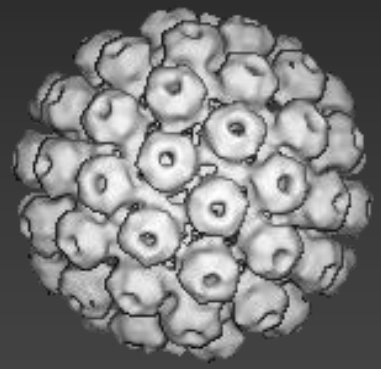
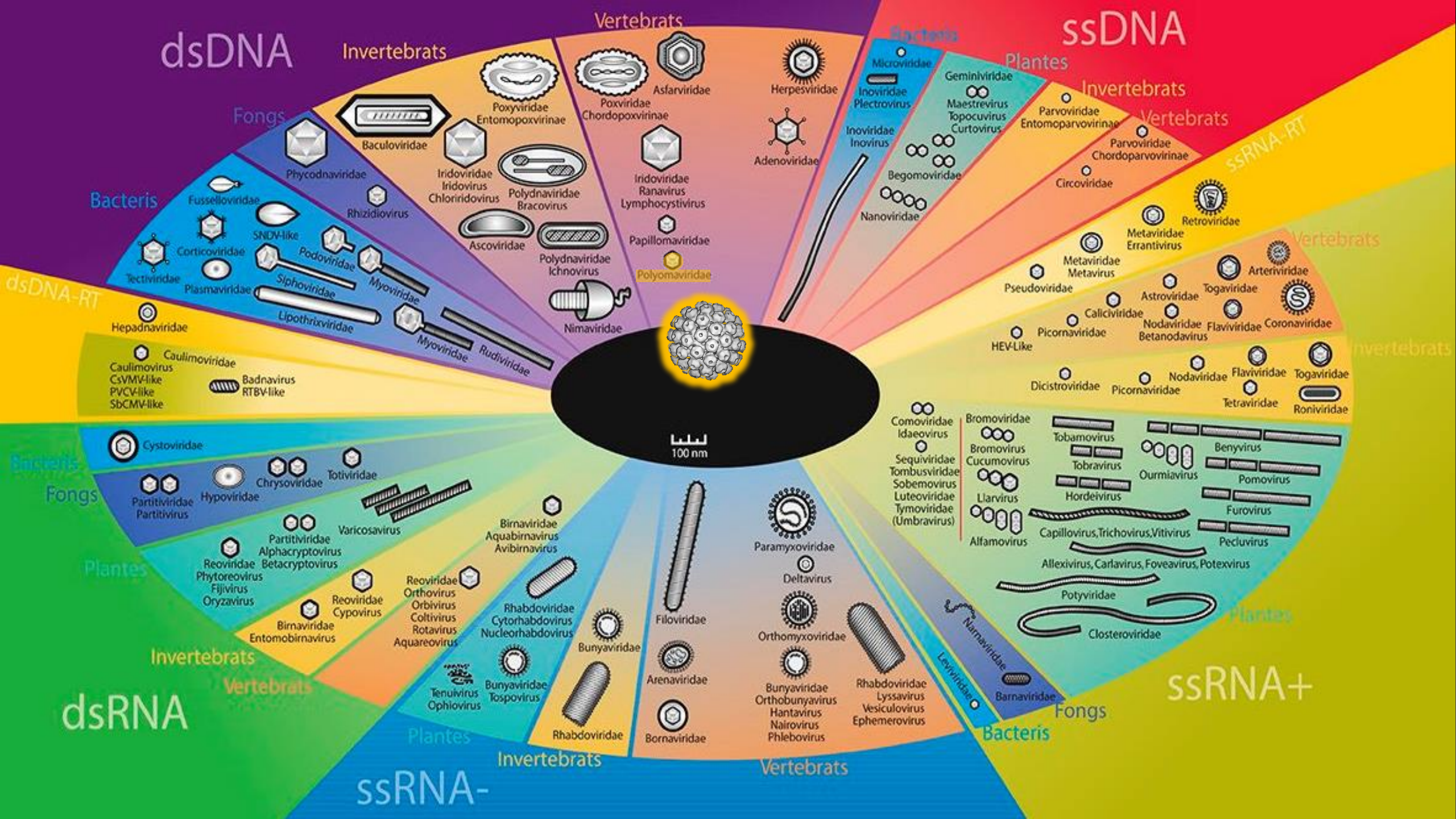
Human polyomaviruses in blood donors



