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Anti-Hepatitis A Virus Immunoglobulin M Antibodies in Urine Samples for Rapid Diagnosis of Outbreaks

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The main goal of this study was to test the feasibility of using urine for diagnosing hepatitis A virus (HAV) infections. A correlation of 90.78% between the test results of urine and serum samples was obtained. Four outbreaks of hepatitis A were confirmed by testing only urine samples. The levels of anti-HAV immunoglobulin M (IgM) antibodies in urine samples remained stable during 6 months of storage at −70°C but decreased when the samples were stored at 4°C. The results of tests of samples obtained 2 and 6 months after infection suggested that IgM levels decline more rapidly in urine than in serum.

Hepatitis A is a disease that is endemic in Cuba. Viral hepatitis outbreaks are frequently observed in preschool day care centers, among schoolchildren and young adults, and within closed institutions (8).

Frequently, it is difficult to collect blood samples, especially from infants, children, and individuals to whom access is limited. Urine samples are easier to collect, the collection method is not invasive, and collection does not require qualified staff. In addition, urine samples can be tested without previous concentration or treatments by using a class-specific antibody capture assay (1).

Urine is a body fluid with low concentrations of immunoglobulins. It has been postulated that large macromolecules such as immunoglobulin M (IgM) antibodies cannot pass through the glomerular filter under normal conditions. However, monomeric IgM proteins (67,000 kDa) have been detected in postrenal sources and not in the glomerular filter (3, 6, 11). The utility of urine for diagnostic testing has been reported for many viral infectious diseases (2, 5, 6, 9). Particularly for hepatitis A, Joshi et al. have found that urine appears to be comparable to serum as a clinical specimen for the diagnosis of recent and past infections (4).

This study provides evidence that rapid confirmation of the etiology of hepatitis in an outbreak situation can be obtained by using an accessible sample (urine) with minor modifications of an existing enzyme-linked immunosorbent assay (ELISA) (8).

Thirty serum and urine samples from healthy individuals and 217 serum and urine samples from patients infected randomly or during seven acute viral hepatitis (AVH) outbreaks were collected on the same day. Sixty urine samples were taken from patients infected during four AVH outbreaks. To study the stability of anti-HAV IgM antibodies, 16 positive urine specimens collected during an AVH outbreak were stored at 4° or −70°C for up to 6 months. Single serum and urine specimens were taken from seven HAV-infected patients at the beginning of an outbreak, and new specimens were collected from the same patients 6 months later for studying IgM kinetics in urine.

A class-specific capture ELISA was used to detect anti-HAV IgM antibodies in both serum and urine samples (8). On the basis of the method of Perry et al., we expressed the results of the assays of the urine samples in test-negative (T:N) values, which were calculated by dividing the optical densities (OD) of the samples by the mean OD of four replicates of the HAV-negative control serum sample. HAV-positive and HAV-negative urine specimens were discriminated by using a cutoff value determined by a histogram method (6).

Statistical analysis was performed by using the Statistica statistics package. The Kolmogorov-Smirnov test, the Student *t* test, analysis of variance, and Fisher's exact test were used to analyze the data.

The results for the urine and serum samples from healthy persons, which were used as negative controls, demonstrated that using urine samples did not decrease thespecificity of the ELISA. The results for both urine and serum samples were adjusted to a normal distribution without significant differences. The Student *t* test results for the urine and serum samples showed no statistically significant difference between their mean OD values ($P < 0.05$). The potency of the Student *t* test was 85%, so it was possible to use serum samples successfully as controls in the urine test. The T:N values for 60 (negative and positive) urine samples were used to establish the cutoff level as 1.2. Using this cutoff value, we expected to get better sensitivity and specificity. Some studies have used serum samples as controls in urine-based immunoassays, with very good results (6, 9).

