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Review Article

Urinary immunoglobulins in viral diagnosis: An overview

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Antibody detection by serological methods gained a lot of interest in recent years and has become the backbone of virological diagnosis. Despite the detection of all five classes of immunoglobulins in urine, not much attention has been paid to the use of urine as a diagnostic sample to detect viral antibodies. Unlike venipuncture, this non-invasive mode of sample collection can help cover all age groups, especially paediatric and old age patients, where blood collection is difficult. Using urine as a sample is also economical and involves lesser risk in sample collection. The antibodies are found to be stable in urine at room temperature for a prolonged period, which makes the sample transport management easier as well. A few recent studies, have also shown that the detection limit of antibodies in urine is at par with serum or other clinical material. So, the ease in sample collection, availability of samples in large quantity and stability of immunoglobulins in urine for prolonged periods can make urine an ideal sample for viral diagnosis.

Key words Antibody detection - diagnostics - immunoglobulins - urine - viral diseases

Introduction

Antibody detection has become a valuable tool in viral diagnostics and is accomplished by several serological techniques *viz*. complement fixation, neutralization test, haemagglutination inhibition, ELISA (Enzyme Linked Immunosorbent Assay), *etc*. All of these techniques are well established in virology laboratories and have been the backbone of diagnostic virology since before the advent of molecular tools[1,2]. Despite the availability of newer molecular techniques, serological techniques still remain the mainstay of viral diagnosis in many laboratories due to their specificity and economy for mass screening. Serological techniques have played an important role in the diagnosis of aetiological agents during the emergence and re-emergence of several diseases of viral origin globally in the past two decades[3,4]. Advancements in the techniques over the years have made many of these rapid and sensitive[2] with most of these tests available in a ready-to-use kit format[5]. This has improved the efficiency of diagnoses that lead to the proper management and control of viral outbreaks.

The utility of urinary antibodies as a diagnostic tool has been investigated for certain viral diseases due to the inexpensive and non-invasive nature of sample collection[6,7]. During viral or bacterial infections, antibodies to immunoglobulins are excreted through urine, which have been found to be stable

Table I. Stability of urinary immunoglobulins against viral pathogens

Temperature	Virus and type of Igs	Stability status
Room temperature	HIV-1, IgG	Stable for 55 days[8]
	Rubella, IgG	For five months at 25°C with 0.1% sodium azide[9]
	HCV, IgG	Stable for 20 days[10]
At 4°C	HIV-1, IgG	Stable for one year at 2°C-8°C[8]
	Rubella, IgG	Stable for five months with 0.1% sodium azide[9]
	HAV, IgM	Stable for 48-72 h[11]
	HAV, IgM	Stable for five months[12]
At 20°C and below	HAV, IgM	Significant reduction in titre (to 81.25% and 76.08% after three and six months, respectively, when stored at −70°C) [11]
	HAV IgM	Stable for six months at −70°C[12]
	HCV	Stable at −25°C and −80°C for longer periods[10]

HCV, hepatitis C virus; HAV, hepatitis A virus; Ig, immunoglobulin

for considerable periods (Table I)[8-12]. The antibodies can be detected in urine using class-specific assays or Western blot[13]. The use of urine for antibody detection also reduces the risks involved in sample collection and large quantities could be collected from all age groups, especially paediatric patients and elderly people where blood collection is relatively difficult. The present review focuses on the available literature, advantages and limitations of using urine as a diagnostic sample for the detection of viral immunoglobulins.

Presence of viral antibodies in urine

Lerner *et al*[14] were the first to report the presence of viral antibodies in human urine when they observed neutralizing antibodies (NAbs) to poliovirus in patient's urine. The investigators also demonstrated positive association of NAbs in urine with that of serum. In the same year, Ashkenazi and Melnick[15] demonstrated the presence of NAbs to SV40 (simian virus) in urine samples of experimentally inoculated monkeys, which appeared with the waning of viruria. Similarly, another group in 1969 observed SV40 Nabs in urine and correlated it with high titre in the serum[16]. Subsequently, antibodies, either alone or in combination with several viruses were detected in patient's urine. Table II[14-41] depicts the different viral antibodies detected in urine.

