Characteristics of Different Solid-Phase Immunoassay Formats for the Measurement of BK Virus Immunoglobulin M in Sera of Patients on Renal Dialysis or with Kidney Allografts

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BK virus (BKV) was first isolated in 1971 from the urine of a renal allograft patient (13). Since then, it has become apparent that BKV and another papovavirus, JC virus, are independently circulating in the human population. Serological surveys have shown that both viruses are found worldwide and that primary infection occurs most commonly in childhood (7, 12, 18, 23). Urine is the only known route of excretion of human papovaviruses, although the virus has been found in brains of patients with progressive multifocal leukoencephalopathy (1). Studies of renal allograft and bone marrow transplantation patients have shown that BKV is reactivated in patients with reduced immunocompetence (5, 16, 22, 25). BKV has been found in the urine of immunocompromised patients (2), including patients with congenital immunodeficiencies (26, 31), acquired immunodeficiency syndrome patients (8), and patients receiving therapy for malignancy (10, 25). Recently, BKV DNA has been found in Kaposi’s sarcoma tissue (3), and receptors for BKV have been found on lymphocytes (24). Serological studies have shown that pregnant women may have a primary or reactivated BKV infection (6), and this has raised the question of in utero papovavirus infection. Polyomavirus is transmitted transplacentally in mice (19), but evidence for transplacental transmission of polyomavirus in humans is contradictory (6, 14, 26, 28, 30) and there have been no confirmed reports of in utero infection in humans.

Infections with human polyomaviruses are difficult to diagnose since wild-type strains of human polyomaviruses require passage before they will grow in conventional cell culture, making primary isolation an impractical approach. To date, serology has been much more useful than virus isolation. Seroconversion, fourfold increases in immunoglobulin G (IgG) antibody titers between acute- and convalescent-phase sera, and detection of specific IgM antibody have been used for diagnosing BKV infections. Detection of papovavirus-specific IgM was first performed by sucrose density gradient (SDG) separation followed by hemagglutination inhibition (HI) or by indirect immunofluorescence using virus-infected cell cultures (10, 27). Both of these methods are time consuming and cumbersome and do not lend themselves to testing large numbers of serum samples. Solid-phase immunoassays have recently been developed for the measurement of BKV IgM antibody (9, 32). We have developed sensitive and specific antigen capture and antibody capture enzyme immunoassays (EIAs) for the detection of BKV IgM antibody and have used these assays to diagnose BKV infections in immunocompromised patients.

MATERIALS AND METHODS

Sera. Sera were obtained from renal dialysis and allograft patients attending the Regional Renal Dialysis Unit at St Joseph’s Hospital. Additional sera submitted to the Regional Virology Laboratory, St. Joseph’s Hospital, for viral studies were obtained from patients with recent rubella virus, cytomegalovirus, or hepatitis A or B virus infections. Paired serum samples from two patients with JC virus infection were obtained from K. Shah (National Institutes of Health, Bethesda, Md.).

Treatment of sera. Sera were treated to remove IgG and rheumatoid factor (RF) by using Staphylococcus aureus protein A (Sigma Chemical Co., St. Louis, Mo.) or IgG-coated latex particles (RF reagent was obtained from Behringwerke AG, Marburg, Federal Republic of Germany) as described previously (32). Confirmation of IgG removal was obtained by Ouchterlony immunodiffusion with goat anti-human IgG antisera (Organon Teknika, Malvern, Pa.).

Separation of IgG and IgM. SDG fractionation was performed by using conventional procedures (32). Separation of...
IgM and IgG by ion-exchange chromatography was performed by using quaternary amioethyl Sephadex columns (Isolab Inc., Akron, Ohio) according to the instructions of the manufacturer. IgM fractions were adjusted to pH 7 before being tested for BKV IgM antibody.

**Purification of BKV antigen.** The laboratory-adapted BKV (strain Mad) was obtained from E. Major (National Institutes of Health, Bethesda, Md.) and was propagated in Vero cells. Cultures were harvested 10 to 12 days after infection, and cells were disrupted by three cycles of freezing and thawing followed by sonication. Cell debris was removed by centrifugation at 2,000 rpm for 20 min, and virus was collected by centrifugation for 3 h at 4°C in a rotor (SW27; Beckman Instruments, Inc., Fullerton, Calif.) at 21,000 rpm. Virus was extracted three times with Freon (E. I. du Pont de Nemours & Co., Inc., Wilmington, Del.), collected by centrifugation onto a cushion of saturated CsCl, and further purified by isopycnic centrifugation in a CsCl gradient. The virus banded at a density of 1.34 g/ml and was collected and then used as the antigen in solid-phase assays. Control antigen was prepared by processing uninfected Vero cells in the same manner. In some experiments, BKV was grown in human embryonic kidney (HEK) cells. Fourth-passage HEK cells (M. A. Bioproducts, Walkersville, Md.) were infected with BKV, and cells showing 4+ cytopathic effect were harvested on day 10 postinfection and lysed by four cycles of freezing and thawing. Virus was then purified as described above.