Mariet Feltkamp

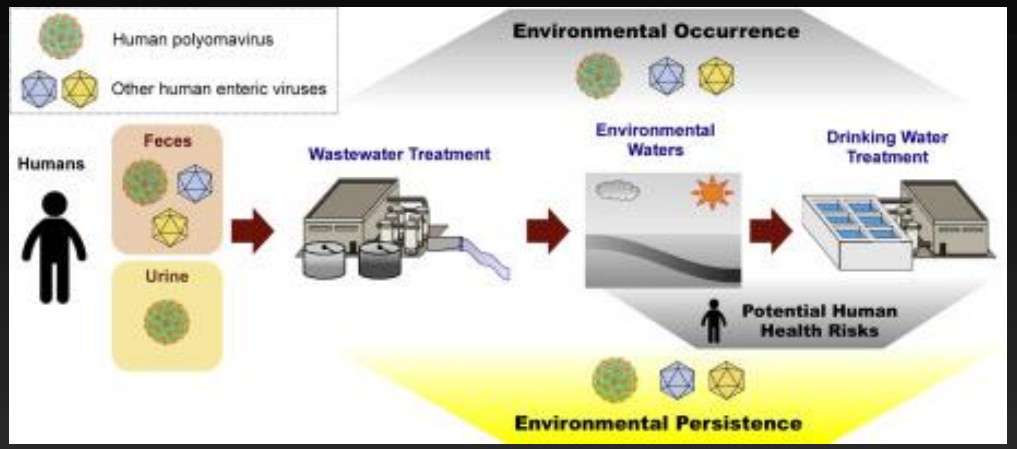
Sanquin

Leiden University Medical Center


dsDNA
Invertebrats
Vertebrats
Fongs
Bacteris
Fuselloviridae
Phycodnaviridae
Baculoviridae
Poxyviridae
Entomopoxvirinae
Rhizidiovirus
Iridoviridae
Iridovirus
Chloriridovirus
Polydnaviridae
Bracovirus
Poxviridae
Chordopoxvirinae
Asfarviridae
Herpesviridae
Adenoviridae
Iridoviridae
Ranavirus
Lymphocystivirus
Papillomaviridae
Polyomaviridae
Ascoviridae
Polydnaviridae
Ichnovirus
Nimaviridae
SNDV-like
Corticoviridae
Tectiviridae
Plasmaviridae
Podoviridae
Siphoviridae
Myoviridae
Lipothrixviridae
Myoviridae
Rudiviridae
dsDNA-RT
Hepadnaviridae
Caulimoviridae
Caulimovirus
CsVMV-like
PVCV-like
SbCMV-like
Badnavirus
RTBV-like
ssDNA
Bacteria
Plantes
Invertebrats
Vertebrats
Microviridae
Inoviridae
Plectrovirus
Inoviridae
Inovirus
Geminiviridae
Maestrevirus
Topocuvirus
Curtovirus
Begomoviridae
Nanoviridae
Parvoviridae
Entomoparvovirinae
Parvoviridae
Chordoparvovirinae
Circoviridae
ssRNA-RT
Retroviridae
Metaviridae
Errantivirus
Metaviridae
Metavirus
Pseudoviridae
Arteriviridae
Togaviridae
Astroviridae
Caliciviridae
Nodaviridae
Betanodavirus
Flaviviridae
Coronaviridae
Picornaviridae
HEV-Like
Dicistroviridae
Picornaviridae
Nodaviridae
Flaviviridae
Togaviridae
Tetraviridae
Roniviridae
dsRNA
Cystoviridae
Bacteris
Fongs
Plantes
Partitiviridae
Partitivirus
Hypoviridae
Chrysoviridae
Totiviridae
Partitiviridae
Alphacryptovirus
Betacryptovirus
Varicosavirus
Reoviridae
Phytoreovirus
Fijivirus
Oryzavirus
Reoviridae
Cypovirus
Birnaviridae
Entomobirnavirus
Invertebrats
Vertebrats
Reoviridae
Orthovirus
Orbivirus
Coltivirus
Rotavirus
Aquareovirus
Birnaviridae
Aquabirnavirus
Avibirnavirus
Rhabdoviridae
Cytorhabdovirus
Nucleorhabdovirus
Tenuivirus
Ophiovirus
Bunyaviridae
Tospovirus
Bunyaviridae
Rhabdoviridae
Plantes
Invertebrats
ssRNA-
Filoviridae
Arenaviridae
Bornaviridae
Paramyxoviridae
Deltavirus
Orthomyxoviridae
Bunyaviridae
Orthobunyavirus
Hantavirus
Nairovirus
Phlebovirus
Rhabdoviridae
Lyssavirus
Vesiculovirus
Ephemerovirus
Vertebrats
Comoviridae
Idaeovirus
Sequiviridae
Tombusviridae
Sobemovirus
Luteoviridae
Tymoviridae
(Umbravirus)
Bromoviridae
Bromovirus
Cucumovirus
Llarvirus
Alfamovirus
Tobamovirus
Tobravirus
Hordeivirus
Capillovirus, Trichovirus, Vitivirus
Allexivirus, Carlavirus, Foveavirus, Potexvirus
Potyviridae
Closteroviridae
Ourmiavirus
Benyvirus
Pomovirus
Furovirus
Pecluvirus
Plantes
ssRNA+
Narnaviridae
Levivirideae
Barnaviridae
Fongs
Bacteris
100 nm

Background

- Nonenveloped, ds-DNA viruses
  - Resistent particles and ubiquitous
  - Excretion via saliva, droplets, urine, feces
  - Primary infection usually in childhood

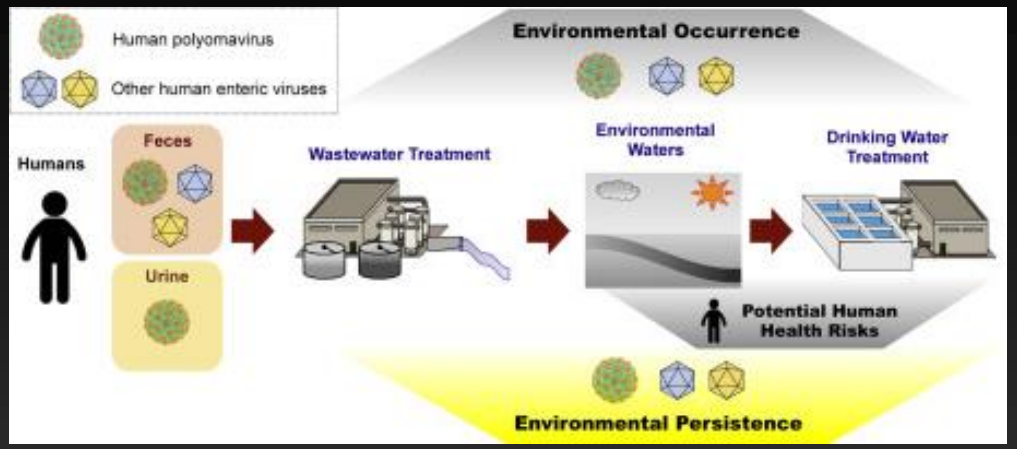


- Infection and disease
  - In healthy people: persistent, latent infection
  - In immunocompromised: symptomatic, lytic infection (reactivation)
  - In naive immunocompromised patients: severe primary infection
  - In elderly: integration in host DNA → carcinoma



- Treatment and prevention
  - No proven effective anti-viral therapy available
  - Tapering of immunosuppressive drugs

Background



- Nonenveloped, ds-DNA viruses
  - Resistent particles and ubiquitous
  - Excretion via saliva, droplets, urine, feces
  - Primary infection usually in childhood (viremia)