Concentration and stability of antibodies in urine

Concentration and stability of antibodies are important while considering a sample for its diagnostic potential. In comparison to serum, concentration of viral antibodies in urine is low[42,43]. It depends on many factors *viz.,* fluid intake, use of diuretics, body posture at the time of collection, physical activity *etc*[25,43]. The concentration can be optimized for tests either by using undiluted samples or by increasing sample volume. Other methods such as concentrating the sample or extension of the incubation time, *etc*. can also be employed[43,44]. High concentration of urine has been found to increase sensitivity in many assays where the total IgG concentration required for saturating the binding sites was optimal[45].

Immunoglobulins are found to be stable in urine for a prolonged period; however, fresh urine or samples stored for a short duration at 4°C are ideal for diagnosis[42]. In a study on the stability of urinary proteins under various conditions, it was found that IgG was stable at room temperature for seven days and at 4°C for a month with and without preservatives, whereas the IgG concentration was found to decrease upon storage at −20°C without preservative[46]. Several viral antibodies have been reported to be stable at ambient temperature, 4°C, −20°C and −80°C conditions for varying periods (Table I). Repeated freeze-thawing of urine has, however, shown a reduction in antibody titres[46,47]. On the contrary, freeze-thawing had no effect on the stability of Hepatitis C virus antibodies in one study[10]. Tencer *et al*[46] have demonstrated the use of benzamidinium chloride, EDTA (ethylenediamine - tetraacetic acid), tris (hydroxymethyl)-aminomethane azide, *etc*. for successful preservation for varying periods of storage. Thimerosal (5 mg/ml) has also been used as a preservative and stability of HIV-1 antibodies for six months at −20°C has been demonstrated with this preservative[43]. Sodium azide at a final concentration of 0.1 per cent was also used as a preservative and found antibodies to be stable at room temperature and 4°C[9].

Table II. Urinary antibodies against viral pathogens

Name of virus	Family	Symptoms	Antibody type detected in urine	Test and antigen used for detection
Poliovirus 1, 3	*Picornaviridae*	Fever, fatigue, headache, vomiting, stiffness of the neck, pain in the limbs, paralysis of limbs	nAb[14], IgA[17]	Plaque reduction combined with immunoinactivation, using the whole virus[14], RIA using purified polio virus[17]
BK virus	*Polyomaviridae*	Renal dysfunction (post-transplant cases)[18]	IgG[19]	Haemagglutination inhibition tests using supernatant fluid/ W138 cells infected with BKV as antigen[19]
SV 40	*Polyomaviridae*	Viral antigen detected in human brain tumours, mesotheliomas, osteosarcomas[20]	nAb[15,16]	Neutralization test using infected green monkey cells culture supernatant as antigen[15], neutralization test[16]
Adeno virus	*Adenoviridae*	In chimpanzees with chronic adenoviral viruria[21]	nAb, IgG, IgA[21]	Neutralization test and immunofluorescence using virus infected WI-38 cell cultures[21]
Bovine leukemia virus	*Retroviridae*	Bovine leucosis in cattle[22]	IgG[22]	Commercial ELISA kit
Dengue virus	*Flaviviridae*	Nausea, vomiting, pain behind eyes, muscle pain, joint pain, rashes, bleeding[23]	IgA[6,7,23], IgG[23]	Commercial Ig A kit against Dengue virus[6], AACELISA and GAC-ELISA using tetravalent dengue virus antigen prepared in suckling mice brain and extracted by acetone–sucrose[7,23]
Hepatitis A	*Picornaviridae*	Fever, nausea, lack of appetite, diarrhoea, dark-coloured urine, jaundice[24]	IgG[25,12] IgM monomer[25,12]	Tissue culture grown antigen[25], RIA using tissue culture-derived HAV Ag[12]
Hepatitis B	*Hepadnaviridae*	Fatigue, nausea, vomiting, abdominal pain, jaundice, dark urine, cirrhosis/ liver cancer (in some cases)[26]	IgG (Anti-HBc), IgM monomer[25,12]	RIA using recombinant HBcAg (gifted)[25], RIA using liver-derived HBcAg[12]
Hepatitis C	*Flaviviridae*	80% asymptomatic, fever, fatigue, vomiting, nausea, abdominal pain, dark coloured urine, grey-coloured faeces, joint pain and jaundice[27]	IgG[10]	Commercial ELISA
HIV-1	*Retroviridae*	Progressive immunosuppression and secondary infections[28]	IgG (antibodies against gp120, gp160, p17, p24, p33, gp41, p51, p55 and p61)[28]	Commercial ELISA
Rubella	*Togaviridae*	Rash, low fever, nausea, conjunctivitis swollen lymph glands, arthritis and painful joints (adults), congenital rubella syndrome[29]	IgA[29], IgG[9,29]	ELISA using commercially available rubella viral antigen[29], commercial ELISA[9]

Contd...

