Journal of Clinical Laboratory Analysis 16:137–142 (2002)

Hepatitis E Antibody Profiles in Serum and Urine

M.S. Joshi, A.M. Walimbe, V.A. Arankalle, M.S. Chadha, and S.D. Chitambar*

Hepatitis Division, National Institute of Virology, Pune, India

The aim of this study was to evaluate anti-HEV antibody profiles in urine specimens in comparison to corresponding serum samples to assess the utility of urine as a clinical specimen. Paired serum and urine specimens from 71 hepatitis E patients, 33 non-E hepatitis patients, 63 patients with nonhepatic diseases, and 26 healthy individuals were tested by recombinant HEV protein (55 kD)-based indirect enzyme-linked immunosorbent assay (ELISA). Uronegativity for anti-HEV IgM was noted in 71 (100%) serologically confirmed patients with hepatitis E. Hepatitis E patients (10/10) showed urinary absence or very low levels of total IgM by capture ELISA, suggesting absence or low levels of filtration, and/or local synthesis, and/or transudation of IgM in urine during infection. When these patients were tested for total IgG and IgA, microquantities of immunoglobulins were noted in all urine samples (10/10 for each). However, the proportions of uropositivity for anti-HEV IgG and IgA in hepatitis E patients were low and indicated only 21.42% and 49.33% concordance with seropositivity, respectively. Control groups also showed low and variable uropositivity for anti-HEV IgG and IgA. Overall, HEV-specific antibodies exhibited by serum in recent and past infections were not found in urine. The study demonstrated the inadequacy of urine specimens for detection of hepatitis E antibodies. J. Clin. Lab. Anal. 16:137–142, 2002.

Key words: hepatitis E; anti-HEV antibodies; urinary antibodies; immunoglobulins; protein

INTRODUCTION

Enterically transmitted hepatitis E virus is the causative agent of outbreaks of hepatitis in different parts of the world. Hepatitis E is highly endemic in India and occurs in a large number of sporadic and epidemic cases. Surveillance reports concerning hepatitis E in India, as well as in other countries, are based on the demonstration of anti-HEV antibodies in serum. Urine has been reported to have several advantages over serum (1). It has also been used to detect specific immunoglobulins against various viral infections, including poliovirus, cytomegalovirus (CMV), Hantan virus, bovine leukemia virus, BK virus, measles virus, rubella virus, human immunodeficiency virus (HIV), hepatitis A virus (HAV), and hepatitis B virus (HBV) (2–10). However, there are no similar reports on its use for hepatitis E. The present study was therefore undertaken to assess urine specimens in comparison to corresponding serum samples from healthy individuals, patients with hepatitis E, non-E hepatitis, and nonhepatic diseases for anti-HEV antibodies, protein, and immunoglobulin profiles, and to explore the possibility of using urine for epidemiologic and diagnostic purposes.

MATERIALS AND METHODS

Subjects and Specimens

Paired serum and urine specimens were collected from 26 healthy individuals, 71 hepatitis E patients, 33 non-E hepatitis patients (17 hepatitis A patients, and 16 non-A non-E hepatitis patients), and 63 patients with nonhepatic diseases (including 37 patients with renal disease).

The 26 healthy individuals (10 males and 16 females) were 23–58 years old, with no history of recent illness. The hepatitis patients included sporadic cases from local hospitals in Pune, and epidemic cases from Aurangabad, Chikhali, and Lonawala that occurred during August 1999, March 2000, and June 2000, respectively, in the state of Maharashtra in India. The cases were clinically examined for characteristic symptoms and signs, and elevated serum ALT activity. The

*Correspondence to: Dr. Shobha D. Chitambar, Assistant Director, Hepatitis Division, National Institute of Virology, 20-A, Dr. Ambedkar Road, Pune 411 001, India. E-mail: chitambars@hotmail.com

Received 2 October 2001; Accepted 19 December 2001

DOI:10.1002/jcla.10027

Published online in Wiley InterScience (www.interscience.wiley.com).