- Infection and disease (viremia)
  - In healthy people: persistent, latent infection
  - In immunocompromised: symptomatic, lytic infection (reactivation)
  - In naive immunocompromised patients: severe primary infection
  - In elderly: integration in host DNA → carcinoma

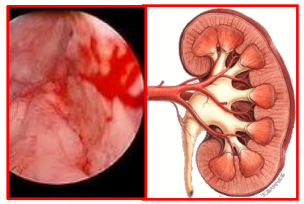
- Treatment and prevention
  - No proven effective anti-viral therapy available
  - Tapering of immunosuppressive drugs



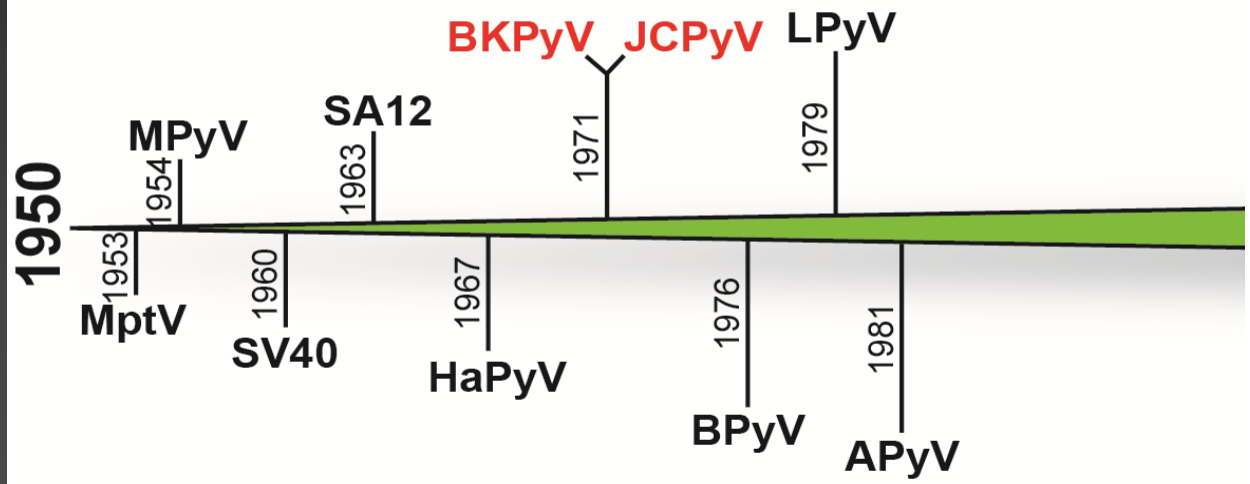
Human polyomaviruses

Nephropathy (KTx)
Hemorrhagic cystitis (HSCTx)

PML encephalopathy
(AIDS, multiple sclerosis)







Immunosuppression

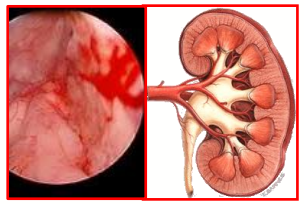
- SOTx ↑
- HSCTx ↑
- Biologicals ↑
- Ageing

Technical advances

- Next-generation sequencing

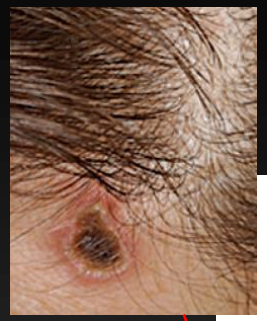
Human polyomaviruses

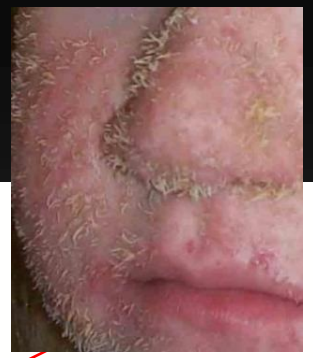
Nephropathy (KTx)
Hemorrhagic cystitis (HSCTx)

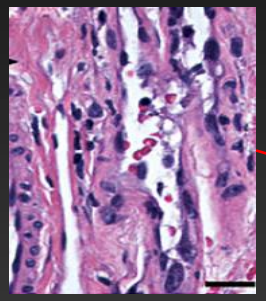
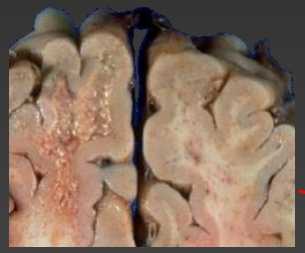


PML encephalopathy
(AIDS, multiple sclerosis)









Feltkamp, Kazem, Van der Meijden, Lauber and Gorbalenya, J Gen Virol 2013

Is HPyV infection a danger for blood safety?

(considering 50% of our recipients is immunocompromised)

- Is our blood donor population infected with HPyVs?
  - *Determine seroprevalence in blood donors*
- Are HPyVs present in donor blood, and are they infectious?
  - *Determine presence and load of HPyV-DNA in donor blood*
  - *Determine integrity and infectivity of HPyV particles*
- If so, which blood components/products confer the highest risk?
  - *Compare HPyV-DNA loads between components*
- Is HPyV transmitted to recipients; do they develop HPyV-related disease?
  - *Detect and compare HPyV-DNA sequences between donor and receptor*
  - *Analyze recipients for HPyV-related signs and symptoms*

Is HPyV infection a danger for blood safety?

