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Development and evaluation of a BK polyomavirus serotyping assay using Luminex technology

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ABSTRACT

Background: The BK polyomavirus (BKPyV) is subdivided into four genotypes. The consequences of each genotype and of donor-recipient genotype (mis)match for BKPyV-associated nephropathy (BKPyVAN) in kidney transplant recipients (KTRs) are unknown.
Objectives: To develop and evaluate a genotype-specific IgG antibody-based BKPyV serotyping assay, in order to classify kidney transplant donors and recipients accordingly.
Study design: VP1 antigens of six BKPyV variants (Ib1, Ib2, Ic, II, III and IV) were expressed as recombinant glutathione-s-transferase-fusion proteins and coupled to fluorescent Luminex beads. Sera from 87 healthy blood donors and 39 KTRs were used to analyze seroreactivity and serospecificity against the different BKPyV genotypes. Six sera with marked BKPyV serotype profiles were analyzed further for genotype-specific BKPyV pseudovirus neutralizing capacity.
Results: Seroreactivity was observed against all genotypes, with seropositivity rates above 77% comparable for KTRs and blood donors. Strong cross-reactivity ($r > 0.8$) was observed among genotype I subtypes, and among genotypes II, III and IV. Seroresponses against genotypes I and IV seemed genuine, while those against II and III could be out(cross)competed. GMT (Luminex) and IC_{50} (neutralization assay) values showed good agreement in determining the genotype with the strongest seroresponse within an individual.
Conclusions: Despite some degree of cross-reactivity, this serotyping assay seems a useful tool to identify the main infecting BKPyV genotype within a given individual. This information, which cannot be obtained otherwise from nonviremic/nonviruric individuals, could provide valuable information regarding the prevalent BKPyV genotype in kidney donors and recipients and warrants further study.

1. Background

BK polyomavirus-associated nephropathy (BKPyVAN) is one of the major causes of graft dysfunction and loss in kidney transplant recipients (KTRs). BKPyV DNA is detected in urine and blood in 50–70% and 20–30% of KTRs after transplantation, respectively [1–6]. BKPyVAN generally develops in 1–10% of KTRs, usually in those with sustained viremia and viral DNA-loads above 10^4 genome copies/ml [3,6–8]. Unfortunately, the burden of BKPyVAN continues to increase, as the population of KTRs is still growing [9–12].

Despite the clinical need, BKPyV-specific antiviral drugs are not available, and reduction of immunosuppression is the only effective evidence-based treatment [6,13–15]. Therefore, current guidelines recommend regular screening of KTRs to detect BKPyV viremia and guide timely reduction of immunosuppression [3,4,6,14], which improves BKPyV immunity, but at the same time increases the risk of acute rejection [6,14,15]. This makes management of BKPyV infection challenging for transplantation physicians and calls for reliable pre-transplantation predictive markers that identify KTRs at risk. Such markers could, for example, guide physicians toward more frequent monitoring of BKPyV viremia or use of a lower viremia threshold for at-risk patients.

The overall seroprevalence for BKPyV exceeds 90% [16,17], and it is believed that nearly all adults are persistently infected with at least one BKPyV genotype [18,19]. Recently, we provided compelling evidence that the level of BKPyV-directed IgG seroreactivity measured before kidney transplantation (KTx), especially in donors, predicts the risk of BKPyV infection, in KTRs after transplantation [20]. In line with

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previous studies that showed associations between donor and recipient seroreactivity and recipient BKPyV infection risk [21–23], our results showed that strongly BKPyV-seroreactive kidney donors conferred an approximately 10-fold increased risk of viremia to their recipients. In the prior study, BKPyV genotype Ib1 VP1 antigen was used to analyze seroresponses. To learn more about the specificity of BKPyV-directed seroresponses and to investigate the impact of BKPyV genotype disparity between donors and recipients [9], we set out to complement our BKPyV-immunoassay with the most common circulating BKPyV subtypes. The availability of a high-throughput BKPyV serotyping assay could overcome the limitation of BKPyV genotyping, requiring a certain amount of viral DNA, which is usually not detectable in healthy donors without viruria and viremia.

