

**Real-time quantitative PCR  
assays for the detection and  
monitoring of pathogenic  
human viruses in  
immunosuppressed pediatric  
patients**

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and Development of Genetic Diagnostics,  
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1. General information on the occurrence of viruses in the SCT setting and composition of our virus panel
2. Methodological aspects of the assays
3. Different applications of the technology
4. Summary

# Panel of RQ-PCR assays

## DNA-VIRUSES:

### ▪ **HERPES-VIRUSES:**

**Cytomegaly V** (major immediate early prot.)

**Epstein-Barr V** (non-glycosylated membrane prot.)

**Herpes Simplex V 1** (US 4)

**Herpes Simplex V 2** (glycoprot. D gene)

**Varicella Zoster V** (ORF 38)

**Human Herpes V 6** (DNA-Polymerase)

**Human Herpes V 7** (major capsid prot.)

**Human Herpes V 8** (ORF 26 gene)

▪ **Adeno V** (hexon gene, fiber gene)

▪ **Parvo V B19** (VP1, VP2)

▪ **Polyoma V** (VP3) (BKV, JCV, SV-40)

## RNA-VIRUSES:

▪ **Entero V** (5'UTR gene)

includes: Polio V (PV), Coxsackie V (CV),

Echo V (ECV) and Entero V 68-71 (EV 68-71)

▪ **Respiratory-Syncytial V** (N-gene)

▪ **Influenza A V** (M gene)

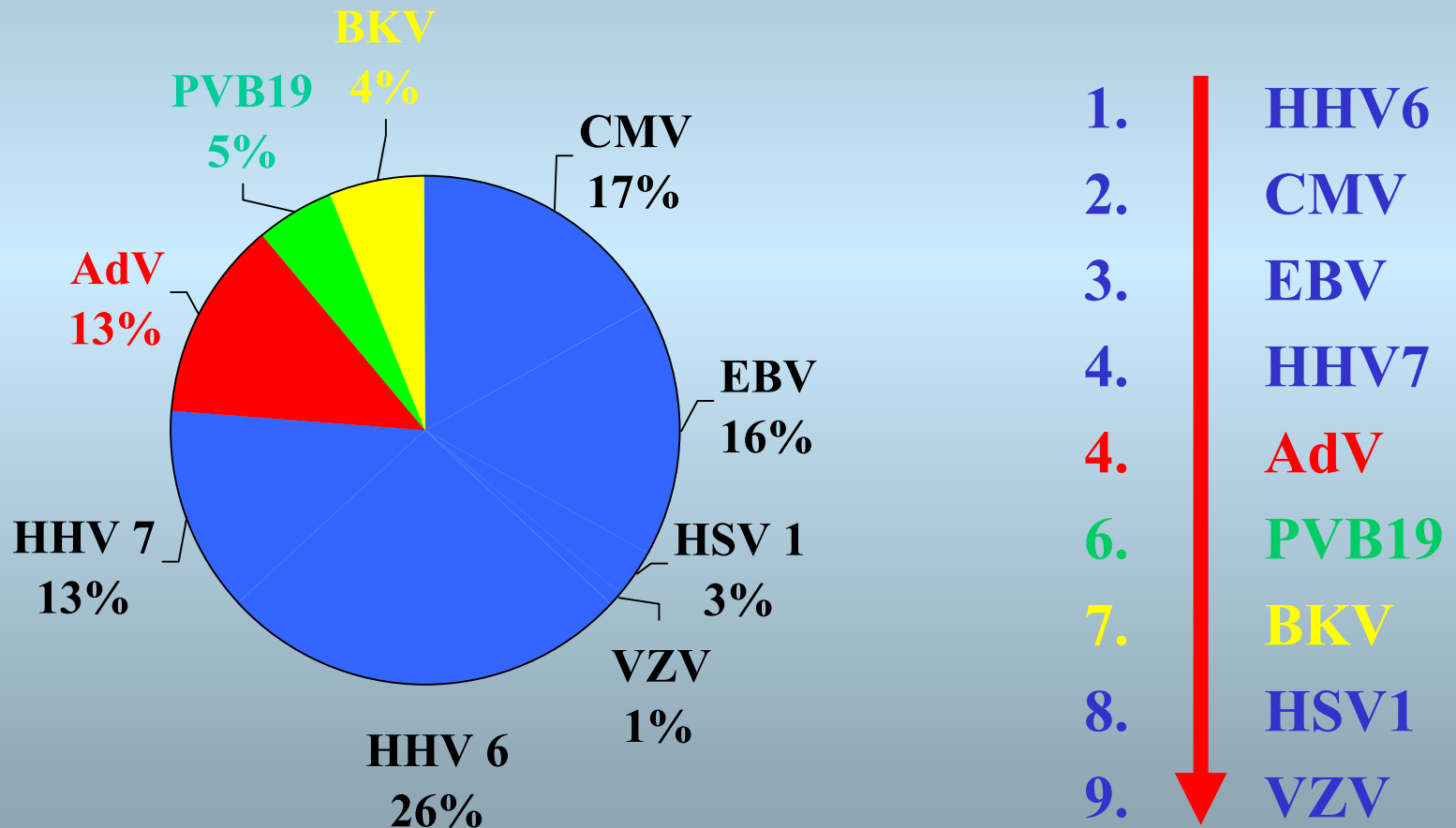
▪ **Influenza B V** (HA-gene)