**Antigen capture EIA.** Purified BKV antigen was used to coat 96-well microtiter plates as described previously for the radioimmunoassay (RIA) (32). Culture supernatant fluid with a hemagglutination titer of 1:4,096 was also used to coat microtiter plates for some experiments. All reagent volumes were 50 μl. HEK cell lysate was used at an equivalent protein concentration (~1 μg/ml) to coat parallel wells to assess nonspecific antibody binding. Doubling dilutions of sera starting at 1:200 were prepared by using buffer G as the diluent (17), and sera were incubated for 3 h at 37°C. BKV IgM antibody was detected with horseradish peroxidase-conjugated goat anti-human IgM antibody diluted 1:400 in buffer G (1 h at 37°C). ortho-Phenylendiamine substrate and 3 N HCl (50 μl) stop solution were used as described previously (17). Plates were read with an automated Microelisa reader (model MR580; Dynatech Industries, Inc., McLean, Va.). The cutoff for positivity was established as twice the mean of 12 serum samples which were IgM negative by RIA.

**Antibody capture EIA.** Wells of microtiter plates were coated with sheep anti-human IgM antibody diluted 1:200 in carbonate buffer, pH 9.6 (2 h at 37°C). All reagent volumes were 50 μl. Serum dilutions starting at 1:200 were prepared by using buffer G as the diluent with or without 2% normal rabbit serum, and plates were incubated overnight at 4°C. Purified BKV antigen diluted 1:100 in buffer G was added, and the plates were incubated at 37°C for 3 h. A mouse monoclonal antibody to BKV, designated 2D6 (M. Zapata et al., manuscript in preparation), was added at a dilution of 1:800 in buffer G and incubated for 1 h at 37°C. Horseradish peroxidase-conjugated goat anti-mouse IgG diluted 1:400 in buffer G was added for 1 h at 37°C, followed by ortho-phenylenediamine substrate. The cutoff of positivity was established as twice the mean absorbance of 12 RIA IgM-negative serum samples. BKV IgM antibody titers were determined as the highest serum dilution with an absorbance greater than the cutoff.

**RIA.** BKV IgM RIA was performed as described previously without modification (32). Briefly, purified virus was used to coat polystyrene chloride plates, and serum dilutions in buffer G were incubated overnight at 4°C. Bound IgM antibody was detected with iodinated goat anti-human IgM antibody, and binding ratios were determined as described previously (32).

**HI.** Antibody to BKV was measured by a standard microtitration technique following treatment of sera with receptor-deactivating enzyme and absorption with type O erythrocytes as described previously (12). HI titer was the highest dilution of serum showing less than 50% agglutination of erythrocytes.

**RF.** RF was measured by latex agglutination with the RF reagent (Behringwerke AG).

## RESULTS

All reagents for the antigen capture EIA were titrated to determine optimal concentrations for each. The concentration of solid-phase BKV antigen had the greatest effect on the signal strength, and the highest signal/noise ratios (>30:1) were obtained with a 1:100 dilution of purified BKV antigen, corresponding to 1.9 μg of viral protein per ml. Experiments with crude unpurified virus (in culture medium) showed that unpurified antigen could be used in place of purified virus without a decrease in sensitivity. The optimal amount of unpurified virus was 102 hemagglutination units per microdilution well, determined by testing an IgM-positie serum sample by using serial dilutions of culture medium containing 2,048 hemagglutination units of virus. This amount of virus also gave a signal/noise ratio of ≥30:1. The antibody capture EIA used solid-phase anti-human IgM antibody, BKV antigen, and anti-BKV monoclonal antibody. The results obtained for three serum samples are shown in Fig. 1. BKV IgM titers were 1:1,600, 1:800, and 1:200 for antigen capture (Fig. 1A) and 1:25,600, 1:1,600, and 1:1,600 for antibody capture EIA (Fig. 1B).