BKPyV is classified into four genotypes based on single nucleotide polymorphisms (SNPs) [24,25]. Genotype I is the most prevalent and widespread BKPyV genotype worldwide, genotype IV accounts for most of the remaining subjects, while genotypes II and III are rarely detected in all geographic regions [26,27]. Phylogenetic sequence analysis has been used to classify BKPyV strains, resulting in further subdivision of genotypes I and IV into subtypes Ia, Ib1, Ib2, Ic, and IVa1, IVa2, IVb1, IVb2, IVc1, and IVc2, respectively [26–29]. Ia is most prevalent in Africa, Ib1 in Southeast Asia, Ib2 in Europe, America and West Asia, and Ic in Northeast Asia [26,27,30,31]. All subtypes of genotypes IV except IVc2, are prevalent in East Asian populations, with subtype IVc2 occurring mainly in Europe, America and Northeast Asia [32].

It is generally believed that each BKPyV genotype represents a distinct serotype, which fits with the majority of SNPs being located in the VP1 capsid protein [9,33,34]. Genotype-specific vaccination studies in mice have confirmed this [34], and indicated antibody-mediated genotype-specific BKPyV neutralization. Furthermore, it was shown that subtypes Ib1 and Ib2 can behave as distinct serotypes in some individuals, while Ib2 and Ic seem to represent a single serotype, as are all subtypes of genotype IV [33,34].

2. Objectives

The aim of this study was to develop and evaluate a serological BKPyV IgG genotyping assay with the help of separate cohorts of healthy blood donors and KTRs, that detects BKPyV genotype-specific IgG antibody responses. This could be useful to detect pretransplantation BKPyV genotype-specific IgG antibody responses in kidney transplant donors and recipients. Availability of such a serotyping system could shed light on previously reported, sometimes conflicting results regarding associations between specific BKPyV genotypes and pathogenic replication [28,35–39]. Furthermore, BKPyV serotyping could reveal whether the current BKPyV genotype distribution pattern deduced from viremic KTRs reflects that of asymptomatic immunocompetent people. Finally, if BKPyV serotyping can reliably differentiate between genotypes, this method could be used to analyze and predict the clinical impact of BKPyV genotype mismatch between donor and recipient.

3. Study design

3.1. Study population

For evaluation of the BKPyV multiplex serotyping immunoassay, anonymized serum samples from a cohort of 87 adult Dutch HBDs [40,41], and a cohort of 39 adult Dutch KTRs [42,43] were tested. Basic demographic details, such as age, sex and year of collection can be found in the references. The study adhered to the Declaration of Helsinki Principles and all participants gave informed consent.

3.2. BKPyV VP1 bead-based immunoassay

To detect IgG seroresponses against the BKPyV major viral capsid protein (VP1), an antibody-binding assay using Luminex xMAP technology [44] was previously developed, equipped with the VP1 antigen of BKPyV subtype Ib1 as described [16,20]. The assay showed a good intra (r 0.963 – 0.999, $p < 0.001$) and intertest variability (r 0.891, $p < 0.001$) [20]. To detect seroresponses against other BKPyV genotypes, synthetic VP1 gene fragments (gBlocks, IDT, San Jose, CA, USA) of BKPyV Ib2, Ic, II, III and IVb1, were cloned into pGEX-5x-3 vectors (GE Healthcare Life Sciences, Chicago, IL, USA) and expressed as GST-VP1.tag fusion proteins in BL21 Rosetta bacteria. The synthetic gene fragments used in this study were reported previously [45]; BKV-Ib2 (PittVR2; DQ989796), BKV-Ic (RYU-2; AB211377), BKV-II (GBR-12; AB263920), BKV-III (KOM-3; AB211386), and BKV-IVb1 (THK-8; AB211390). The different GST-VP1 fusion proteins were individually coupled to differently coloured Luminex bead sets. BKPyV Ia was not included in the antigen set, because BKPyV Ia and Ib1 differ by only three synonymous SNPs in VP1, and are identical in their amino acids [46]. Only one genotype IV VP1 antigen was included in the analyses, BKPyV IVb1, since all BKPyV genotype IV subtypes are thought to belong to one serotype [33,34].

Serostatus (positive or negative) was identified and interpreted according to the calculated cut-off values, Ib1 763 MFI, Ib2 515 MFI, Ic 475 MFI, II 446 MFI, III 366 MFI and IV 298 MFI, as described in the supplemental information.

A high agreement was observed between test plates for all 6 BKPyV variants as described in supplementary information together with further information regarding the previously described BKPyV VP1 bead-based immunoassay.