(considering 50% of our recipients is immunocompromised)

- Is our blood donor population infected with HPyVs?
  - *Determine seroprevalence in blood donors*
- Are HPyVs present in donor blood, and are they infectious?
  - *Determine presence and load of HPyV-DNA in donor blood*
  - *Determine integrity and infectivity of HPyV particles*

Van der Meijden et al., J Infect Dis. 2017;215:1080-1084

Kamminga et al., J Clin Microbiol. 2018; 56:e01566-17

Kamminga et al., PLoS One. 2018; 3:e0206273

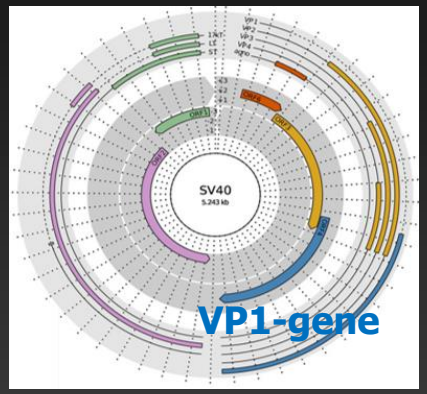
Kamminga et al., Transfusion. 2019; 59:3689-3697

Kamminga et al., J Clin Virol. 2021;143:104944



Sergio Kamminga

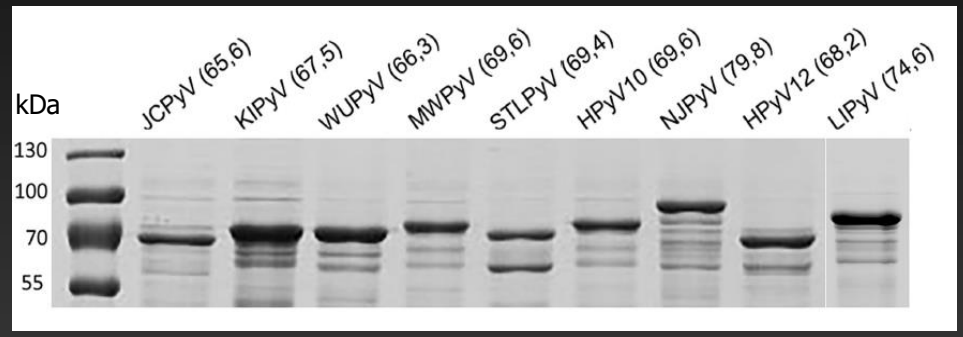
Seroprevalence of the HPyVs



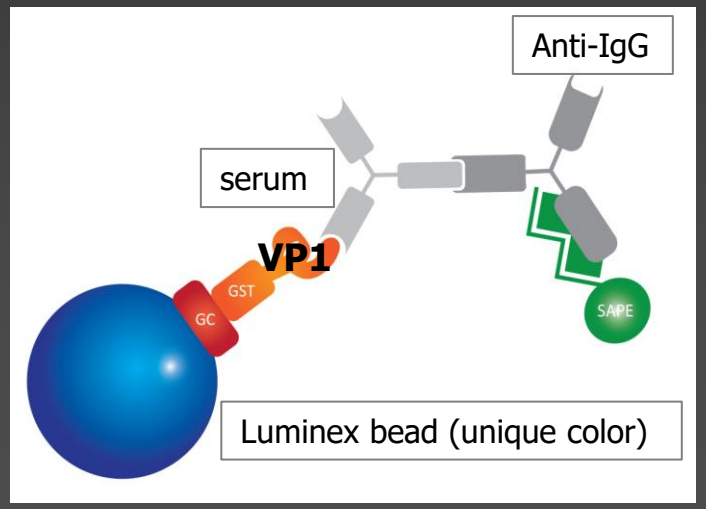
→ Clone VP1 into pGex vector (GST)

→ Express VP1-GST fusion protein (Ag)