The sensitivity and specificity of the urine-based ELISA were 88.98 and 92.92%, respectively. A good correlation (90.78%) between the results of the urine and serum assays was obtained. The positive and negative predictive values were 93.75 and 87.61%, respectively, which is an acceptable proportion between positive and negative results and between results for infected and healthy individuals. The positive and negative likelihood ratios were 12.56 and 0.11, respectively. This high

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TABLE 1. Discordant OD and T:N values for serum and urine samples from 20 patients

Sample	OD value for:		T:N value for urine sample[a]	Discordant results (serum/urine)
	Serum sample	Urine sample		
1	1.869	0.085	1.16[a]	+/−
2	0.771	0.101	1.13[a]	+/−
3	0.665	0.051	0.57	+/−
4	0.339	0.068	0.77	+/−
5	1.603	0.090	0.71	+/−
6	0.488	0.094	0.74	+/−
7	0.554	0.098	0.77	+/−
8	0.696	0.089	0.70	+/−
9	1.089	0.122	0.97[a]	+/−
10	0.525	0.105	1.1[a]	+/−
11	0.29	0.14	1.05[a]	+/−
12	1.61	0.11	0.84	+/−
13	0.360	0.093	1.13[a]	+/−
14	0.113	0.698	9.56	−/+
15	0.091	0.111	1.52	−/+
16	0.079	1.556	21.32	−/+
17	0.141	0.415	5.68	−/+
18	0.082	0.099	1.37	−/+
19	0.078	0.182	2.49	−/+
20	0.15	0.31	2.46	−/+

[a] T:N result localized in the "gray gone" (i.e., inconclusive).

TABLE 2. T:N values for urine samples stored at 4 and −70°C

Urine sample	T:N value for urine sample at:								
	Time zero	1 mo		2 mo		3 mo		6 mo	
		4°C	−70°C	4°C	−70°C	4°C	−70°C	4°C	−70°C
1	4.8	3.3	3.91	5.29	4.51	4.8	3.15	3.2	3.7
2	2.5	3.51	1.62	4.6	2.49	3.9	1.75	1[a]	2.9
3	5.6	4.84	4.53	5.31	6.05	5.8	5.47	5	6.8
4	5.03	3.38	2.83	3.16	3.78	2.9	2.5	3.5	3.2
5	5.8	4.21	3.85	5.11	4.53	6.4	4.6	1.7	4.0
6	6.7	3.46	4.2	5.21	6.18	4.7	4.9	7	5.0
7	6.8	4.8	4.29	4.41	5.67	6.04	3.3	5	6.5
8	3.1	2.47	2.12	3.68	2.74	2.8	1.5	4.2	7.5
9	5.9	4.68	4.69	6.01	6.36	5.7	5.9	7.5	9.2
10	6.2	4.6	4.57	5.89	6.11	6.8	6.03	0.7[a]	2.1
11	4.8	3.24	3.29	4.42	4.47	4.5	3.5	0.9[a]	1.4
12	3.6	2.84	2.39	3.6	4.23	3.3	2.1	6.5	7.0
13	3.09	1.92	1.76	2.5	2.66	2.3	1.8	0.6[a]	1.9
14	2.5	2.5	2.59	3.15	2.43	2.23	3.12	2.5	3.4
15	6.03	6.03	8.5	1.21	5.37	0.75	8.9	0.6[a]	3.3
16	7.47	7.47	7.19	4.13	5.2	4.07	7.72	4.2	8.2
Median	5.00	3.95	3.90	4.23	4.55	4.19	4.14	3.38	4.76
SD	1.46	1.42	1.73	1.31	1.21	1.65	2.09	1.85	2.35

[a] Sample tested negative after being stored for 6 months at 4°C.

likelihood ratio indicates that the test can be used to diagnose the disease.

A wide range in T:N values (24.91 to 0.53; median, 3.9) was obtained from the results for the urine samples. Twenty discordant results between urine and serum samples were found (Table 1). Thirteen persons tested positive for HAV when serum samples were used and negative when urine samples were used. However, results for urine samples from 6 of these 13 patients were inconclusive (within 10% below the cutoff value). The remaining seven patients whose results were discordant tested positive when urine samples were used but negative when serum samples were used.