109 hepatitis patients (86 males and 23 females) were 2–55 years old. The 63 pairs of serum and urine samples included in the study were collected from hospitalized patients with nonhepatic diseases. This group consisted of 42 males and 21 females (<1 year to 75 years old) suffering from viral gastritis, bronchitis, anemia, diarrhoea, renal disease, or renal failure. Prior to sample collection, informed consent was obtained from the healthy adults and the patients with nonhepatic diseases (and in the case of children, from the parents).

The pairs of serum and urine samples were stored in a refrigerator (+4°C) and tested within 24 hr after collection.

Anti-HEV ELISA

Anti-HEV IgM, IgG, and IgA in serum and urine samples were determined by enzyme-linked immunosorbent assay (ELISA) tests as described previously (11–13). Recombinant ORF2 protein of HEV (55 kD) coated on the wells was allowed to bind to anti-HEV Igs (IgM/IgG/IgA from the test samples), which was probed by antihuman Ig HRP conjugate [anti-μ HRP (1:10,000) (Sigma, USA)/anti-γ HRP (1:10,000) (Sigma, USA)/anti-α HRP (1:2,000) (Dako, Denmark)]. The bound HRP was allowed to react with substrate (phosphate citrate buffer, pH 5.0, containing O-phenylene diamine and urea peroxide). The development of color (yellow-orange) directly indicated the presence of HEV-specific Igs (IgM/IgG/IgA) in the test sample.

Calculations for cut-off optical density (OD) and interpretation of results were carried out according to the method described by Arankalle et al. (14). Serum specimens known to be negative and positive for anti-HEV antibodies were included as negative and positive controls, respectively, in the test. The cut-off value to detect anti-HEV IgM and IgA was calculated as mean negative control OD value X3. For anti-HEV IgG detection the cut-off value was calculated as mean negative control OD value X2.1.

Determination of Protein and Total Immunoglobulin M, G, and A Content in Paired Serum and Urine Samples

The method described by Lowry et al. (15) was used to determine the protein content of serum and urine samples. An aliquot of 10 μl, each of serum (1:10 dilution) and urine specimen (neat) was employed in the assay.

Total IgM, IgG, and IgA in serum and urine specimens were determined by IgM, IgG, and IgA capture ELISAs, respectively. Briefly, microwell ELISA plates (Maxisorb; Nunc, Naperville, IL) were coated (125 μl/well) at room temperature overnight with rabbit antihuman IgM (μ chain specific; Dako, Denmark)/IgG (γ chain specific; Dako, Denmark)/IgA (α chain specific; Dako, Denmark) antibodies at 1:1,000, 1:250, and 1:400 dilutions, respectively. Test serum and urine samples were then added in different dilutions in identified wells. Standard human IgM (Dako, Denmark) and IgG (Lupin, India) were added in different concentrations in the range of 0.078–10 ng/well, and IgA (Lupin, India) in the range of 0.078–12 ng/well in duplicate in identified wells. The captured human antibody was probed using antihuman immunoglobulin HRP [anti-μ HRP (1:10,000)/anti-γ HRP (1:10,000)/anti-α HRP (1:2,000)] conjugates as per requirement of the assay. The bound HRP was allowed to react with substrate (phosphate citrate buffer, pH 5.0, containing O-phenylene diamine and urea peroxide). The development of color (yellow-orange) directly indicated the presence of Igs (IgM/IgG/IgA) in the test sample.

Statistical Analysis

The data was analyzed by linear regression analysis to fit a standard line of regression between the OD value of standard protein/Ig and concentration, which was then used to estimate the total protein and immunoglobulin concentration of the serum and urine specimens.