3.3. Serum competition analysis

To study the cross-reactivity between the different BKPyV serotypes, VP1 antigen competition experiments were performed, where a fixed amount of unbound competitor VP1 antigen is added to a serum dilution series, in addition to the bead-bound targeted VP1 antigen, as described previously [16,42]. Selected serum samples were diluted from 1:100 up to 1:409.600 and incubated with regular blocking buffer containing either GST or GST-VP1 fusion proteins (~2 mg/ml).

3.4. BKPyV neutralization assay

Of the 39 KTR samples, six were independently analyzed for serological confirmation with a BKPyV genotype-specific pseudovirion based neutralization assay (PVNA) (dilution 1:100 to 1:39.062.500), as described [45]. The neutralization titer was defined as the half maximal inhibitory concentration (IC_{50}) and was calculated using Prism Software (Graphpad) by fitting a variable-slope sigmoidal dose-response curve for each serum dilution series. The IC_{50} values of the PVNA were compared with the geometric mean titers (GMT) determined on seroreactivities of the six serum samples measured in the BKPyV serotyping multiplex immunoassay.

3.5. Statistical analysis

Data were analyzed with IBM SPSS Statistics software version 21. Differences between HBDs and KTRs were assessed using the chi-square test, Fisher exact test or Student t-test, as appropriate. Pearson correlation coefficients were calculated to determine intertest reliability. Correlation between assessed BKPyV serotypes was further examined by calculating Spearman rank correlation coefficients.

4. Results

4.1. BKPyV genotype-directed seroreactivity

VP1 antigens of the common BKPyV subtypes, Ib1, Ib2, Ic, II, III and IVb1 were analysed for seroreactivity BKPyV Ia was not included in the

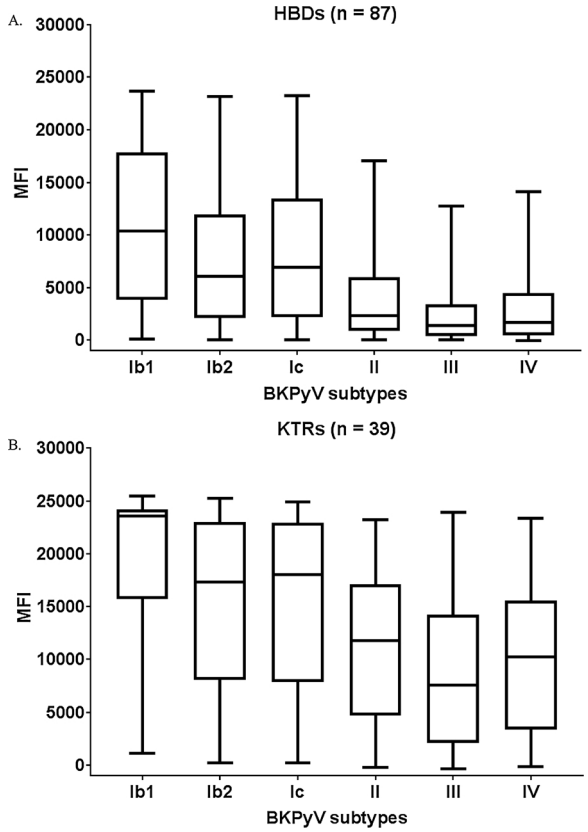


Fig. 1. Seroreactivity against BKPyV genotype-specific VP1 antigens measured by the Luminex multiplex immunoassay in healthy blood donors (A) and kidney transplant recipients (B).

Seroreactivities against BKPyV serotype specific VP1 antigens were measured in healthy blood donors (HBDs, n = 87, panel A) and in kidney transplant recipients (KTRs, n = 39, panel B). Results are depicted as mean fluorescence intensity (MFI), and are shown in box plots. The bottom and top of the boxes represent the first and third quartiles, the band inside the boxes represents the median, and the end of the whiskers represents the minimum and maximum seroreactivities. The differences between the seroreactivities against BKPyV genotype-specific VP1 antigens were statistically significant: BKPyV Ib1 HBDs mean 10,976 MFI, standard deviation 7586 MFI and KTRs mean 19,163 MFI, standard deviation 7019 MFI, $p < 0.001$; BKPyV Ib2 HBDs mean 7631 MFI, standard deviation 6419 MFI and KTRs mean 14,996 MFI, standard deviation 8468 MFI, $p < 0.001$; BKPyV Ic HBDs mean 8201 MFI, standard deviation 6615 MFI and KTRs mean 15,850 MFI, standard deviation 8016 MFI, $p < 0.001$; BKPyV II HBDs mean 4428 MFI, standard deviation 4768 MFI and KTRs mean 10,867 MFI, standard deviation 7307 MFI, $p < 0.001$; BKPyV III HBDs mean 2543 MFI, standard deviation 3028 MFI and KTRs mean 8859 MFI, standard deviation 7649 MFI, $p < 0.001$; BKPyV IV: HBDs mean 2961 MFI, standard deviation 3209 MFI and KTRs mean 10,370 MFI, standard deviation 7365 MFI, $p < 0.001$.