→ Couple VP1-Ag to Luminex bead



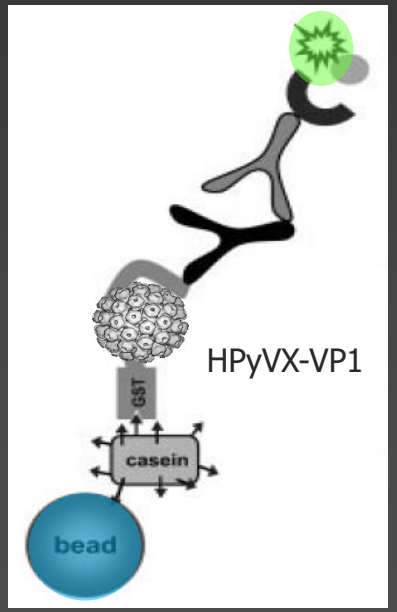
↓



→



→

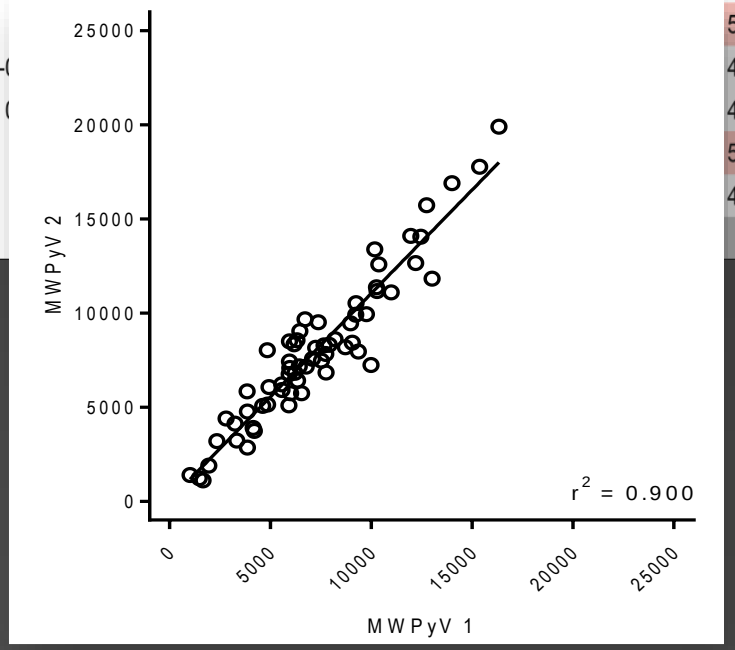


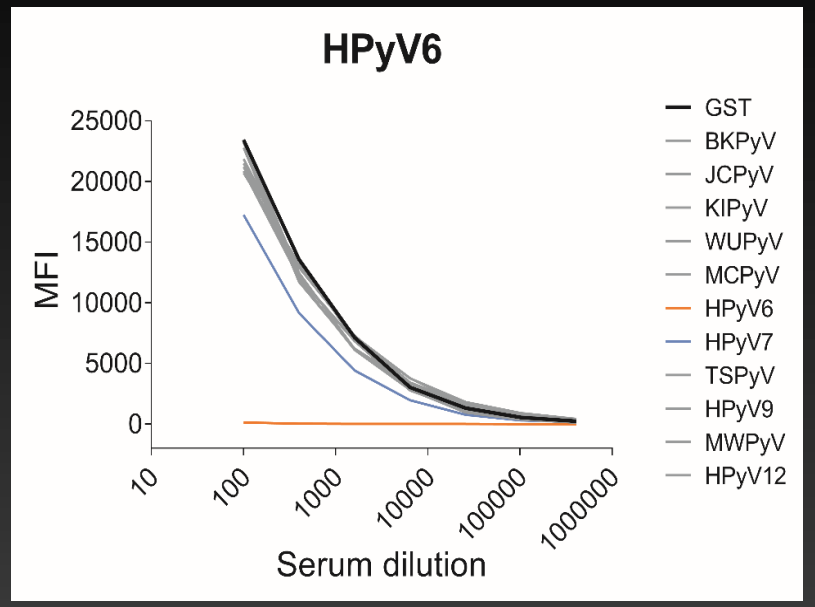
Assay validation

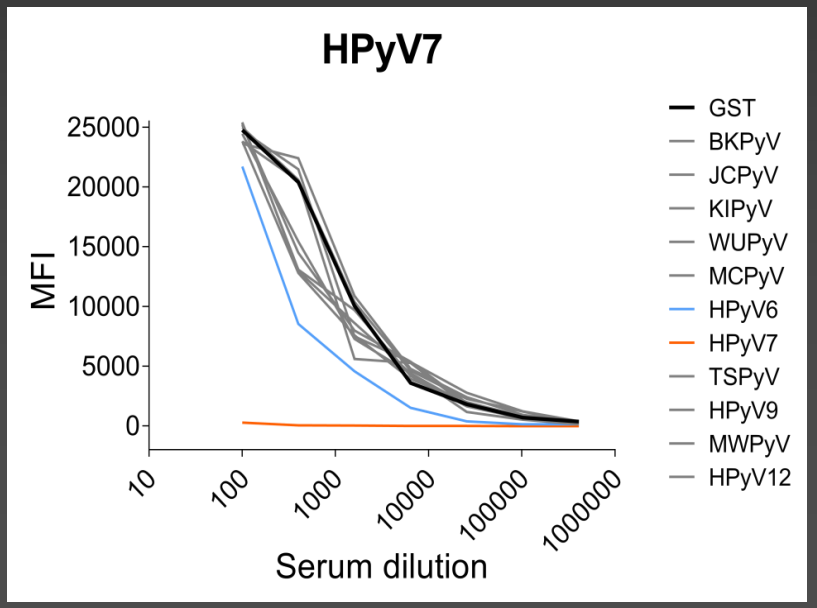
VP1 sequence similarity (%)

Spearman correlation coefficient (ρ)

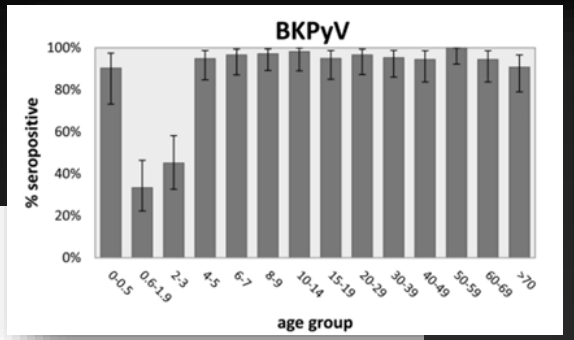
	BKPyV	JCPyV	KIPyV	WUPyV	MCPyV	HPyV6	HPyV7	TSPyV	HPyV9	MWPyV	STLPyV	HPyV12	NJPyV	LIPyV
BKPyV		78.5	28.2	29.1	43.9	27.7	26.4	54.1	53.8	43.3	41.4	53.8	47.4	44.3
JCPyV	-0.14		28.4	28.3	46.6	26.3	26.5	52.4	54.4	45.0	40.6	55.1	45.4	42.4
KIPyV	-0.02	-0.13		66.1	27.2	33.5	36.9	23.9	26.2	24.4	26.3	25.4	22.7	24.2
WUPyV	0.06	0.16	0.03		29.5	37.4	37.8	24.6	27.1	28.3	26.4	27.6	21.6	26.9
MCPyV	0.02	0.20	0.08	0.14		27.5	25.1	56.1	57.4	39.1	40.0	56.2	49.3	54.8
HPyV6	0.00	-0.01	-0.02	0.06	0.03		68.0	27.2	27.7	24.9	29.1	29.9	22.7	26.6
HPyV7	-0.12	0.23	-0.03	0.12	0.11	0.49		25.7	27.8	26.3	30.1	27.7	24.1	23.9
TSPyV	0.09	0.24	0.23	0.21	0.16	-0.06	0.01		60.2	43.9	43.2	60.3	55.1	46.3
HPyV9	nd	nd	nd	nd	nd									50.1
MWPyV	0.14	0.09	0.14	0.20	0.17									41.7
STLPyV	-0.12	0.20	0.15	0.25	0.07									40.1
HPyV12	nd	nd	nd	nd	nd									52.5
NJPyV	nd	nd	nd	nd	nd									46.1
LIPyV	nd	nd	nd	nd	nd									