RESULTS

Anti-HEV Antibodies in Serum and Urine Samples

Freshly collected paired serum and urine samples from 71 hepatitis E patients, 26 healthy controls, 33 non-E hepatitis patients (12 hepatitis A, 21 non-A non-E hepatitis) and patients with nonhepatic diseases (63, which included 37 patients with renal disease) were subjected to indirect ELISA for detection of anti-HEV IgM, IgG, and IgA antibodies. The results obtained for urine samples were compared with those of corresponding serum samples.

IgM Anti-HEV

Table 1 presents the comparative data obtained for anti-HEV IgM. All patients with confirmed seropositivity (71/71) for anti-HEV IgM showed an absence of uropositivity

TABLE 1. Comparison of paired serum and urine specimens for anti-HEV IgM

	Serum		Urine	
Group	No. +ve/ no. tested	S/Co (mean ± SD)	No. +ve/ no. tested	S/Co (mean ± SD)
Hepatitis E patients	71/71 (100%)	3.71 ± 2.23	0/71 (0%)[a]	0.10 ± 0.12
Healthy controls	1/26 (3.84%)	0.43 ± 0.27	0/26 (0%)	0.03 ± 0.02
Non-E hepatitis patients	0/33 (0%)	0.49 ± 0.31	0/33 (0%)	0.06 ± 0.08
Patients with non-hepatic diseases	1/63 (2.38%)	0.31 ± 0.27	0/63 (0%)	0.08 ± 0.11

[a]Highly significant difference when compared with corresponding serum value.

S/Co, sample OD/cutoff OD.

(0/71). Control groups were negative for both serum and urine anti-HEV IgM.

IgG and IgA Anti-HEV

Among anti-HEV IgM seropositive patients, 91.54% (65/71) and 85.91% (61/71) were positive for serum anti-HEV IgG and IgA, respectively. Comparative data obtained from paired serum and urine specimens for different groups for anti-HEV IgG and IgA are shown in Tables 2 and 3, respectively.

Among anti-HEV IgG seropositive hepatitis E patients, only 21.53% (14/65) showed uropositivity. In healthy individuals, non-E hepatitis patients, and patients with nonhepatic diseases, uropositivity was still low and variable (20% (1/5); 6.2% (1/16); 41.66% (5/12). Sensitivity and specificity were found to be 21.42% and 100%, respectively. Percent uropositivity for anti-HEV IgA was 47.54% (29/61) among seropositive hepatitis E patients. In the other groups investigated, low and variable sero- and uropositivity were noted (Table 3). Sensitivity and specificity were found to be 50% and 92.42%, respectively.

Overall, no statistical correlation was noted between serum and urine positivity for anti-HEV antibodies.

To investigate the lack of concordance in sero- and uropositivity for anti-HEV antibodies, the urine and corresponding serum samples were tested for protein and immunoglobulin levels.

Protein and Total Immunoglobulin M, G, and A Content in Paired Serum and Urine Samples

Protein concentration was determined in freshly collected paired serum and urine samples from 26 healthy controls, 17 hepatitis A patients, 24 hepatitis E patients, 15 patients with non-A non-E hepatitis, 10 patients with nonhepatic diseases, and 10 patients with renal disease. Eight runs were performed

TABLE 2. Comparison of paired serum and urine specimens for anti-HEV IgG

	Serum		Urine	
Group	No. +ve/ no. tested	S/Co (mean ± SD)	No. +ve/ no. tested	S/Co (mean ± SD)
Hepatitis E patients[a]	65/71 (91.54%)	5.06 ± 3.62	14/71 (19.71%)	0.68 ± 0.87
Healthy controls[b]	5/26 (19.23%)	1.07 ± 1.70	1/26 (3.84%)	0.59 ± 1.79
Non-E hepatitis patients[b]	16/33 (48.48%)	1.92 ± 1.90	1/33 (3.03%)	0.34 ± 0.31
Patients with non-hepatic diseases[b]	12/63 (19.04%)	0.86 ± 1.21	5/63 (7.93%)	0.57 ± 1.07

[a]Anti-HEV IgM +ve.
[b]Anti-HEV IgM –ve.
S/Co, sample OD/cutoff OD.

