique. In addition stools from 2 owl monkeys experimentally infected with HAV were also studied by similar methods. The stool samples from the patient revealed an irregular shedding of HAV upto 3 weeks after the onset of jaundice as detected by SPRIA and molecular hybridisation techniques. In the light of present work and other reported studies, the stools of a patient of hepatitis A should be considered infectious to other persons upto 3—4 weeks after the onset of appropriate measures adopted (JPMA 38: 96, 1988).

## INTRODUCTION

Hepatitis A infection is global in distribution with high prevalence in the developing countries<sup>1,2,3</sup>. The causative agent of hepatitis A was identified less than 15 years ago<sup>4</sup>. More recently the successful propagation of hepatitis A virus (HAV) in cell culture<sup>5</sup> and molecular cloning of HAV genome<sup>6</sup> have led to better understanding of HAV — host cell interaction and the nature of hepatitis A virus. The pathogenesis of hepatitis A remains largely unknown but some understanding has been gained from a limited number of experimental infections involving humans<sup>7,8,9</sup> and several susceptible non-human primate species i.e., chimpanzees, marmosets and owl monkeys<sup>10,11,12</sup>. A brief period of viraemia has been shown to precede the onset of hepatitis and faecal shedding of overt symptoms of hepatitis and appropriate precautions found to be maximum during the late incubation period, just prior to or shortly after the onset of liver disease<sup>13,14,15</sup>. Some of the later reports have shown that HAV may be detected for a variable period of upto 3 weeks in stools even after the appearance of jaundice<sup>16,17,18</sup>. This study was conducted to assess the faecal shedding of hepatitis A virus in stools after the appearance of overt symptoms of jaundice in a patient suffering from hepatitis A.

## MATERIALS AND METHODS

### Materials:

Ten stool samples were collected from a patient suffering from hepatitis A. The stools were collected daily from 26 February to 5 March 1986 and kept at —20°C until studied. The patient was first seen in a hospital on February 13, 1986 He appeared jaundiced and had abnormal liver function tests with raised transaminases. The serology for acute hepatitis A was positive (1gM anti-HAV) while the serological markers of acute hepatitis B were negative. Twelve stool samples of 2 owl monkeys experimentally infected with hepatitis A were also studied similarly. These animals had previously been infected with HAV strain HM-175. The faecal matter included in this study represents the stool

specimens collected from these two owl monkeys upto 3 weeks after inoculation of virus.

### **Methods:**

Ten percent stools dilutions were prepared in normal saline and clarified at 10,000 rpm. On the 10 K clarified stools following tests were carried out:-

#### **1. Solid Phase Radioimmunoassay (SPRIA):**

SPRIA was performed on all stool samples to assess the presence of hepatitis A antigen. The test was done as previously reported by Purcell et al.<sup>1</sup> Briefly, flexible microtitre plastic plates with wells after washing were pre-coated with anti-HAV positive serum. After another washing, 25 µl of diluted stool samples were loaded in the wells and incubated overnight at 4°C. Next morning, after washing of plates with PBS-Tween 80: 25 µl of <sup>125</sup>I-labelled anti-HAV was added in each well. The wells were cut and counted individually in a gamma counter (Rack Gamma Counter, LKB Instruments). Any reading > 2.1 of P/N value was considered positive for the presence of HAV antigen in the stool samples.

#### **2. Cesium Chloride (CsCl) Purification:**

Six of the 10 stools were processed individually to assess the hepatitis A virus density in cesium chloride density gradient preparations. The procedure was done as previously reported by Coulepis et al.<sup>20</sup> Briefly 11 ml gradients were prepared by addition of 1.0 ml of 10% stools to 10 ml phosphate buffered saline (PBS) containing 4.4 g of CsCl to give a pre-density of 1309 g/cm<sup>3</sup>. The gradients were loaded in a SW 41 rotor and centrifuged for 48 hours at 30,000 rpm in a L55 OB ultra-centrifuge (Beckman Instruments, California). After centrifugation, 0.5 ml fractions were collected from bottom of the tubes. Cesium chloride density was measured in every other fraction and hepatitis A antigenicity was determined in these fractions by SPRIA as described above.