▪ **Parainfluenza 1 V** (HN-gene)

▪ **Parainfluenza 2 V** (HN-gene)

▪ **Parainfluenza 3 V** (HN-gene)

# Relative frequency of virus detection after allo-SCT



# Virus detection in:

## largely cell free material

DNA/RNA-Viruses

Serum/Plasma

Tracheal Secretion

Urine

Stool

BAL

CSF

Nasal Secretion

## cell material

DNA-Viruses

Leukocytes

Tissue Samples

## Sensitivity

(1-2 virus particles/PCR Reaction)

$1 \times 10^2$ /ml

$10/10^6$  cells

## Quantifiability

$>10^3$ /ml

$> 10/10^6$  cells

# Controls & Precautions

## *Positive controls*

PHHV

Efficiency of extraction and RQ-PCR

PHHV

Presence of PCR inhibitors

Virus-specific control

Virus PCR and inter-assay variability

B2 MG

Quality and amount of DNA

## *Negative controls*

NAC

Non-specific primer binding

NTC

Contamination

## *Precautions*

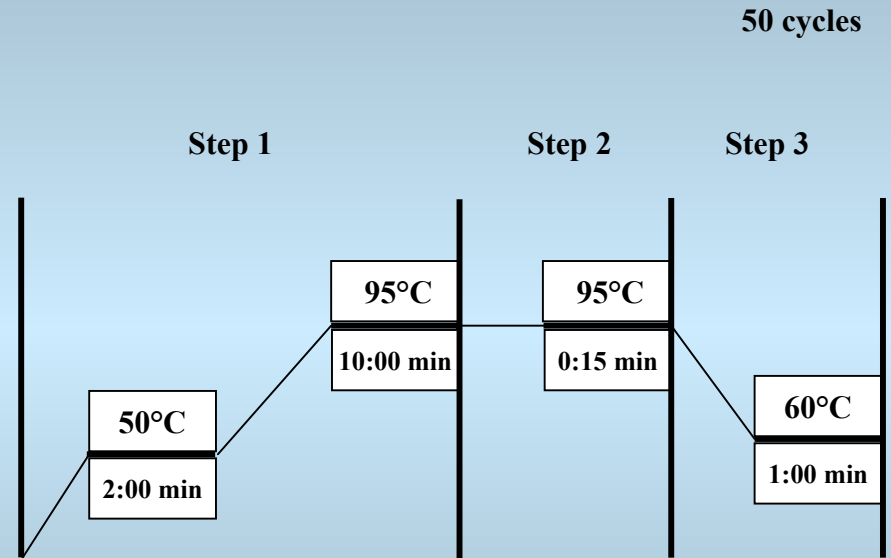
UNG

Contamination with PCR products

## RQ-mix composition

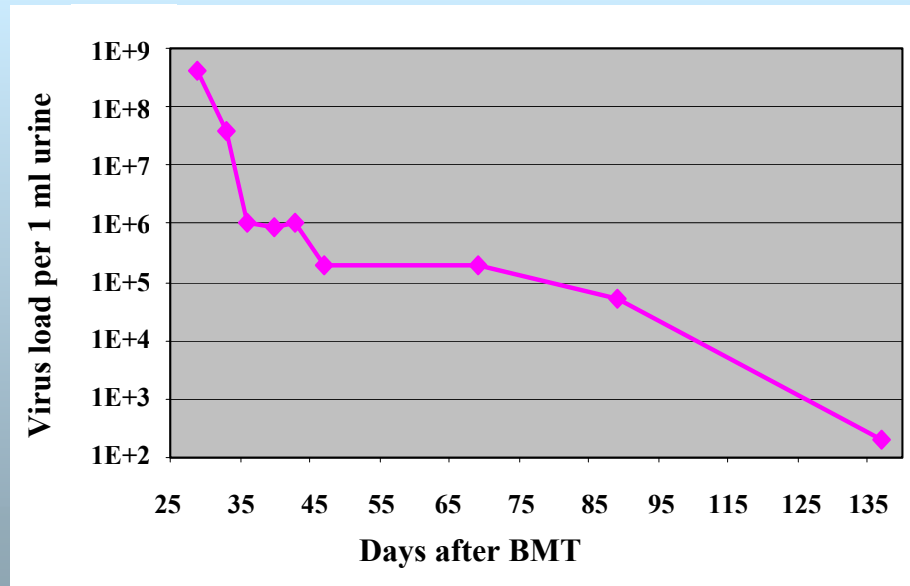
Master Mix (ABI)	2x conc.
Primers	50-900nM
Probe	200nM
DNA/c-DNA template	6 ul
<hr/>	
Total volume	25 ul