To verify that the IgM EIA was specific for BKV IgM and were not affected by the presence of BKV IgG antibody, sera were separated by SDG fractionation, and the fractions were tested for BKV IgG and IgM by antigen capture and antibody capture EIA. Only fractions containing IgM antibody as assessed by Ouchterlony immunodiffusion with class-specific reagents were positive by antigen capture or antibody capture IgM EIA. These results were similar to those obtained previously for the antigen capture RIA (32). Similarly, the addition of BKV IgG antibody to IgM antibody fractions did not affect the measurement of BKV IgM antibody in either EIA (data not shown).

Since the presence of RF has been shown to give false-positive IgM antibody results in the presence of specific IgG antibody when tested in antigen capture assays, we conducted experiments to determine the effect of RF on the antigen capture EIA. Serial dilutions of a serum sample containing RF (titer, 1:10,240) were added to aliquots of a BKV IgM-negative (titer <1:50). BKV IgG-positive (titer, 1:102,400) serum, and the resultant serum samples were tested for BKV IgM by antigen capture EIA. A combination of RF at a titer of ≥1:40 and high levels of BKV IgG antibody (1:10,240) or BKV IgG-positive (1:102,400) serum and the resultant serum samples were tested for BKV IgM by antigen capture EIA. A combination of RF at a titer of ≥1:40 and high levels of BKV IgM antibody (1:10,240) or a combination of high RF levels (≥1:10,240) together with BKV IgG titers of ≥1:40 by HI was sampled in false-positive IgM antibodies in the antigen capture EIA (Table 1). The presence of RF false-positives could be eliminated by treatment of sera with sheep anti-human IgG antiserum or IgG-coated latex particles. The presence of RF false-positives could be eliminated by treatment of sera with sheep anti-human IgG antiserum or IgG-coated latex particles.
ence of RF did not, however, give false-positive results in the antibody capture EIA (data not shown).

The specificity of the EIAs for BKV IgM was assessed by testing sera from patients with other viral infections, including four with rubella virus, two with cytomegalovirus, three with hepatitis B virus, three with hepatitis A virus, and two with JC virus. All 14 serum samples containing IgM antibody to these other viruses were negative for BKV IgM antibody by both EIAs (data not shown).

The performance of the two EIAs was compared with RIA by testing sera from renal allograft recipients with BKV infections. Of 37 serum samples, 15 had BKV IgM antibody; 14 were positive by RIA and 11 were positive by each EIA (Table 2). Thirteen sera were negative by all three tests (eight of these were included in Table 2). The geometric mean titer of positive sera tested by antibody capture EIA was 9,657, compared with 11,745 for RIA and 2,718 for antigen capture EIA. Using RIA as the standard, the sensitivities (78.6%), specificities (100%), positive predictive values (100%), and negative predictive values (88.5%) for both EIAs were identical.

**DISCUSSION**

We have developed antigen capture and antibody capture EIAs for the detection of BKV IgM antibody. Both EIAs detected BKV IgM antibody in sera from renal allograft recipients with reactivated BKV infections, and results for both EIAs were comparable with results obtained with RIA. RF produced false-positive results in both antigen capture EIA and RIA but not in the antibody capture EIA. The RIA appeared to be more sensitive than both EIAs, detecting three additional sera with low levels of BKV IgM antibody.

The development of solid-phase antigen capture EIAs and RIAAs for the measurement of specific IgM antibody has provided a significant advantage over the measurement of viral IgM antibody by traditional methods, including SDG fractionation followed by HI; however, these assays are not without problems. Sera from patients with immunological abnormalities, including anti-nuclear antibody, anti-smooth muscle antibody, or multiple myeloma, have been shown to cause false-positive rubella virus IgM results with antigen capture assays (4). The sensitivity of these assays depends upon a high proportion of virus-specific IgM antibody in sera, since other IgM-class antibody, including RF, can reduce the sensitivity of antibody capture EIAs by competing with virus-specific IgM for binding sites on the solid phase. RF bound to the solid phase has been shown to produce false-positive results in a number of different assays (11, 15, 20, 32). This may occur as a result of RF binding enzyme-labeled antiviral antibodies directly or by complexing with serum antiviral IgG antibodies which bind viral antigen (11). RF interference has therefore necessitated the removal of IgG antibody by treatment with either staphylococcal protein A, aggregated IgG, or IgG-coated latex particles prior to testing (4, 11, 29, 32). Additional approaches for improving specificity have included the use of purified viral antigen to eliminate false-positive IgM results obtained with crude antigens (11, 28, 29) and the use of a control

![Figure 1](http://jcm.asm.org/) Measurement of BKV IgM antibody by antigen capture and antibody capture EIA. Three serum samples tested by antigen capture EIA (A) had titer of 1:1,600 (●), 1:800 (○), and 1:200 (■), while the same serum samples had titer of 1:25,600 (●), 1:6,400 (○), and 1:1,600 (■) when tested by antibody capture EIA (B). The cutoff value (-----) was determined by taking twice the mean of 12 IgM-negative serum samples (□).
antigen to allow identification of false-positive reactions in antibody capture assays (11).