antigen set, because BKPyV Ia and Ib1 VP1 are identical [46]. Only one genotype IV antigen was included in the analyses, BKPyV IVb1, since all BKPyV genotype IV subtypes are thought to belong to one serotype [33,34].

Each selected VP1 was analyzed for antigenicity using serum samples from 87 immunocompetent (blood donors) and 39 immunocompromised (immunosuppressed KTRs) individuals. Fig. 1 shows boxplots of the measured MFI values obtained for each BKPyV genotype at 1:100 dilution. The seroreactivity measured against any BKPyV genotype was significantly stronger in KTRs compared to HBDs ($p < 0.001$), indicated by higher median MFI values, probably as the result of boosted seroresponses by replicating virus under immunosuppression, as we have shown previously [41]. The highest median seroreactivities in HBDs and KTRs, were observed for BKPyV

Table 1
Seropositivity against BKPyV geno(sub)types measured in sera from 87 healthy blood donors and 39 kidney transplant recipients.

BKPyV genotype	HBDs n (%)	KTRs n (%)
I, subtype b1	81 (93%)	39 (100%)
I, subtype b2	79 (91%)	37 (95%)
I, subtype c	81 (93%)	38 (97%)
II	75 (86%)	35 (90%)
III	67 (77%)	34 (87%)
IV	70 (80%)	37 (95%)
Mean number of seropositive geno(sub)types per individual	5.24 (1.50)	5.64 (1.01)
Mean number of seropositive genotypes per individual	3.41 (1.08)	3.72 (0.79)

BKPyV, BK polyomavirus; HBDs, healthy blood donors; KTRs, kidney transplant recipients.

subtypes belonging to genotype I. The seropositivity rate was high for all BKPyV serotypes, ranging from 91 to 100% for genotype I, 86–90% for genotype II, 77–87% for genotype III and 80–95% for genotype IV, and comparable between HBDs and KTRs (Table 1). On average, both HBDs and KTRs were seropositive against at least five of the six BKPyV subtypes and three of the four genotypes (Table 1).