Name of virus	Family	Symptoms	Antibody type detected in urine	Test and antigen used for detection
Hepatitis E	*Hepeviridae*	Fever, anorexia, nausea, vomiting, jaundice[30]	IgG, IgA[31]	ELISA using recombinant ORF2 protein of HEV[31]
HCMV	*Herpesviridae*	In immunosuppressed individuals causes retinitis, oesophagitis, encephalitis, myelitis, radiculopathy, colitis[32]	IgA, IgG[33]	ELISA using CMV complement fixing antigen[33]
Hantavirus	*Bunyaviridae*	Headache, abdominal pain, fever, chills, nausea, blurred vision, rash, kidney failure[34]	IgA, IgG[35]	TR-FRET using baculovirus-expressed–N PUUV, immunofluorescence using PUUV infected acetone-fixed Vero E6[35]
H1N1	*Orthomyxoviridae*	Fever, chills, sore throat, cough, headache, coryza, myalgia, prostration[36]	IgG[37]	Purified haemagglutinin from influenza A/Taiwan (H1N1)[37]
RSV	*Paramyxoviridae*	Fever, cough, sore throat, shortness of breath, coryza[38]	IgG[37]	RSV CF antigen[37]
Zika virus	*Flaviviridae*	Fever, headache, rash, joint pain, muscle pain, conjunctivitis (red eyes)[39]	IgM[39]	Detected using whole genome-fragment phage display libraries with binding to antigenic sites in E, NS3 and NS5[39]
HPV	*Papillomaviridae*	Cervical carcinoma	IgM, IgG, IgA[40]	Using HPV-L1-fusion protein antigens in a glutathione S-transferase multiplex assay[40] and virus-like particle-based ELISA[41] to detect HPV-6, -11, -16 and-18

HCMV, human cytomegalovirus; HPV, human papilloma virus; nAb, neutralizing antibodies; IgG, immunoglobulin G; IgA, immunoglobulin A; IgM, immunoglobulin M; RIA, radioimmunoassay; HAV Ag, hepatitis A virus antigen; HEV, hepatitis E virus; CMV, cytomegalovirus; TR-FRET, time-resolved forster resonance energy transfer; PUUV, puumala orthohantavirus; RSV, respiratory syncytial virus; CF, cystic fibrosis; ELISA, enzyme-linked immunoassay; GAC-ELISA, IgG antibody capture ELISA; ORF2, isoform capsid protein; AACELISA, IgA antibody capture ELISA

Origin of urinary immunoglobulins

A urine proteome analysis reportedly showed >1500 different proteins[48]. These proteins were evenly distributed in urine as compared to other body fluids. A healthy person excretes ~150 mg protein through urine in a day and more than half of which is contributed by glomerular filtration of blood[49]. Healthy individuals can excrete 1.1 mg of IgA and ~3 mg of IgG during a 24 h period[50]. Due to its larger size, IgM is not filtered through kidneys; however, monomeric forms have been detected in urine[51].

Immunoglobulins reach urine either through glomerular apparatus or by the local synthesis in the urinary tract. Glomerular apparatus comprises endothelial lining, basement membrane and podocytes that play a major role in filtration, and injury to any of these layers can alter the balance of filtration[52]. It largely depends on the molecular size, shape, charge of solute, concentration in serum and renal function[53]. The glycosialoprotein coating of the endothelium imparts a negative charge to the glomerular membrane and this explains how the negatively charged plasma proteins are retained in circulation. The small percentage of immunoglobulins, which manage to enter the filtrate, are reabsorbed by the tubule and catabolized. Immunoglobulin filtration from blood circulation to urine was experimentally proven by intravenous injection of radio-labeled human gamma globulin and its recovery in the urinary globulin fraction[54]. The FcRn

receptor, which is expressed on the podocytes and the proximal convoluted tubule, aids in IgG transport across the membrane, which may be the reason why antibodies are not found in many infections[55].