TABLE 3. Comparison of paired serum and urine specimens for anti-HEV IgA

	Serum		Urine	
Group	No. +ve/ no. tested	S/Co (mean ± SD)	No. +ve/ no. tested	S/Co (mean ± SD)
Hepatitis E patients[a]	61/71 (85.91%)	3.05 ± 2.11	29/71 (40.81%)	1.77 ± 2.15
Healthy controls[b]	1/26 (3.84%)	0.40 ± 0.23	0/26 (0%)	0.32 ± 0.26
Non-E hepatitis patients[b]	8/33 (24.24%)	1.20 ± 0.23	5/33 (15.15%)	0.57 ± 0.81
Patients with non-hepatic diseases[b]	5/63 (7.93%)	0.52 ± 0.65	3/63 (6.97%)	0.41 ± 0.46

[a]Anti-HEV IgM +ve.
[b]Anti-HEV IgM –ve.
S/Co, sample OD/cutoff.

with bovine serum albumin employed as standard protein. The OD values were averaged over eight runs, and a standard curve was plotted. A line of regression was fitted to this standard curve using linear regression analysis. The linearity of the standard curve was confirmed by computing the correlation coefficient between average OD values and concentration ($r^2 = 0.984$, $P < 0.01$) The equation of this regression line was used for estimating the protein concentration in the test samples. The range of protein concentration, mean ± SD values, and serum protein to urine protein ratios are presented in Table 4. For comparison of mean serum and urine protein levels in different groups, Student's *t*-test was used. The mean serum protein levels in different categories of patients were significantly lower than that of the healthy group ($P < 0.01$ for every comparison). The mean urine protein levels in all groups of patients showed no significant difference with that of the healthy group ($P > 0.05$ for every comparison), except for the group of renal disease patients, which showed a significantly high concentration ($P < 0.01$). The protein content in urine specimens was significantly low as compared to the corresponding serum samples in all groups (paired *t*-test, $P < 0.01$). Among the healthy individuals, the range of serum protein to urine protein ratios was high compared to all groups of patients.

Freshly collected paired serum and urine specimens from 10 healthy individuals, 10 hepatitis A patients, 10 hepatitis E patients, 10 non-A non-E hepatitis patients, five patients with nonhepatic diseases, and five patients with renal disease were subjected to capture ELISA tests to determine the total content of IgM, IgG, and IgA.

Eight runs for each of the three capture ELISA tests were performed, with human IgM, IgG, and IgA employed as standard immunoglobulins. Taking appropriate concentrations of each of the standards (IgM, IgG, and IgA), a curve was plotted for OD value on the Y axis and corresponding concentration on the X axis. A line of regression was fitted by using

TABLE 4. Total protein content in paired serum and urine specimens

Serial number	Group (n)	Range of protein concentration (mean ± SD)		Range of serum protein/ urine protein ratio (S/U)
		Serum mg/ml	Urine mg/ml	
1	Healthy controls (26)	76.68–230.05 (146.8 ± 42.33)	1.05–6.71 (3.17 ± 1.67)	17.69–168.12
2	Hepatitis A patients (17)	26.46–196.69 (107.3 ± 49.39)	1.19–11.69 (4.16 ± 2.58)	6.25–136.77
3	Hepatitis E patients (24)	80.52–230.05 (107.6 ± 30.87)	1.15–19.17 (4.39 ± 3.72)	5.0–100.0
4	Non-A non-E hepatitis patients (15)	23.39–115.02 (84.74 ± 33.65)	0.92–9.2 (2.95 ± 2.11)	4.28–75.00
5	Patients with non-hepatic diseases (10)	47.93–98.54 (66.75 ± 15.30)	0.92–7.74 (3.28 ± 1.96)	6.49–67.50
6	Patients with renal disease (10)	65.95–124.99 (102.95 ± 15.0)	0.77–11.35 (6.61 ± 3.46)	5.81–131.00

linear regression analysis. The linearity was confirmed from the correlation coefficient between OD value and concentration ($r^2 = 0.9659$, 0.9871, and 0.9792 for IgM, IgG, and IgA, respectively; $P < 0.05$ for each). Using the regression line equation, the concentrations of IgM/IgG/IgA antibodies in the test samples were estimated.