## RQ-PCR temperature profile



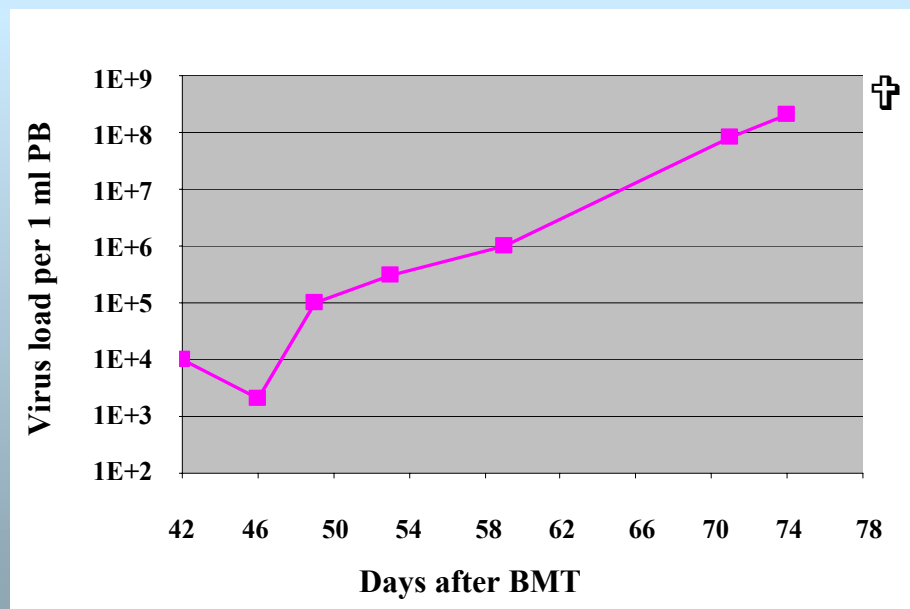
# Monitoring of virus load (1)

**Kinetics of BKV (polyoma virus) load during hemorrhagic cystitis**



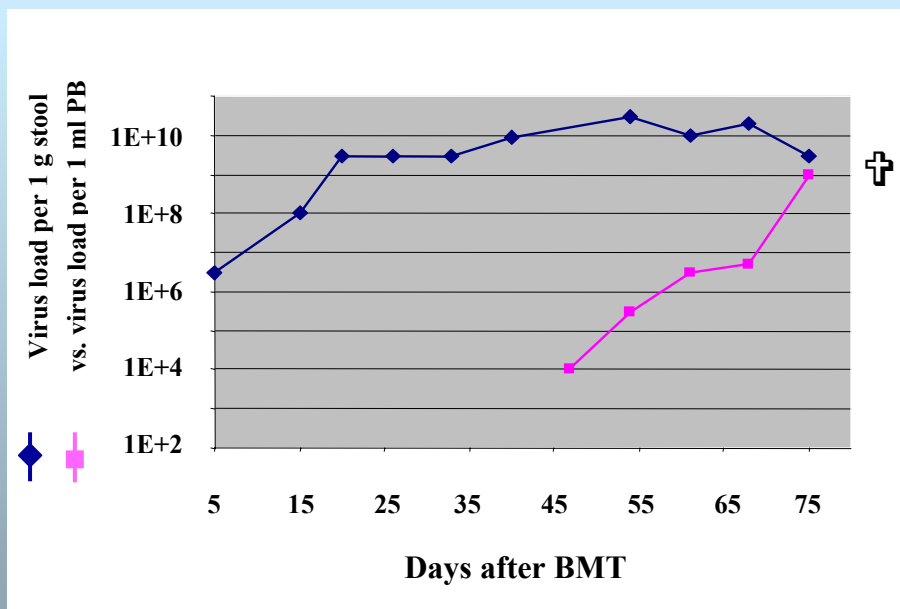
# Monitoring of virus load (2)