The antibody capture EIA for the measurement of BKV IgM antibody has important advantages over the antigen capture EIA and RIA. Although the presence of BKV IgG antibody alone did not affect the measurement of BKV IgM antibody in either assay, when RF was present together with BKV IgG antibody the antigen capture EIA and RIA gave false-positive IgM results, whereas the antibody capture EIA did not. Either high levels of BKV IgG together with low levels of RF or the converse resulted in IgM false-positives in both the antigen capture EIA and RIA. RF false-positives could be eliminated by pretreating the sera to remove IgG. The second advantage of the antibody capture EIA over the antigen capture EIA was increased sensitivity. The geometric mean titer of BKV IgM antibody was higher for the antibody capture EIA than for the antigen capture EIA (9.657 compared with 2.718). The sensitivity of the antibody capture EIA appeared to be slightly lower than that of the RIA as seen by the detection by RIA of low levels of BKV IgM antibody in four serum samples which were negative by antibody capture EIA. Flaegstad and Traavik compared a solid-phase antigen capture enzyme-linked immunosorbent assay (ELISA) and antibody capture EIA with the HI test (9). They also found that high levels of RF and BKV IgG antibody caused false-positives in the antigen capture EIA but not in the antibody capture EIA. In contrast to our findings, they found that a solid-phase antigen capture ELISA appeared to be more sensitive than the antibody capture ELISA or SDG fractionation followed by HI; solid-phase antigen capture ELISA detected 17 positive specimens, compared with 9 by antibody capture ELISA and 10

**TABLE 1.** False-positive BKV IgM results due to the presence of BKV IgG and RF measured by antigen capture EIA

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<tr>
<th>RF or BKV IgG antigen antibody titer</th>
<th>BKV IgM titer</th>
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<sup>a</sup> An aliquot of serum containing BKV IgG antibody (titer 1:102,400) and lacking IgM antibody was mixed with an equal aliquot of serial dilutions of a serum containing RF (titer 1:10,240). The resultant mixtures were tested for RF and IgM by antigen capture EIA with or without pretreatment as described in Materials and Methods.

<sup>b</sup> Neg. No BKV IgM detected.

<sup>c</sup> An aliquot of serum lacking BKV antibody and containing RF (titer 1:10,240) was mixed with an equal aliquot of serial dilutions of a serum containing BKV IgG antibody but no IgM antibody. The resultant mixtures were tested by HI and IgM antigen capture EIA with or without pretreatment as described in Materials and Methods.

by SDG fractionation followed by HI. Their results may not reflect the true relative sensitivities of the three assays, however, since these authors adjusted the sensitivity of both ELISAs to give results comparable with the SDG fractionation-HI test. The maximum IgM titer reported by Flaegstad and Traavik was 1:10,240, compared with 1:51,200 obtained with our antibody capture EIA, suggesting that their assays were not giving maximum sensitivity or that the sera they evaluated contained lower levels of BKV IgM antibody than did our sera.

In the absence of suitable cell cultures for the isolation of BKV from clinical specimens of patients with primary or reactivated BKV infections, serology has been useful for making a diagnosis. Our results and those of others suggest that the antibody capture EIA may be the method of choice for diagnosing BKV infections in immunocompromised patients since antibody capture EIA has good sensitivity, no requirement for pretreatment of sera to eliminate RF false-positives, and none of the disadvantages associated with the use of radioisotopes. The availability of commercial kits employing antibody capture ELISA technology for the detection of BK papovavirus IgM antibody should make this test available to more virology laboratories for the diagnosis of BK papovavirus infections in immunocompromised individuals. The appearance of rubella virus IgM antibody in sera of patients with other viral infections, such as Epstein-Barr virus, which may cause polyclonal stimulation of memory B lymphocytes (21) should alert virology laboratories to interpret IgM serology results in conjunction with other clinical and laboratory findings, especially when assisting physicians with clinical decision making.

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LITERATURE CITED