The range of concentrations for each of the IgM, IgG, and IgA markers obtained in all groups for the serum and urine specimens were analyzed by Student's *t*-test. The data are presented in Table 5. In the serum samples the lower concentration of Ig levels, when compared with the healthy control group, was noted to be statistically significant. However, it was not observed in all groups (significant only in non-A non-E hepatitis patients, nonhepatic and renal disease patients for IgM; in hepatitis E patients, non-A non-E hepatitis patients, and nonhepatic patients for IgG; and in hepatitis A patients, non-A non-E hepatitis patients for IgA ($P < 0.05$ for all markers)).

As reported previously by other investigators (16), the urine levels of immunoglobulins were very low—in microquantities in comparison to serum levels. These are, therefore, expressed in nano- or microgram per milliliter of the sample (Table 5). The mean values for urine IgM, IgG, and IgA in hepatitis E patients, non-A non-E hepatitis patients, and patients with nonhepatic diseases did not show a significant difference with the corresponding values of healthy group ($P > 0.05$ for each). In a similar comparison, hepatitis A patients and renal disease patients showed significantly high mean IgM levels ($P < 0.05$ for each). These groups of patients also showed high mean values for urine IgG and IgA; however, no statistically significant difference was noted with that of the healthy group ($P > 0.05$).

DISCUSSION

Widely used immunological assays currently are based on examination of blood specimens. However, the obvious difficulties and disadvantages inherent in blood collection have also been described (1). Alternative body fluids, such as saliva and urine, were investigated for this purpose, and were shown to contain low immunoglobulin concentrations (10,17). In the present study we evaluated urine, as it can be easily obtained in sufficient quantities without any hesitation from the subjects.

We investigated urine specimens for anti-HEV antibodies. This is the first report to document detection of IgM, IgG, and IgA classes of urinary antibodies against HEV by ELISA. Uronegativity was observed for anti-HEV IgM in all (71/71) hepatitis E patients. Furthermore, when these patients were tested for the presence of urinary anti-HEV IgG and IgA, the proportions of positivity were low.

The inadequacy of urine specimens for detection of hepatitis E antibodies prompted us to determine the protein and immunoglobulin levels of the test (serum and urine) samples. Significantly low levels ($P < 0.01$) of serum proteins were observed in all groups of patients as compared to the healthy individuals (Table 4). Similar to the reduced serum protein levels, serum IgM, IgG, and IgA levels were also noted to be decreased ($P < 0.05$) in all categories of patients (Table 5). However, this observation was not consistent for all markers in all groups—probably due to the small size of the samples.

A wide range of protein levels was detected in urine samples of healthy individuals. Urine protein levels detected in patients with hepatitis and nonhepatic diseases were closer to that of healthy individuals ($P > 0.05$). Patients with renal disease, however, showed significantly high protein levels ($P < 0.01$). These observations confirm earlier reports documenting increased urinary excretion of protein as a common sign of renal disease (18).

In our study, the absence or very low levels of total IgM in urine specimens of healthy individuals, hepatitis E patients, non-A non-E hepatitis patients, and patients with nonhepatic diseases was noted. Total IgG and IgA content of urine specimens in the same groups was not statistically different from that of healthy individuals. Jankowski (19) reported the absence of total IgM, over fourfold increase in IgA, and twofold increase in IgG content of urine in children diagnosed as viral hepatitis patients with and without HBsAg antigenemia. Tencer et al. (20) also postulated that transport of macromolecules such as IgM is highly restricted under normal conditions, and that the urine concentration of other macromolecules could be extremely low. However, in our study, we noted increased urinary IgM and a wide range of IgG and IgA levels