#### **3. Molecular Hybridisation:**

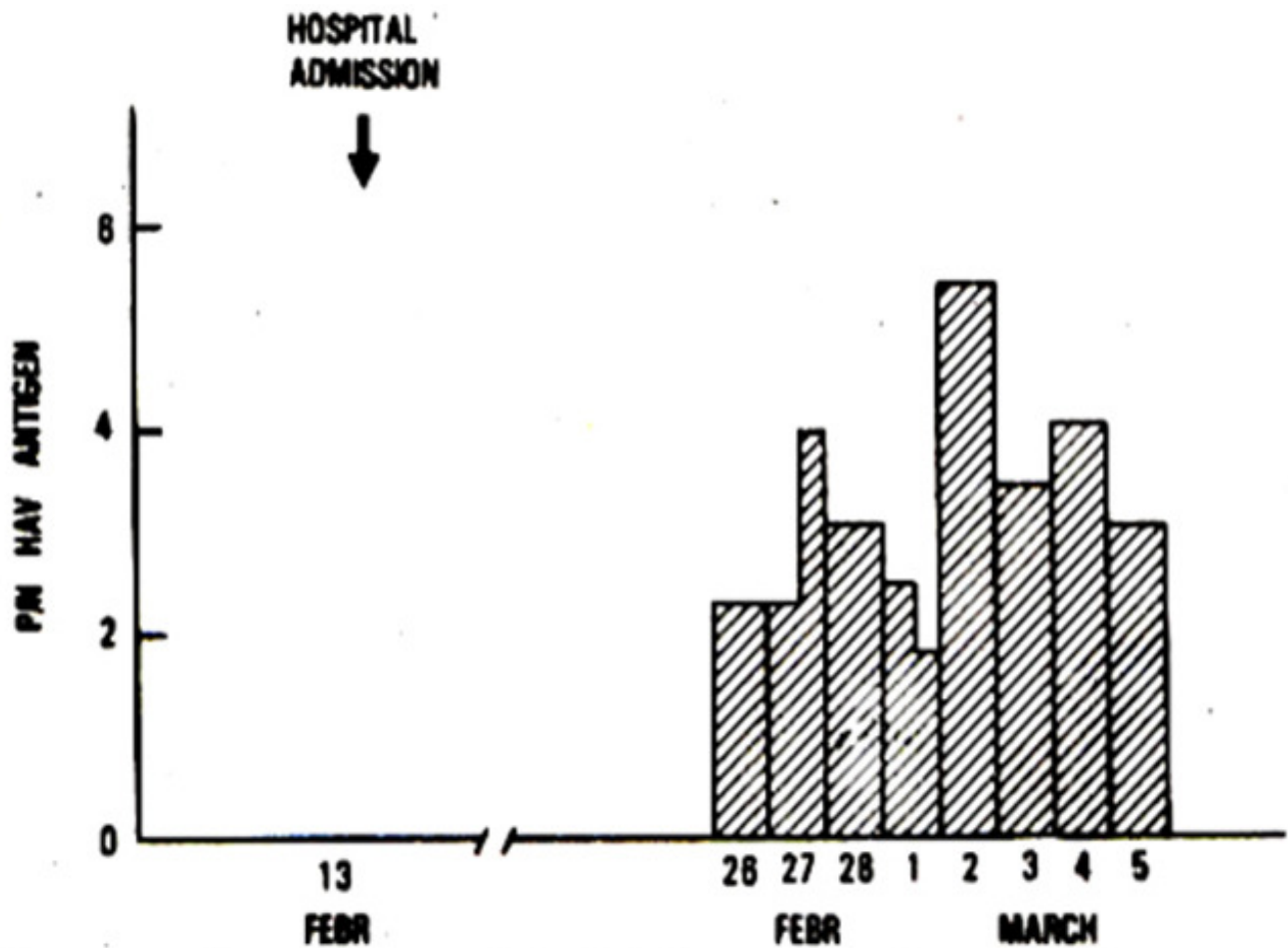
The cDNA-HAV molecular hybridisation was carried out by direct extraction of from stool samples and with monoclonal antibody, according to the combined immunoaffinity cDNA-RNA hybridisation method as described by Jansen et al.<sup>21</sup> Briefly, either direct extraction of HAV-RNA from 10% suspension of 10 K clarified stools or with monoclonal antibody method, the samples were blotted on nitrocellulose filter. After removing the filter from slot blotter, it was dried under a heat lamp for 15 minutes and baked at 80°C for 2 hours. The baked nitrocellulose filter was first sealed in a plastic bag with pre-hybridisation solution and then hybridisation solution containing <sup>32</sup>P-labelled cDNA-HAV probe, for 20 hours at 42°C on a shaking water bath. The washed filter was blotted dry and exposed to XAR-5 film in a cassette at 70°C for 20 hours, after which autoradiography was performed.