4.2. Correlation between seroresponses against individual BKPyV variants

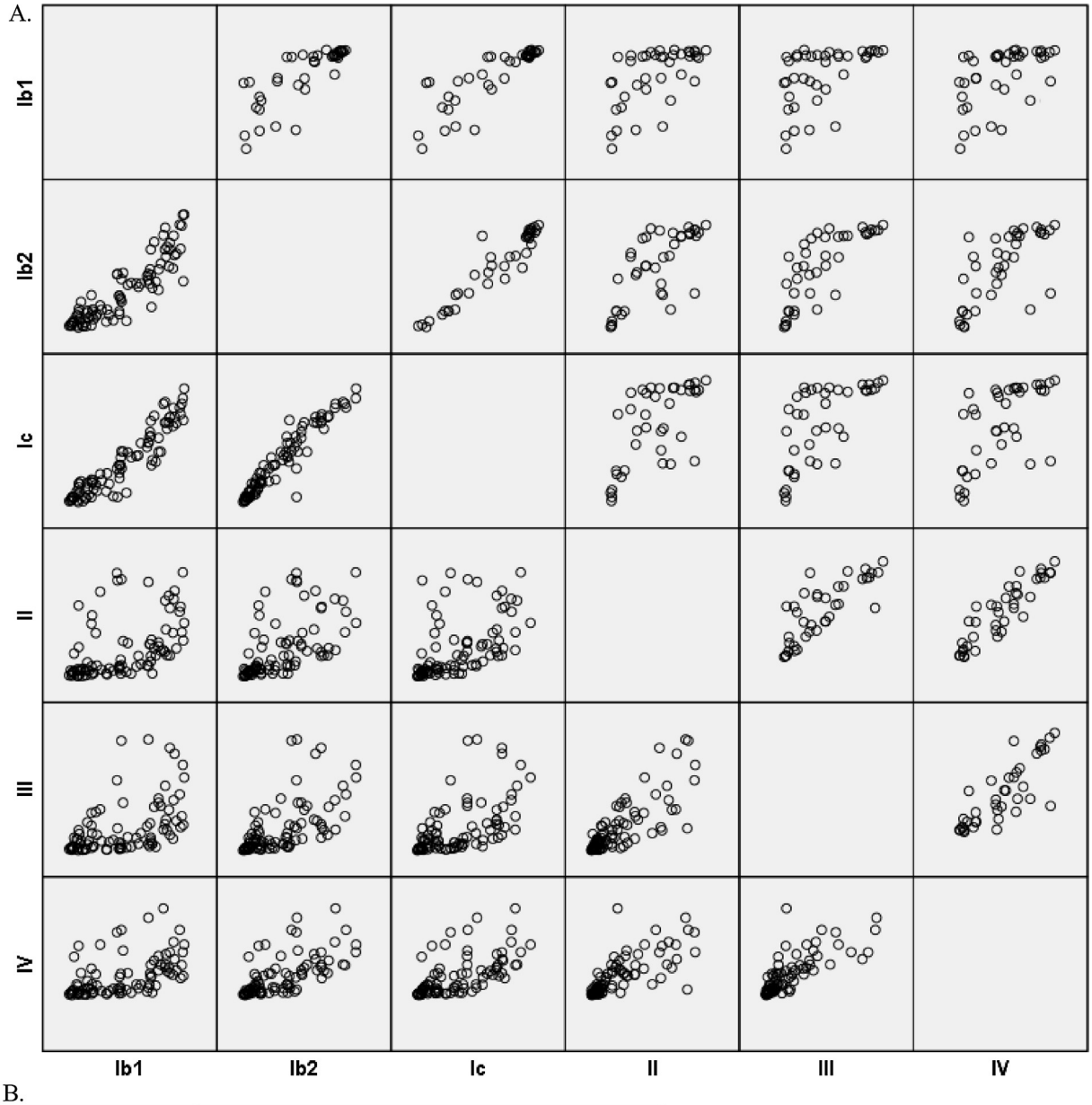
To learn about crossreactivity between the different BKPyV genotypes and subtypes analyzed in our assay, a correlation matrix of the serotype-specific seroresponses was generated for the HBDs and KTRs (Fig. 2A). Spearman rank correlation coefficients were calculated for each BKPyV serotype combination (Fig. 2B). Strong correlations were observed between BKPyV subtypes belonging to genotype I, and between genotypes II, III and IV. Between the seroresponses against genotype I and genotypes II–IV, moderately strong correlations were generally observed. The observed cross-reactivity pattern matched with the VP1 amino acid sequence similarity between the genotypes, with strong correlations among BKPyV genotypes with ≥95% similarity (data not shown).

4.3. Cross-reactivity of seroresponses against BKPyV

To explore the BKPyV crossreactivity in more detail, six serum samples with a high seroreactivity (> 15.000 MFI) to at least one of the genotypes were selected, diluted and tested against each VP1 antigen to calculate their GMT for each genotype (Table 2A). Furthermore, soluble, competing heterologous VP1-antigens were added to the serum titration series, while assaying seroreactivity against bead-bound VP1 antigen of the relevant BKPyV variants. Fig. 3 shows a selection of these analyses for each analyzed genotype, while a comprehensive overview of the VP1-antigen inhibition experiments can be found in supplementary Figure S2.

Although each serum sample proved different in these analyses, seroresponses against BKPyV genotype Ib1 were only efficiently blocked by the homologous Ib1 VP1 antigen. Pre-incubation with other genotype I subtype VP1 antigens caused only a slight reduction in seroreactivity, comparable to the inhibition caused by the more distant genotypes II, III and IV (Fig. 3A). Seroresponses against genotype Ib2 and Ic, in most of the cases could be inhibited by any genotype I VP1 antigen, but not by the other genotypes (Fig. 3B–C). Seroresponses against BKPyV VP1 of genotypes II and III were inhibited by all of the heterologous VP1 antigens (Fig. 3D–E), whereas responses against genotype IV VP1 were not inhibited by the heterologous VP1 antigens (Fig. 3F). Altogether, these data indicate that the immunoassay detects seroreactivity against BKPyV genotype I subtypes, especially Ib1, and genotype IV with little chance of cross-reactivity, while seroresponses against genotypes II and III often seemed to lack specificity as they were

A.



B.