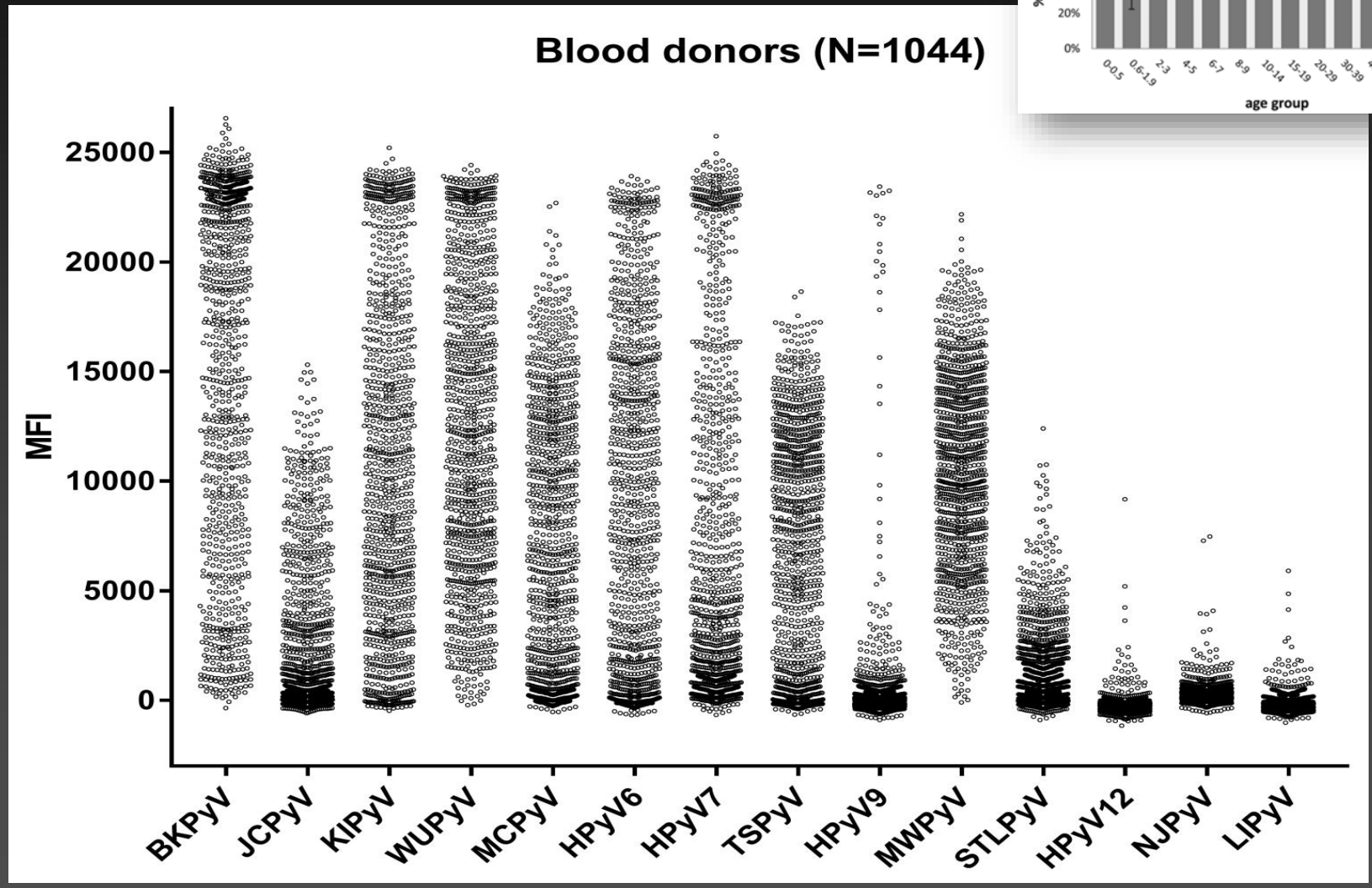


Seroresponses

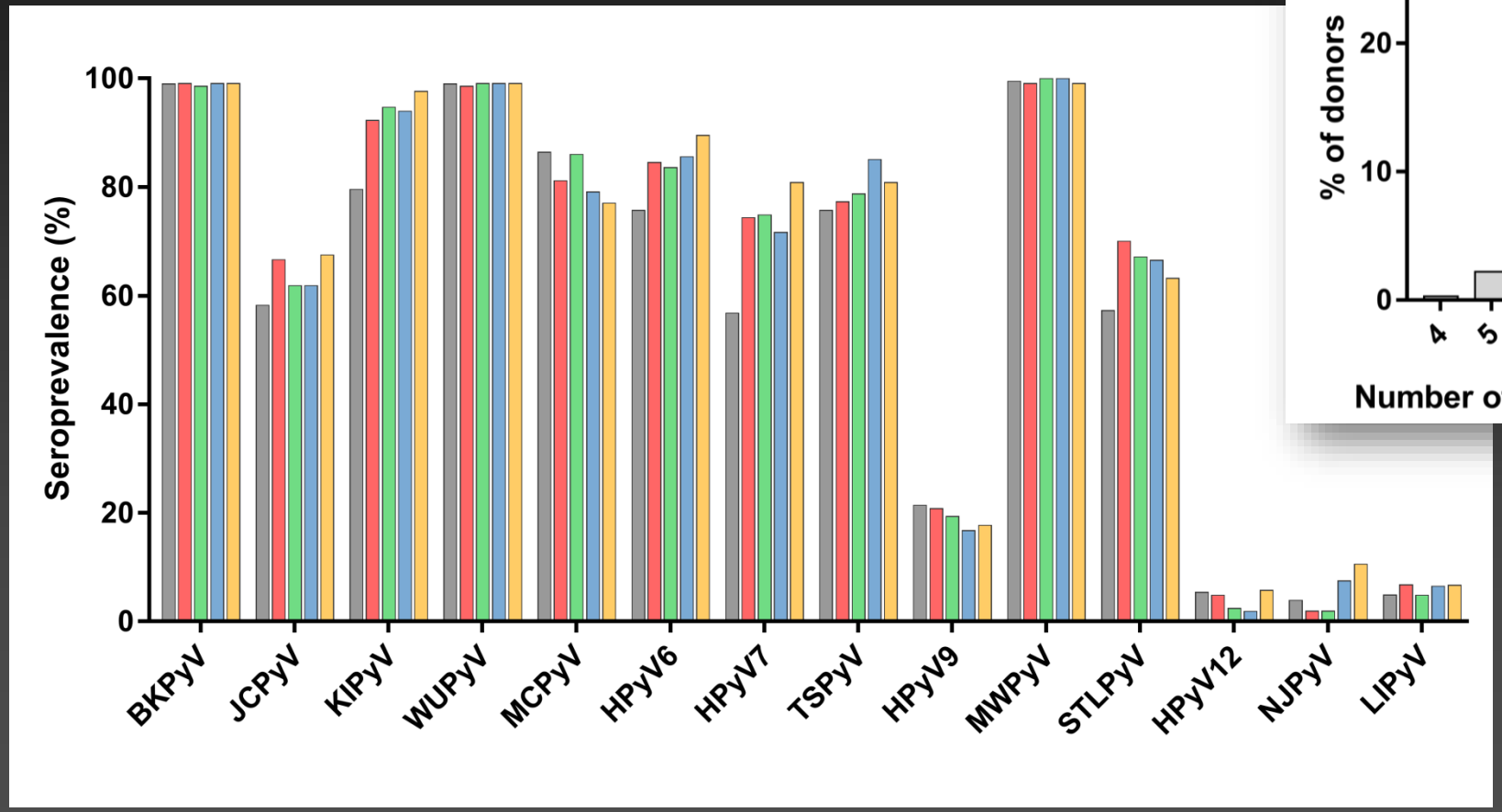


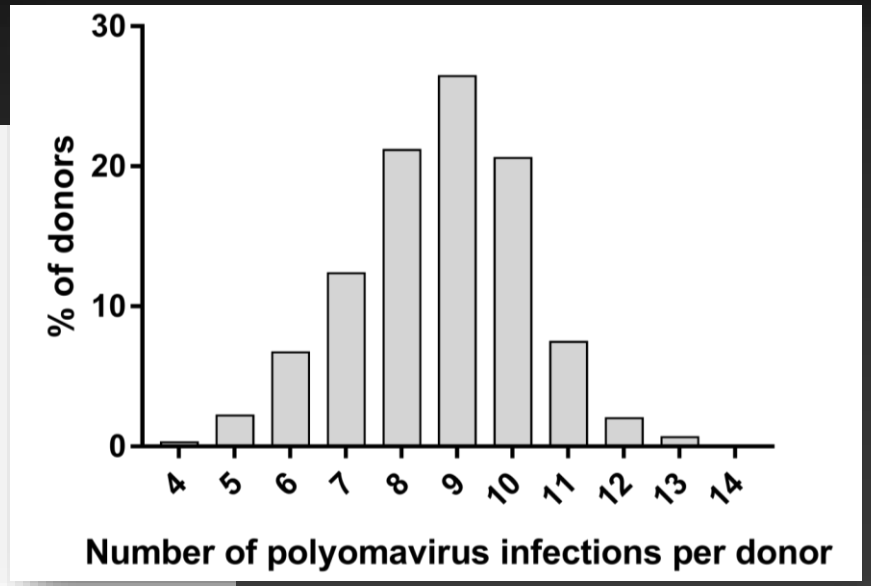


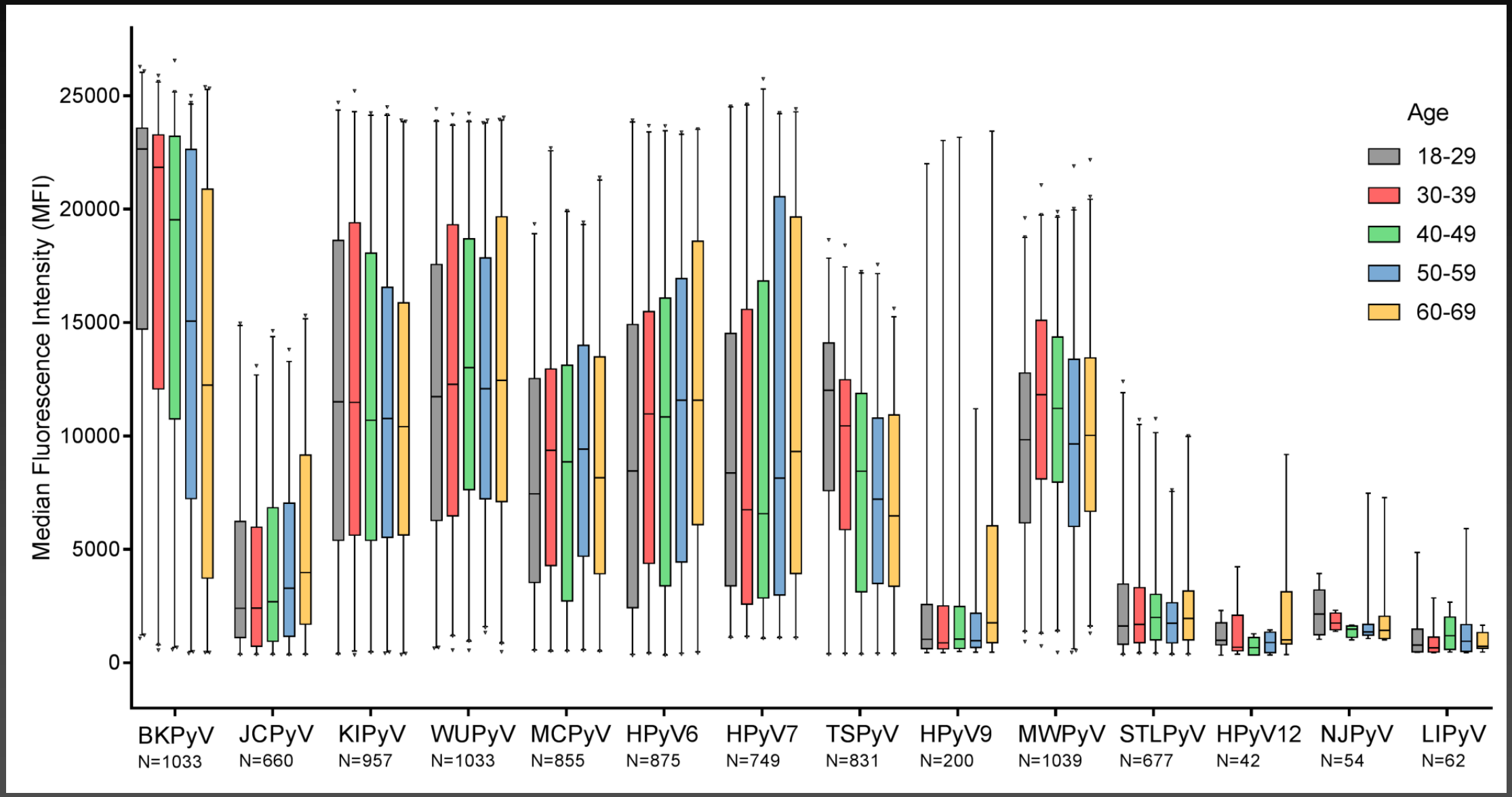
Netherlands



Seroprevalence and frequency






Median Fluorescence Intensity (MFI)
25000
20000
15000
10000
5000
0
Age
18-29
30-39
40-49
50-59
60-69
BKPyV N=1033
JCPyV N=660
KIPyV N=957
WUPyV N=1033
MCPyV N=855
HPyV6 N=875
HPyV7 N=749
TSPyV N=831
HPyV9 N=200
MWPyV N=1039
STLPyV N=677
HPyV12 N=42
NJPyV N=54
LIPyV N=62

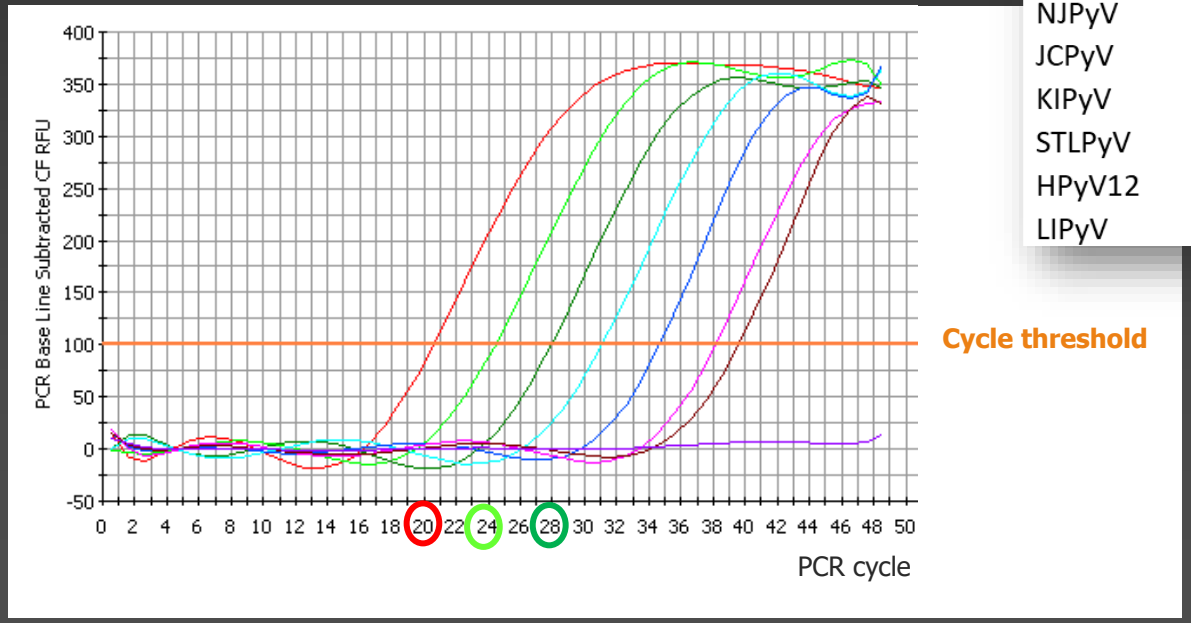
How about the prevalence in donors?

→ Develop species-specific RT-PCR (qPCR)

→ Combined primer/probes (multiplexing)

→ Checked specificity and sensitivity

Target HPyV species	Gene	Multiplex	Product (bp)	Limit of detection (copies/reaction)