TABLE 5. Total IgM, IgG, and IgA content in paired serum and urine specimens

Serial number	Group (n)	Range of IgM concentration (mean ± SD) Serum mg/ml	Urine μg/ml
1	Healthy controls (10)	0.32–1.41 (0.72 ± 0.30)	0.00–12.26 (1.93 ± 3.74)
2	Hepatitis A patients (7)	0.30–2.85 (0.80 ± 0.84)	10.29–54.09 (34.27 ± 16.53)
3	Hepatitis E patients (10)	0.17–1.32 (0.58 ± 0.29)	0.00–19.66 (3.49 ± 6.36)
4	Non-A non-E hepatitis patients (10)	0.13–0.88 (0.37 ± 0.21)	0.00–38.23 (7.06 ± 13.24)
5	Patients with non-hepatic diseases (5)	0.08–0.37 (0.18 ± 0.10)	0.00–0.00 (0)
6	Patients with renal disease (5)	0.15–0.41 (0.32 ± 0.09)	2.49–87.14 (38.47 ± 31.96)

Serial number	Group (n)	Range of IgG concentration (mean ± SD) Serum mg/ml	Urine μg/ml
1	Healthy controls (10)	10.06–20.61 (13.72 ± 3.11)	0.01–0.69 (0.22 ± 0.20)
2	Hepatitis A patients (10)	6.09–17.39 (10.32 ± 4.01)	0.006–40.73 (7.05 ± 12.82)
3	Hepatitis E patients (10)	3.42–16.55 (8.59 ± 3.90)	0.009–1.22 (0.50 ± 0.36)
4	Non-A non-E hepatitis patients (10)	6.51–15.94 (10.29 ± 2.88)	0.053–3.59 (0.86 ± 1.01)
5	Patients with non-hepatic diseases (5)	10.06–12.49 (11.10 ± 0.99)	0.05–0.43 (0.26 ± 0.13)
6	Patients with renal disease (5)	4.87–11.18 (8.71 ± 2.18)	3.30–60.30 (19.14 ± 20.91)

Serial number	Group (n)	Range of IgA concentration (mean ± SD) Serum mg/ml	Urine μg/ml
1	Healthy controls (9)	1.24–6.72 (2.76 ± 1.50)	0.12–5.48 (1.64 ± 1.41)
2	Hepatitis A patients (10)	0.48–3.38 (1.31 ± 0.78)	0.11–85.97 (12.98 ± 25.57)
3	Hepatitis E patients (10)	0.19–6.34 (2.20 ± 1.53)	0.15–4.08 (1.78 ± 1.39)
4	Non-A non-E hepatitis patients (10)	0.89–2.03 (1.39 ± 0.34)	0.18–11.41 (3.01 ± 3.72)
5	Patients with non-hepatic diseases (5)	0.14–2.48 (1.25 ± 0.94)	0.34–2.09 (0.90 ± 0.64)
6	Patients with renal disease (5)	1.47–3.17 (2.10 ± 0.76)	3.27–108.90 (40.22 ± 36.87)

in hepatitis A patients with mild proteinuria, and in renal disease patients with excessive proteinuria.

Globulinuria could result from overflow and/or defective glomerular retention, and/or defective tubular reabsorption, and/or increased filtration, and possibly decreased reabsorption (hemodynamic) (18). Evidence for transudated and locally synthesized gamma globulins in urine has been provided both in pyelonephritis and lower urinary tract infections (21,22). It is also possible that active excretion of immunoglobulins by kidneys could be facilitated by specific or non-specific stimulation. The excretion of IgA and IgG noticed in hepatitis patients was thought to be due to stimulation by the viremia (19). In the present study, high-level immunoglobulinuria was observed in hepatitis A and renal disease patients, but not in patients with hepatitis E, thus indicating undisturbed glomerular/tubular functions during HEV infection. It may be noted that in spite of the low-level presence of total IgG and IgA in urine, concordance between sero- and uropositivity for anti-HEV IgG and IgA was only 21.42% and 49.33%, respectively.