		Spearman correlation serotiters of KTRs (n = 39)					
		Ib1	**Ib2**	**Ic**	**II**	**III**	**IV**
Spearman correlation serotiters of HBDs (n = 87)	**Ib1**		0.850	0.911	0.618	0.621	0.615
	Ib2	0.893		0.940	0.742	0.854	0.766
	Ic	0.926	0.953		0.682	0.746	0.700
	II	0.630	0.740	0.678		0.819	0.912
	III	0.601	0.734	0.680	0.823		0.826
	IV	0.612	0.745	0.711	0.831	0.858	

Fig. 2. Cross-reactivity matrices (A) and Spearman rank correlation coefficients (B) between the VP1 antigens from different BKPyV genotypes and subtypes in serum samples of HBDs and KTRs.

In panel A correlation graphs are shown as scatter plots for the healthy blood donor population (lower left part, n = 87) and for the kidney transplant recipients (upper right part, n = 39), with each circle representing one serum sample. In panel B, the numbers in the lower left part of the Table show the Spearman correlation coefficients calculated between seroresponses measured against VP1 of the BKPyV genotypes and subtypes tested in the healthy blood donor population and the upper right part of the kidney transplant recipients. The color of the cells represents the degree of correlation between the different BKPyV variants; red = high correlation (r ≥ 0.8), yellow = moderate correlation (r = 0.6 - 0.8).

Titrated serum samples were pre-incubated with crude bacterial extract containing GST only (in black), or containing GST-VP1 of the autologous BKPyV geno(sub) type (target subtype shown on top of each graph) or non-target heterologous BKPyV geno(sub)types. Results are depicted as median fluorescence intensity (MFI) and are shown for the seroresponses to Ib1 (A), Ib2 (B), Ic (C), II (D), III (E) and IV (F) measured in each serum sample.

Table 2
Comparison between results obtained with the BKPyV Luminex multiplex serotyping immunoassay and the pseudovirion based neutralization assay for six selected sera.

A.

Test method	BKPyV serotypes						
	Serum sample	Ib1	Ib2	Ic	II	III	IV
Luminex assay (MFI values)	101	17717	8433	8376	20434	5985	22321
	150	**24875**	24009	24443	20532	22753	22109
	256	**24853**	23334	24084	19259	20531	19804
	258	12994	4464	7780	14526	7726	**17326**
	278	**25243**	24143	22973	20414	21081	20017
	312	**17318**	170	1876	−243	−385	1087
Luminex assay (GMT values)	101	187	58	55	624	32	**1403**
	150	**2233**	1265	1848	302	434	511
	256	1225	929	**1265**	459	568	526
	258	490	125	186	487	144	**887**
	278	**5874**	2667	2602	1834	1602	1722
	312	**476**	2	8	1	1	2
Neutralization assay (PVNA IC_{50} values)	101	4825	195	333	20939	497	**36516**
	150	**73673**	27286	21557	24346	20477	10942
	256	**20672**	2925	5856	8921	5580	2894
	258	5452	260	683	**20407**	4810	14587
	278	**26768**	925	1051	5513	896	311
	312	**4974**	0	0	237	0	O

B.

	Luminex assay(MFI value)	Luminex assay (GMT value)	Neutralization assay (IC_{50} value)
Main genotype serum samples			
101	IV	IV	IV
150	I	I	I
256	I	I	I
258	IV	IV	II
278	I	I	I
312	I	I	I

Panel A shows the MFI values of six serum samples with a 1:100 dilution from kidney transplantation recipients, the geometric mean values of the serial dilutions (1:100 up to 1:409.600) of these six serum samples, and the IC_{50} values of the serial dilutions (1:100 up to 1:39.062.500) of six selected serum samples. The highest values per serum sample are depicted in bold. Panel B shows for each serum sample the BKPyV genotype that reached the highest MFI, GMT and IC_{50} value determined in the Luminex and the neutralization assay, respectively.

completely inhibited by the heterologous VP1 antigens.

4.4. Virus neutralization by BKPyV genotype VP1-specific sera identified in the bead-based assay

To further evaluate the performance of the BKPyV serotyping immunoassay with regard to specificity and capacity to detect seroresponses with neutralizing activity, the selected serum samples were also tested in a previously described BKPyV PVNA [33,34]. The PVNA IC_{50} values obtained for each BKPyV genotype and each serum are shown in Table 2A, in comparison with the Luminex-obtained MFI and GMT values mentioned above.