## **RESULTS AND OBSERVATIONS**

### **Serological Result:**

The serum samples from the patient at the time of admission to the hospital were positive for IgM anti-HAV and negative for hepatitis B surface antigen (HBsAg) and antibody to hepatitis B core antigen (IgM anti-HBc) by ELISA technique.

### **Detection of HAV by Solid Phase Radioimmunoassay:**



**Figure 1. Detection of HAV in stools by radioimmunoassay (Positive:  $P/N > 2.1$ ). The stool samples after Feb. 27, revealed variable degree of positivity for HAV.**

The figure 1 depicts the HAV antigen P/N values in 10 stool samples of the patients studied in this work. It was interesting to observe a wide variation of P/N levels in the sequential stool specimens. Indeed, on 27 February and 1 March, 86 when instead of one sample two stool specimens were collected from the patient on the same day a variability of P/N values was observed in these stool specimens collected on the same day.

**CsCI Gradient Purification:**

**DETECTION OF HAV IN CESIUM CHLORIDE FRACTIONS  
BY SOLID-PHASE RADIOIMMUNOASSAY**

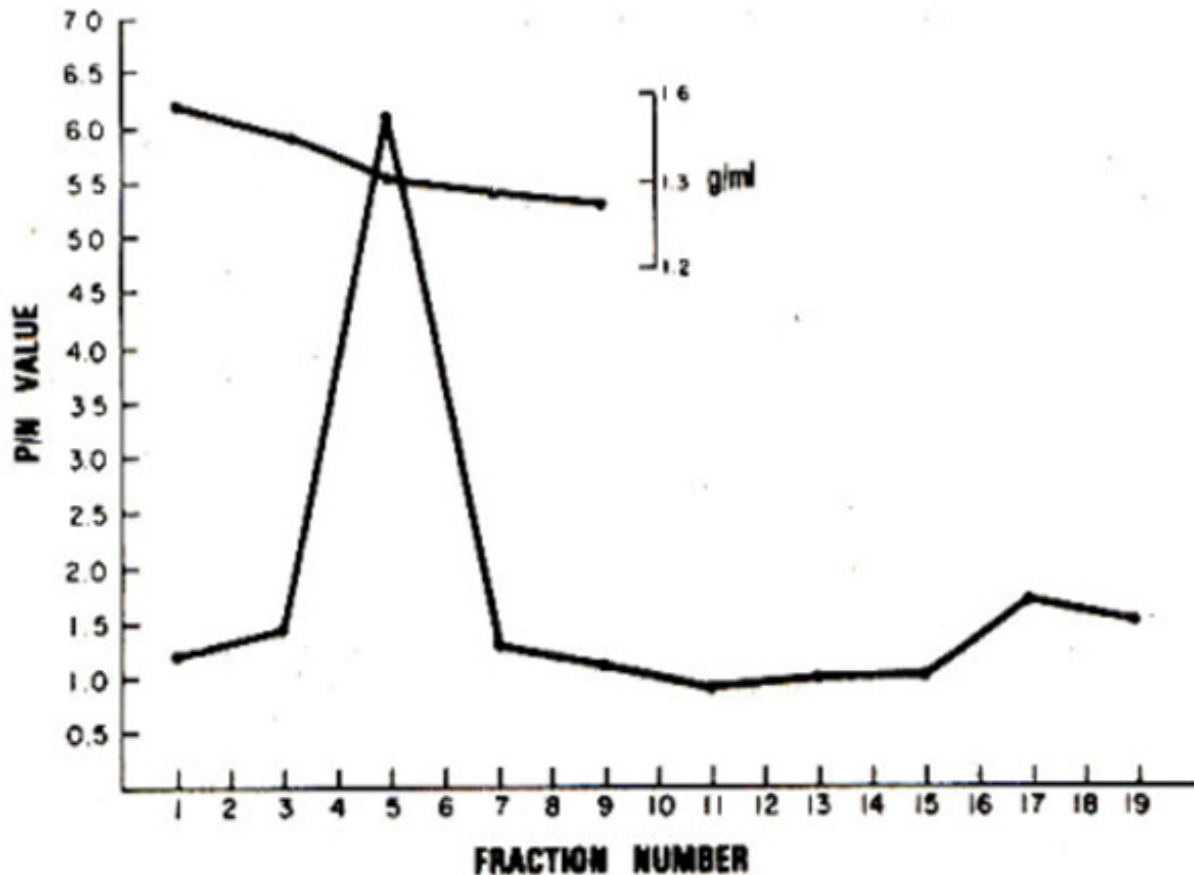
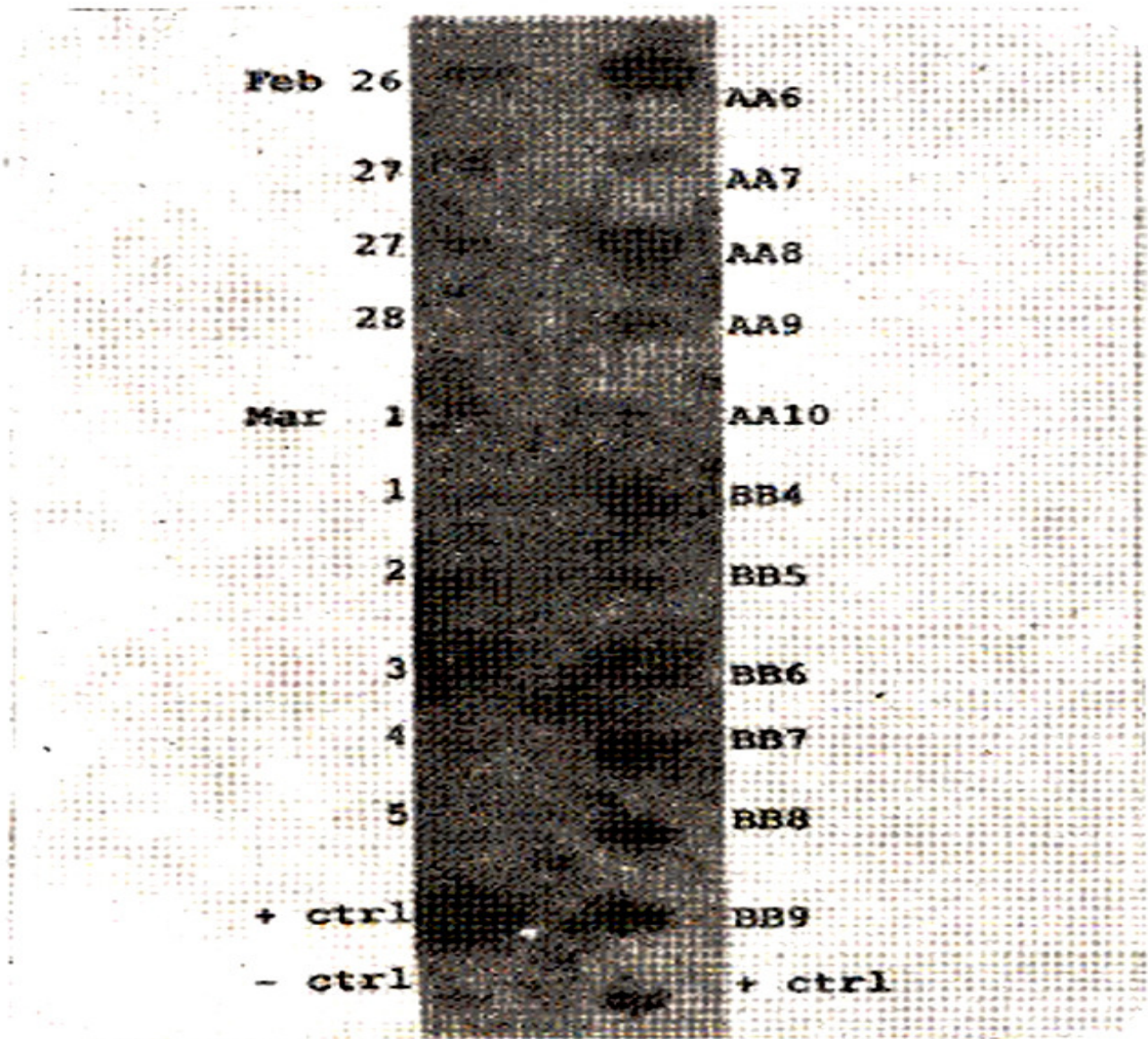