MCPyV	LT	1	149	100
HPyV6	VP1	1	150	15
HPyV7	VP1	1	116	15
TSPyV	VP1	1	104	10
HPyV9	VP1	1	109	15
BKPyV	VP1	2	90	10
WUPyV	VP1	2	74	15
MWPyV	VP1	2	86	15
NJPyV	VP1	2	135	15
JCPyV	LT	3	129	10
KIPyV	VP1	3	148	15
STLPyV	VP1	3	101	15
HPyV12	VP1	3	139	15
LIPyV	VP1	3	83	15



HPyV-DNA detection and viral load in donors

PCR target	PCR-positive Any Ct N (%)	 Ct <40 N (%)	 Product sequence confirmation*	Viral load copies/ml
BKPyV	5 (0.5)	1 (0.1)	1/1 (100)	55
JCPyV	14 (1.4)	5 (0.5)	5/5 (100)	9-37
KIPyV	2 (0.2)	1 (0.1)	0/1	46
WUPyV	2 (0.2)	2 (0.2)	1/2 (50)	8-30
MCPyV	63 (6.2)	39 (3.8)	7/9 (78)	24-452
HPyV6	4 (0.4)	1 (0.1)	1/1 (100)	13
HPyV7	7 (0.7)	0	-	-
TSPyV	7 (0.7)	5 (0.5)	5/5 (100)	9-81
HPyV9	6 (0.6)	4 (0.4)	1/3 (33)	7-68
MWPyV	3 (0.3)	1 (0.1)	1/1 (100)	15
STLPyV	1 (0.1)	1 (0.1)	0/1	39
HPyV12	11 (1.1)	10 (1.0)	0/10	2-13
NJPyV	3 (0.3)	1 (0.1)	0/1	123
LIPyV	6 (0.6)	2 (0.2)	2/2 (100)	13-31

Conclusions and consequences

- HPyV infection is common in blood donors
  - Seroprevalence is high for most HPyVs
  - Average donor is infected with 9 different HPyV species
- HPyV-viremia
  - Found in a smal proportion (<5%) of blood donors
  - JCPyV and TSPyV, perhaps MCPyV
  - Viral load is low, and integrity and infectivity of the particles is still unknown
- Transfusion risk: unknown
  - Unaware of the possibility
  - Discriminate between autologous (reactivation) and transfusion-transmitted virus (*de novo*)
  - Learn from SOTx-medicine (BKPyV viremia/nephropathy is donor derived)