For comparison, in Table 2B we show for each selected serum the BKPyV genotype that generated the highest MFI, GMT and IC_{50} value in each test method. Genotype ranking based on MFI and GMT Luminex values showed a 100% concordance. Comparing MFI/GMT ranking values with the PVNA IC_{50} value ranking, revealed one discrepant result (serum 258), since Luminex indicated the highest MFI/GMT values for genotype IV, while the PVNA indicated genotype II. In both cases, however, the obtained MFI, GMT, and IC50 values were rather close to each other, and the second-best response for the relevant serum was directed against the reciprocal genotype, being IV with PVNA, and II with Luminex (Table 2).

5. Discussion

Little is known about the distribution of BKPyV genotypes among kidney donors and recipients and their association with the risk, course and severity of BKPyV infection and complications after KTx. Studies assessing this association in KTRs have reported conflicting results. These studies were mainly focused on isolates obtained from recipients with manifest BKPyV infection (viremia or viruria) and thus may not represent the distribution of BKPyV genotypes circulating in the general population, including kidney donors [28,35–39].

Current BKPyV genotyping mainly relies on sequencing of BKPyV DNA in clinical samples. In healthy subjects however, BKPyV DNA is seldom detectable in sufficient amounts to allow sequence analysis [24,25,46–48]. This makes BKPyV genotyping of donor-recipient pairs before KTx almost impossible. Serum neutralization assays to detect infecting BKPyV genotypes have been described using pseudovirion systems [9,33,34], but these are not suitable for routine use, as they are laborious in terms of production of the infectious pseudovirus and in the conduct of the neutralization assays. The BKPyV serotyping Luminex immunoassay could potentially fill this gap, as it creates the possibility of a fast and efficient high-throughput assay detecting multiple BKPyV genotype-specific VP1 antigens at the same time in only a small amount of sample, eventually saving time and costs. A previous comparison between BKPyV GST-VP1 antigen presented on a bead and BKPyV VP1 VLP antigen showed good agreement between the two [20,42].

Based on our results with this new serotyping assay, the prevalence of BKPyV genotype I infections was high (> 90%) in both blood donors and KTRs, which is in accordance with literature [16,17]. The measured seroprevalence of serotypes II and III ranged between 77–90% in both groups. These percentages are higher than generally reported in the literature [9,26,27,45]. However, as most serotype II and III seroresponses were completely inhibited by heterologous VP1 antigens, we believe the high serotype II and III seroprevalence could be a reflection of the cross-reactivity with heterologous serotypes and should be interpreted with extreme caution. Whether this caution applies as well to BKPyV genotype II and III seroprevalences obtained in other studies we do not know. Since genotype IV-directed seroresponses could not be inhibited by other VP1 antigens, we consider the prevalence of infections with this genotype to be genuinely high.

The cross-reactivity analysis showed high correlations between the seroreactivities against subtypes belonging to genotype I, and between genotypes II, III and IV, indicating that cross-reactivity with these two groups is likely. The antigen competition experiments, on the other hand, showed that the immunoassay detects seroreactivity against BKPyV genotypes I and IV with little chance of cross-reactivity, as the responses to the VP1 of these genotypes were not inhibited by the heterologous VP1 antigens. As genotypes I and IV are the most prevalent and widespread BKPyV genotypes worldwide and BKPyV genotypes II and III are only rarely detected in all geographic regions [26,27], BKPyV I and IV serotyping based on this method could be of potential interest to explore the risk of BKPyV (genotype-specific) infection in KTRs. Ideally, further study in that direction should include a direct comparison between serotyping and genotyping results, and therefore can only be performed in viruric or viremic subjects.

Comparison between the MFI and GMT values of the Luminex immunoassay with the IC_{50} values of the neutralization assay showed good agreement in determination of the main genotype. A previous study also showed a good correlation between BKPyV-VLP and BKPyV VP1 antibody responses [49]. The only possible disagreement was a type IV in the Luminex compared to type II with the neutralization assay, which, if interpreted as a discrepancy although MFI and IC_{50} values for both genotypes were rather similar, can be explained by