Figure 2. Detection of HAV antigen in cesium chloride fractions by solid phase radioimmunoassay (Positive: PIN > 2.1). The HAV from stools banded isopycnicly at 1.325 g/ml with small quantities banding at approximately 1.42 and 1.27 g/ml.

The figure-2 shows the cesium chloride density (1.31 g/ml) in the fraction of stools which were positive for HAY antigen as determined by SPRIA. Although the human stool specimen collected on 2 March, 86 has been selected for graphic representation, nevertheless other 5 human stools studied also showed similar results.

**Molecular Hybridisation:**



**Figure 3.** Detection of HAV in stools by cDNA-HAV hybridisation in the patient suffering from hepatitis A. In the left column HAV detection in the stools of the patient is depicted which reveals 1<sup>+</sup> positivity on Feb 27 and March 1, and 3<sup>+</sup> on March 3. The right column shows variable positivity for HAV in stool samples collected from 2 owl monkeys (AA&BB) infected with HAV. (ABBREVIATION : ctrl = control).

The figure-3 depicts the molecular hybridisation results by direct extraction method. The immunoaffinity method also showed similar results (data not shown). In the left column, stools of 27 February 86 (second sample) and 1 March 86 are considered 1<sup>+</sup> positive. The stool sample from 3 March 86 shows a strong (3<sup>+</sup>) positivity similar to the positive control. The other human stool samples

are considered negative. The owl monkeys stools (AA & BB) also revealed a variability of positive and negative hybridisation signals in stool samples collected 3 weeks after inoculation with HAV.

## DISCUSSION

During early infection in chimpanzees, the hepatitis A antigen can be identified by immunofluorescence in the cytoplasm of upto 5 to 10% of hepatocytes<sup>22</sup>. It is currently believed that HAV found in faeces is derived from hepatocytes and reaches the intestine via the bile ducts<sup>23</sup>. A very small amount of HAV has also been detected in human saliva<sup>24</sup>. In a patient infected with hepatitis A virus, a transient period of viraemia occurs preceding the appearance of jaundice which is followed by appearance of viral antigen in hepatocytes and excretion in bile and stool specimen<sup>25</sup>. The shedding of viral particles of HAY in stools has been reported by different workers. Some of these studies revealed that the shedding of hepatitis A antigen was maximum during the late incubation period and terminated after onset of jaundice<sup>13,14,15</sup>. Several other workers have shown that HAY may still be detectable in stools upto 10 days after the appearance of symptoms of jaundice<sup>25</sup>. Our observation of detection of viral antigen of HAY in stool specimen of a patient suffering from hepatitis A tends to agree with some recent studies which have shown the presence of viral antigen of HAY in stools upto 3 weeks after the appearance of jaundice and elevation of aminotransferase levels in serum samples<sup>17</sup>. However, in a large proportion of patients with clinically overt disease, HAY is undetectable in stool specimen collected early in the disease<sup>17,18</sup>. This study also highlights one more feature that the shedding of HAY during illness is not a consistent feature as a wide variation was observed to occur in HAY shedding in sequential stool specimen. These observations imply that contrary to earlier reports, the stool specimen of a patient with hepatitis A should be considered infectious for at least 3 weeks after the onset of jaundice and all precautions must be taken to prevent the spread of this disease to other healthy individuals.

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