Glomerular permeability, in general, could be altered by glomerulonephritis, endothelial cell damage, immune complex deposition in the glomerular basement membrane, podocyte effacement and nephritic syndrome[51,56,57]. Deposition of macromolecules, which happen to escape the endothelial lining and basement membrane, can damage the podocyte. The glomerular damage could be either due to direct infection by viruses or by the inflammatory cytokines released during infection. This has been experimentally shown with HIV-1 and other viruses, *viz*., Parvovirus B19, SV 40, cytomegalovirus (CMV), Epstein–Barr virus, *etc*., which have probable association with glomerulosclerosis[58]. Dengue virus NS1 antigen is known to damage the glycocalyx layer as demonstrated by the increased heparin sulphate excretion, a component of glycocalyx layer in urine of children with severe dengue infection[59]. Antibody excretion seen in Hantaan virus cases with haemorrhagic fever and renal syndrome stopped when the normal kidney function was restored[34]. The absence of urinary dysfunction with the detection of IgG from cattle urine was observed in Bovine Leukaemia virus infection, indicating the origin to be serum[22].

The concept of local IgG synthesis has been confirmed in studies showing urinary immunoglobulins in children with pyelonephritis or urinary tract infections[60] as well as in experimentally infected laboratory animals[61,62]. Secretory IgA antibody has been isolated from urine[50]. IgA- and IgM-positive plasma cells were found in the bladder urothelium in urinary bladder infections, which is an important part of the mucosal immune system[63]. Intact immunoglobulin without albuminuria has been reported in the urine of HIV patients[64]. The ability of renal epithelial cells to act as a reservoir has been studied extensively in HIV infections and compartmentalized immune response was observed in such patients[65]. Urnovitz *et al*[8] analyzed various urinary antibody tests for HIV-1 and hypothesized that the above could be the reason for the urine positive seronegative cases and positivity of urinary IgA in most of the cases strengthen its local origin. The theory of local immune response contributing to antibody production was also suggested in congenital CMV infection by demonstrating CMV specific-secretory IgA in urine of infants[66]. The passive transfer may also cause urine positivity of CMV as it was observed in the urine of the uninfected[34]. Haemorrhages to the urinary tract can also contribute to the presence of antibodies in urine. Bleeding into the urinary tract can also result in antibodies in urine, as shown in individuals with schistosomiasis[67].

Viral nephropathy

Viral infections are known to cause kidney damage. Viruses, *viz*., HIV, HBV, HAV, HCV, Epstein–Barr virus, Parvovirus B19, Hantavirus, SARS-CoV, BK virus and Influenza A virus *etc.*, have shown a direct association with kidney diseases. Dengue, CMV, adeno and Coxsackie B virus have also been found to cause nephropathy[68,69]. A suppositious role has also been attributed to viruses such as mumps, measles, varicella and herpes[68]. Varying degrees of proteinuria have been reported from many of these infections. The mechanisms attributed to virus-related kidney injury have a direct cytopathic effect on glomerular/tubular epithelial cells, inflammatory response to infection by host, injury due to immune complex deposition, therapeutic nephrotoxicity and multi-organ failure[69].

HIV and hepatitis viruses are well studied among the viruses that induce kidney diseases. HIV-associated nephropathy is well established and a wide range of lesions are reported from patients[70,71]. Compartmentalized viral replication has been observed in kidneys[70]. Focal segmental glomerulosclerosis and immune complex-mediated glomerulonephritides are also reported from HIV patients[71]. Renal toxicity has been observed with many antiretroviral agents, which cause damage to the filtration barrier[72]. HBV causes glomerulonephropathy and viral antigen depositions have been demonstrated in the glomeruli[73]. Secondary glomerular disease in the form of membraneous/IgA nephropathy, immune complex-related vasculitis and complement-mediated injury has also been reported with HBV infection[74]. HCV infection inflicts kidney damage by direct renal invasion or by circulating cryoglobulins causing mesangiocapillary glomerulonephritis[75]. Incidences of acute tubular necrosis, interstitial nephritis and glomerulonephritis have been reported in HAV patients[76]. Glomerulonephritis has also been observed in patients with HIV and HCV coinfection[77].