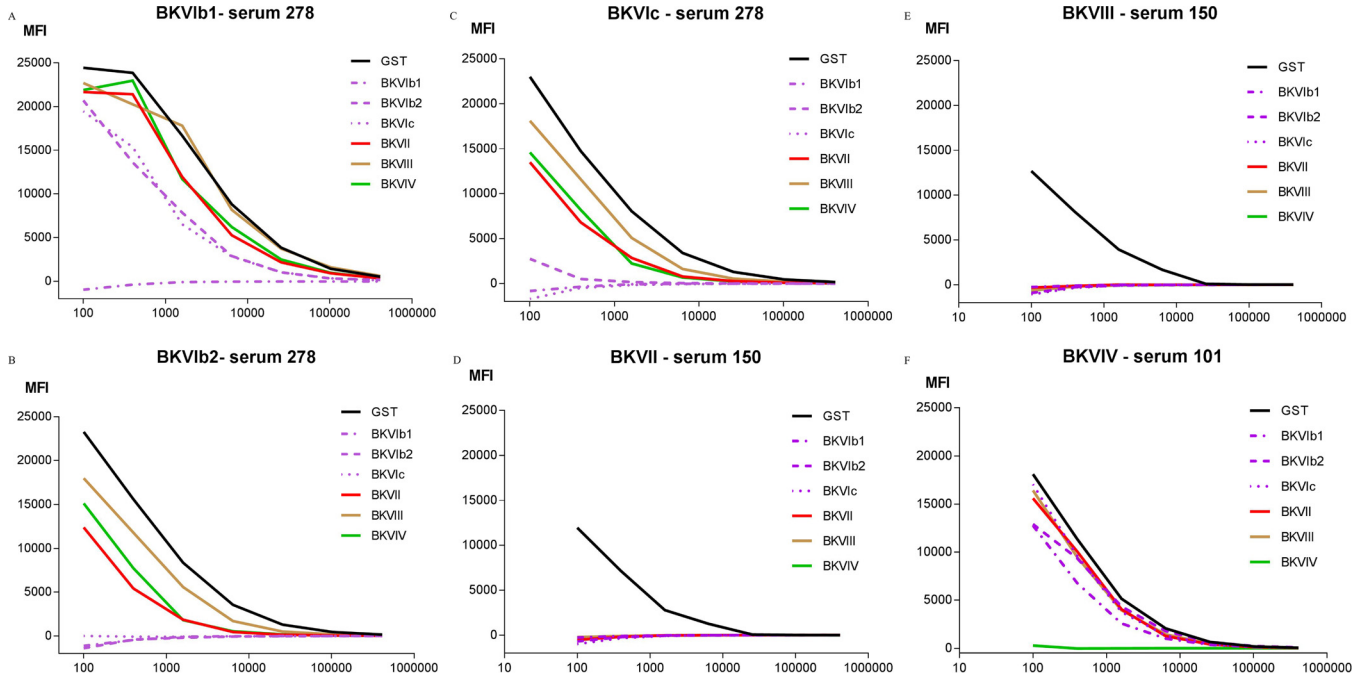


Fig. 3. Cross-reactivity analysis of BKPyV serotype seroresponses by VP1-specific competition.
Titrated serum samples were pre-incubated with crude bacterial extract containing GST only (in black), or containing GST-VP1 of the autologous BKPyV geno(sub) type (target subtype shown on top of each graph) or non-target heterologous BKPyV geno(sub)types. Results are depicted as median fluorescence intensity (MFI) and are shown for the seroresponses to Ib1 (A), Ib2 (B), Ic (C), II (D), III (E) and IV (F) measured in each serum sample.

cross-reactivity between these serotypes in the neutralization assay [34]. Based on this limited comparison, we assume that serum samples that show high seroreactivity in the Luminex assay have BKPyV neutralizing activity, as shown in the PVNA.

To conclude, we described the development and evaluation of a BKPyV genotype-specific VP1 directed IgG immunoassay. The results indicate that this immunoassay is a potentially useful tool for the detection of BKPyV infection with the most prevalent genotypes I and IV, in individuals without detectable viral DNA available. Whether the assay can detect and discriminate genotype II and III-specific seroresponses remains unclear and should be further evaluated with sera from individuals with molecularly proven genotype-specific BKPyV infections.

Declaration of interests

We declare that we have no conflicts of interest

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Role of the funding source

The Dutch Kidney Foundation who supported this study had no role in study design; collection, analysis, and interpretation of data; writing of the report; or in the decision to submit the paper for publication.

Author contributions

HFW and MCWF initiated and designed the study. ACMK provided the infrastructure. CSdB, EvdM, and DVP performed the experiments and gathered the experimental data. HFW analyzed the data. HFW, CBB, and MCWF interpreted the data. HFW and MCWF drafted the manuscript, including figures and tables. All authors reviewed and approved the final report.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jcv.2018.11.009.

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