Overall, the presence of microquantities of proteins and immunoglobulins (G and A) was noted in urine specimens of all individuals. However, HEV-specific antibodies exhibited by serum in recent and past HEV infections were not correspondingly found in urine. Previous studies have reported the utility of urine as a specimen for detection of immunoglobulins specific to viral infections such as polio, CMV, Hantan, bovine leukemia, measles, rubella, HIV, and HBV (2–10). Hepatitis A and E viruses are transmitted enterically and cause diseases that are indistinguishable clinically and biochemically. It was interesting to note that sero- and uropositivity for anti-HAV IgM, IgG, and IgA were >95% in agreement (unpublished data). However, such an observation was not made for hepatitis E in the present study. Of the anti-HEV IgM seropositive patients investigated in this study, 91.54% and 85.81% were positive for serum anti-HEV IgG and IgA, respectively. These observations were similar to those reported by Chau et al. (12), who observed 84.1% and 52.4% positivity for anti-HEV IgG and IgA, respectively, in an outbreak of enterically transmitted hepatitis that occurred in southern Somalia. The uronegativity/low proportion positivity for anti-HEV antibodies in hepatitis E patients noted in the present study could be a result of low-level in vivo replication and/or low immunogenicity of HEV. It has been pointed out previously that anti-HEV titers are considerably low compared to the high titers of anti-HAV (11,14,23,24). The extent of systemic and/or local infection and renal involvement may also play an important role in causing or restricting specific immunoglobulinuria.

Finally, the absence or low levels of total IgM, and the positivity for total IgG and IgA in urine specimens from hepatitis E patients were demonstrated by total immunoglobulin (IgM/IgG/IgA) capture ELISA tests in the present study. Thus, any limitations of the recombinant antigen-based ELISA tests employed in this study were ruled out.

ACKNOWLEDGMENTS

The authors are grateful to Dr. R.H. Purcell, Head of the Hepatitis Viruses Section, NIH, for the HEV ORF-2 antigen. We are thankful to Dr. A.R. Bavdekar, Dr. Kalpana Kulkarni, and the staffs of the Department of Pediatrics, KEM Hospital, and the Nephro ward, Sasson General Hospital, Pune, for

their help in providing clinical samples. The technical assistance of Prakash Jawalkar, Rajesh Kannalu, and Neeta Thorat is gratefully acknowledged.