Urine-based diagnostic assays

ELISA and Western blot-based assays were used initially to detect urinary antibodies. The United States Food and Drug Administration licensed an enzyme immunoassay and Western blot for the detection of

Table III. Accuracy of assays employed to detect urinary antibodies against viral pathogens

Virus	Method of detection	Antigen used for detection	Sensitivity (%)	Specificity (%)	Reference
HIV-1	EIA	Commercial assay	98	99.8	64
	EIA	Commercial assay	89.6	97.3	78
	Rapid assay	Recombinant *env* proteins	97.89	100	79
	GACPAT	Commercial antigen	100	97-99	42
	GACELISA	Recombinant HIV *env-gag* construct antigen	99.4	97-99	42
	GACPAT	Commercial HIV antigen	96.5	98.8	80
	GACELISA		98.8	99.2	
	Immunocomplex transfer enzyme immunoassay (rRT antigen)	rRT	100	100	40
	Western blot	Commercial assay	97.2	100	39
			98.6	100	
	EIA	Commercial assay	99.5	98.3	81
	EIA	Commercial assay	100	88.8	82
	Western blot	Commercial antigen	97.7	100	83
HCV	ELISA	Commercial ELISA and immunoblot	100	100	84
HAV	GACRIA	Tissue culture grown antigen	98.9	99.1	25
	MACRIA	Tissue culture grown antigen	95.8	99.6	25
	ELISA	-	88.98	92.92	12
	IgM capture ELISA	Cell culture grown virus antigen	95.65	100	11
	IgG capture ELISA		97.76	76.47	
	IgA capture ELISA		92.23	88.18	
HBV	GACRIA	Recombinant HBcAg	94.2	100	25
	MACRIA	Recombinant HBcAg	26.19	100	25
Rubella	ELISA, HI	Commercial assay	96	99	9
	ELISA	Commercial RV antigen	100	100	29
Dengue	Rapid diagnostic test (IgG, IgA)	Commercial assay	27.9	100	7
	in house ELISA (IgG, IgA)		10.7		
			40.1		
	GACELISA	Antigen prepared in mouse brains	100	100	23
	EIM		61		
HCMV	ELISA	pp150 expressed in *Escherichia coli*	70	94	66
	ELISA (IgA)	Complement fixing antigen	38.88	92	33
	ELISA (IgG)		76.9	50	

IgG, immunoglobulin G; IgM, immunoglobulin M; IgA, immunoglobulin A; EIA, enzyme immunoassay; GACPAT, IgG antibody capture particle-adherence test; ELISA, enzyme-linked immunoassay; GACELISA, IgG antibody capture ELISA; GACRIA, IgG antibody capture radioimmunoassay; MACRIA, immunoglobulin M capture radioimmunoassay; HCV, hepatitis C virus; HAV, hepatitis A virus; HBV, hepatitis B virus; HCMV, human cytomegalovirus; rRT, recombinant reverse transcriptase; RV, rotavirus; HBcAg, hepatitis B core antigen; HI, haemagglutination inhibition

HIV-1 antibodies in urine in 2019[13]. Advancements in urine-based diagnostics have led to the development of recombinant/synthetic antigen-based assays[8,43]. Many researchers employed specific immunoglobulin capture assays for urinary antibody detection, which showed promising results with good accuracy. The assays

along with their reported sensitivity and specificity are enlisted in Table III[78-84].

Effect of *p*H in urine samples

The *p*H variations in urine samples are wide as compared to serum samples creating a major hurdle in the validation of diagnostics. The studies that attempted to uncover the effect of *p*H variation on some assays have shown contradictory results[10,43,85]. No significant effect on the absorbance value of ELISA could be detected by variations in the *p*H range 5 to 7.5[10,85]. However, Hashida *et al*[43] saw a reduction in immune complex transfer enzyme immunoassay signals in the acidic *p*H range and observed maximum signals at *p*H 7.0-8.0.

Antibody kinetics in urine

Although most of the studies on urine-based diagnostic assays were restricted mainly to antibody detection, a few tried to understand the antibody kinetics in either diseased patients or in vaccinated individuals.