The low sensitivity of our test could have been caused by the following reasons. (i) Urine samples may not have been collected at the optimal time (the anti-HAV IgM kinetics in urine are not well known). (ii) There may have been immunoenzymatic reaction inhibitors present in the urine, due to chemicals, drugs, or toxic products which normally are excreted in urine. (iii) Differences in the amounts of liquids ingested promote fluctuation in the immunoglobulin concentrations in urine (6).

In 1992, Perry and coworkers obtained a high sensitivity (95.8%) by using an antibody capture radioimmunoassay. They analyzed 200 μl of each urine sample and incubated and conjugated the antigens for much longer times (6). However, the main goal of the present study was to evaluate the utility of our ELISA for testing urine samples, with only a few modifications in the original protocol.

False-positive results are probably due to the effect of the pH of the urine, bacterial contamination, or the presence of sediments in the urine of the individuals tested. All of these could interfere with or block the immunochemical reaction in the ELISA (6, 11).

By analyzing 60 urine samples from four AVH outbreaks, we obtained a very good correlation (93.33%) with clinical and epidemiological data. These results strongly confirmed the etiology of the outbreaks.

Sixteen urine samples were used to evaluate the stability of anti-HAV IgM antibodies stored at 4° or −70°C for 6 months (Table 2). Similar qualitative behaviors were found in the samples stored at these two temperatures for up to 3 months. At 6 months, 5 out of the 16 urine samples stored at 4°C tested negative while all of the samples stored at −70°C tested positive. However, there was no significant difference between the median OD values of the urine samples stored at 4° and those stored at −70°C for the first 5 months ($P < 0.05$). For practical purposes, urine samples that have been refrigerated for a week or two are still worth testing. Studies on the effects of freezing and storage conditions on the stability of urine samples have been reported (10, 12).

Preliminary results regarding the kinetics of urinary anti-HAV IgM were obtained by analyzing urine and serum samples from seven HAV-infected patients. Samples were collected and tested at 2 and 6 months after the onset of the disease. The median value OD value of the urine samples collected at 2 months postinfection was 0.968, higher than that for the serum samples (0.669). However, for the specimens collected four months later, the median OD value for the serum samples was at its highest value (0.337), while the median OD value for the urine samples was only 0.131. At 6 months postinfection, all of the serum samples tested positive, while four (57.14%) out of seven urine samples tested negative. The OD median values for the urine samples collected at 2 and 6 months postinfection were significantly different ($P < 0.01$).

The highest levels of anti-HAV IgM antibodies in serum are reached during the acute phase of HAV, and the antibodies often disappear 3 or 4 months after the onset of the illness. However, some studies have demonstrated that anti-HAV IgM antibodies may persist for more than 6 months in 25% of

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patients (7). Our preliminary results have shown that the level of anti-HAV IgM antibodies seems to decrease gradually, but it decreased faster in urine than in serum. It is remarkable that the anti-HAV IgM levels for seven patients were higher in their urine than in their serum at the beginning of the disease. Nevertheless, this anti-HAV IgM reactivity in urine decreased significantly at 6 months postinfection ($P < 0.01$). Further studies of this topic with more specimens may be needed.

These results provide evidence that support the use of urine samples for rapid diagnosis of hepatitis A during outbreaks when venipuncture is inconvenient, difficult, or unacceptable. They also suggest the usefulness of urine samples in achieving a quick response to an epidemiological situation. However, for sporadic clinical cases, the interpretation of test results should be carefully done.

Our practical experience with urine samples and their usefulness as an alternative to serum samples in the diagnosis of AVH during outbreaks have been satisfactory. This study relied on technical advances in the laboratory as well as concordance with clinical and epidemiological data.

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