REFERENCES

1. Mortimer P, Parry JV. Non invasive virological diagnosis: are saliva and urine specimens adequate substitutes for blood? J Med Virol 1991; 1:73–78.
2. Lerner M, Remington JS, Finland M. Neutralizing antibody to poliovirus in normal human urine. J Clin Invest 1962;41:805–815.
3. Koopmans M, Sanchez-Martinez D, Patton J, Stewart J. Evaluation of antigen and antibody detection in urine specimens from children with congenital human cytomegalovirus infection. J Med Virol 1995; 46:321–328.
4. Verta LA, Elisova TD, Voronkova GM. The detection of antibodies to the hantan virus in the urine of patients with hemorrhagic fever with renal syndrome. Vopr Virusol 1993;38:18–21.
5. Carli KT, Batmaz H, Sen A, Minbay A. Comparison of serum, milk and urine as samples in an enzyme immunoassay for bovine leukaemia virus infection. Res Vet Sci 1993;55:394–395.
6. Marrero M, Pascale F, Alvarez M, De Saint Maur G, Rosseau E, Nicolas JC, Garbarg-Chenon A, Bricout F. Dot ELISA for direct detection of BK virus in urine samples. Acta Virol 1990;34:563–567.
7. Minnich LL, Goodenough F, Ray CG. Use of immunofluorescence to identify measles virus infections. J Clin Microbiol 1991;29:1148–1150.
8. Terda K, Niizuma T, Kataoka N, Niitani Y. Testing for rubella specific IgG antibody in urine. Pediatr Infect Dis J 2000;19:104–108.
9. Cao Y, Hosein B, Borokowsky W, Mirabile M, Baker L, Baldvin DF, Poiesz BJ, Friedman-Kien AE. Antibody to human immunodeficiency virus type 1 in the urine specimens of HIV-1 seropositive individuals. AIDS Res Hum Retrovir 1989;5:311–319.
10. Perry KR, Parry JV, Vandervelde EM, Mortimer PP. The detection of urine specimens of IgG and IgM antibodies to hepatitis A and hepatitis B core antigens. J Med Virol 1992;38:265–270.
11. Arankalle VA, Chadha MS, Tsarev SA, Emerson SU, Risbud AR, Banerjee K, Purcell RH. Seroepidemiology of water borne hepatitis in India and evidence for a third enterically-transmitted hepatitis agent. Proc Natl Acad Sci USA 1994;91:3428–3432.
12. Chau KH, Dawson GJ, Bile KM, Magnius LO, Sjogren MH, Mushahwar LK. Detection of IgA class antibody to hepatitis E virus in serum samples from patients with hepatitis E virus infection. J Med Virol 1993;40:334–338.
13. Tsareva SA, Tsareva TS, Emerson SU, Kapikian AZ, Ticehurst J, London W, Purcell RH. ELISA for antibody to hepatitis E virus based on ORF-2 protein expressed in insect cells: identification of HEV infection in primates J Infect Dis 1993;168:369–378.
14. Arankalle VA, Tsarev SA, Chadha MS, Alling DW, Emerson SU, Banerjee K, Purcell RH. Age specific prevalence of antibodies to hepatitis A and E viruses in Pune, India 1982 and 1992. J Infect Dis 1995;171:447–450.
15. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with folin phenol reagent. J Biol Chem 1951;193:265–275.
16. Poortmans J, Jeanloz RW. Quantitative immunological determination of 12 plasma proteins excreted in human urine collected before and after exercise. J Clin Invest 1968;47:386–393.
17. Parry JV, Perry KR, Mortimer PP. Sensitive assays for viral antibodies in saliva: an alternative to tests on serum. Lancet 1987;ii:72–75.
18. Denis VW. Investigations of renal functions. In: Wyngaarden JB, Smith LH, editors. Cecil Textbook of Medicine, 18th ed. Vol. 1. Philadelphia: WB Saunders Company, Harcourt Brace, Jovanovich Inc. 1988; p 520–528.
19. Janakowaski A. Immunoglobulinuria in the acute phase of viral hepatitis in children and the influence of HBAg antigen. Arch Immunol Ther Exp 1976;24:417–421.
20. Tencer J, Frick IM, Oquist BW, Alm P, Rippe B. Size selectivity of the glomerular barrier to high molecular weight proteins: upper size limitations of shunt pathways. Kidney Int 1998;53:709–715.
21. Hand W, Smith JW, Miller TE, Barnett JA, Sandford JP. Immunoglobulin synthesis in lower urinary tract infections. J Lab Clin Med 1970; 75:19–29.
22. Lehmann JD, Smith JW, Miller TE, Barnett JA, Sandford JP. Local immunoresponse in experimental pyelonepheritis. J Clin Invest 1968; 17:2541–2550.
23. Chitambar SD, Murthy-Grewal S, Bokil M, Arankalle VA, Gore MM, Banerjee K. Indigenous anti-hepatitis A virus IgM capture ELISA for the diagnosis of hepatitis A. Ind J Med Res 1994;99:243–251.
24. Chitambar SD, Joshi MS, Arankalle VA, Banerjee K. Sensitive ELISA tests for detection of anti-hepatitis A virus antibodies. Serodiagn Immunother Infect Dis 1996;8:63–65.