Comparison with the serum concentration was presumably helpful in deciding the window period for diagnosis. Connell and Parry[86] demonstrated the simultaneous appearance of urinary and serum antibodies against p24 and gpl60 antigens in HIV patients. Uropositivity in the convalescent phase in paired serum samples by MAC (IgM antibody capture) ELISA has been demonstrated in hepatitis A patients[11]. Conversely, Rodríguez Lay Lde *et al*[12] found a faster degradation of urinary IgM compared to serum in six months in the case of HAV infection. However, the investigators observed higher IgM levels in urine than serum in seven patients at the initial phase of the disease. Zhang *et al*[10] reported a good correlation of anti-HCV (against NS4, NS5, Core 1, 2, 3, 4) detection between serum and urine samples obtained from forensic autopsy cases. Contradictory results were published against the study by Elsana *et al*[87], and the probability of mixing of serum and urine in autopsy cases was cited as a drawback in the earlier study.

Vazquez *et al*[23] have demonstrated the kinetics of urinary IgA and IgG antibodies in comparison to serum antibodies to classify primary and secondary dengue infections. Although no IgM antibody could be detected in urine, IgG antibodies were detected between 3 to 7 days of onset in secondary infections using an in-house antibody capture ELISA (using an antigen mixture of the four dengue serotypes). Zhao *et al*[6] have shown that urinary IgA antibodies detected by a rapid test could be a good marker to determine secondary dengue infections and can be used as a warning signal for clinical management in severe dengue cases. A similar pattern of serum and urinary IgG antibody levels were observed in the initial two weeks of the study in dengue patients, but the latter started decreasing in the following weeks and only 10 per cent showed positivity after three months, while the plasma IgG remained stable[7]. Their analysis suggested that the urinary concentration of antibody depends on the patient's immune status and sampling time. With respiratory syncytial virus and H1N1 virus, a significant rise in antibody titre against RSV CF antigen or purified haemagglutinin from influenza A/Taiwan (H1N1) was observed between acute and convalescent urine samples by GAC (IgG antibody capture) ELISA[37]. However, low concentration of IgG and IgA antibodies against ORF 2 (open reading frame 2) protein was observed in hepatitis E patients, indicating either an undisturbed renal function or inadequacy of urine samples for diagnosis[31]. Urinary IgG antibody titre showed an increasing trend from 3rd/4th wk to 28th wk like that of serum IgG antibodies in a rubella virus vaccine-induced infection study depicting the application of this non-invasive approach for screening young children[9]**.** The study also observed that anti-Rubella virus IgA/IgG ratio in urine could be a useful marker in diagnosing recent infection.

Limitations

Low concentrations of antibodies and limited knowledge on the antibody kinetics/ stability at different physiological conditions are the major limitations in using urine as a diagnostic sample. In addition, the presence of chemicals or drug residues in urine, changes in *p*H, differences in urine matrix components among individuals, *etc.* can also influence immunoassay results[43,88]. The persistence of IgM antibodies in virus carriers can make it difficult to interpretate the results though it can be managed by relating the IgG and IgM values. Demonstration of a significant increase in antibody titre between acute and convalescent samples require quantification of total immunoglobulin[37]. Even with recommended ELISA and Western blot kits for anti-HIV-1 antibody detection in urine, indeterminate results (values) due to the presence of autoimmune self-antigens are a major concern. The complexing of antigens with specific antibodies can also interfere with immunoassays and this should be kept in mind while using urine as a diagnostic sample.

Conclusions

Overall, diagnostic tools can be useful in public health if they are specific, sensitive, cost-effective and user-friendly. Urine may find application as a useful diagnostic sample for the detection of antibodies due to its unique advantages in comparison with invasive procedures. Its potential as a diagnostic sample has not been fully explored so far due to low concentration of antibodies, variations in results due to physiological changes, methods of collection, presence of drug residues and other chemicals, *etc*. However, recent studies have shown the application of urine for antibody detection in many diseases. Urine-based diagnostic assays like specific immunoglobulin capture assays have shown good accuracy at par with serological assays. Most of the investigations reported are on a limited number of samples, which necessitates large-scale studies to explore the potential of urine as a diagnostic sample. Moreover, the detection of antibodies in urine has given new insights into the pathogenesis of HIV infections which points to the potential application of this approach for other microbes also. Screening of urine for antibodies in cases of viruses that are known or suspected to cause nephropathy should also be considered. Little is known about the quantity, site of synthesis and function of virus-specific urinary immunoglobulins. Studies are hence needed to understand the antibody kinetics of different viruses, particularly of public health importance where the urine samples can be used.

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