**Kinetics of EBV load in peripheral blood in a posttransplant lymphoproliferative disorder (LPD)**



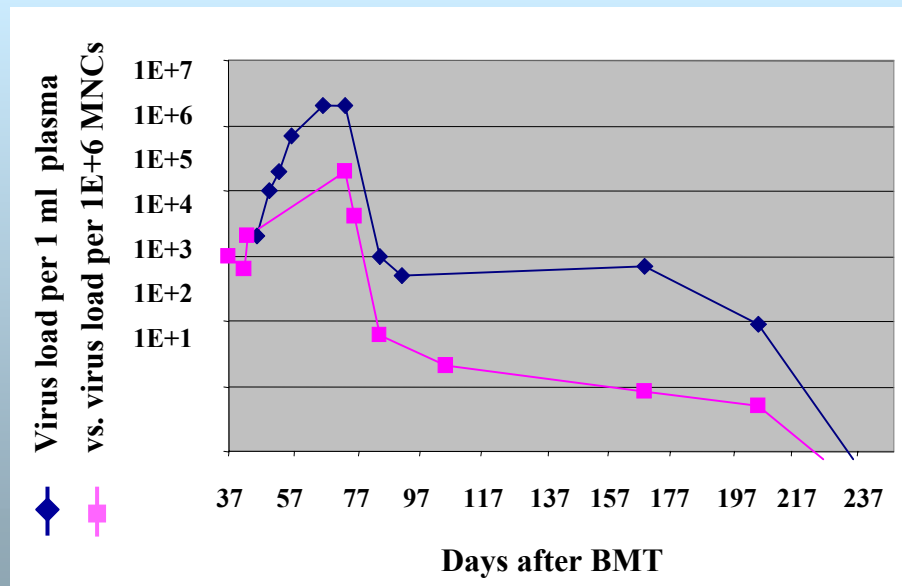
# Monitoring of virus load (3)

**Kinetics of AdV load in stool and PB preceding fatal disseminated disease**



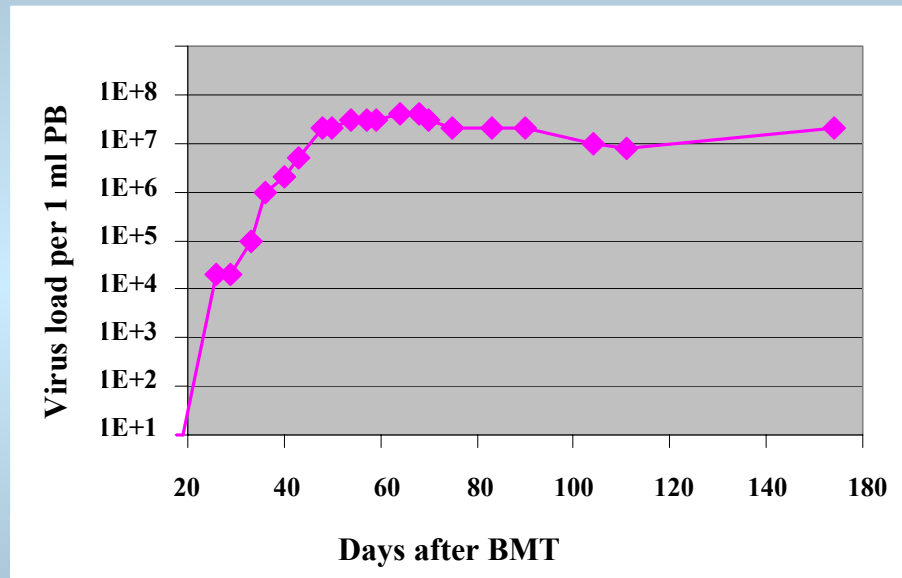
# Monitoring of virus load (4)

**Kinetics of CMV load in plasma and  
PB leukocytes in response to antiviral treatment**



# Monitoring of virus load (5)

## Kinetics of HHV 6 load



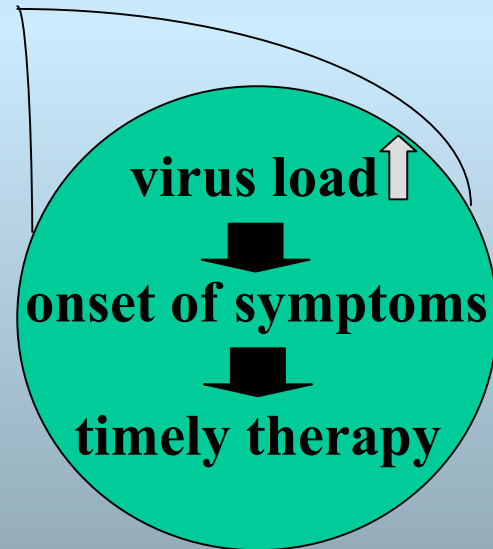
Clinical significance???

# Summary

**RQ-PCR** → sensitive virus detection and quantification

**Serial RQ-PCR** → latent vs active infection

dynamics of infection



monitoring of response to treatment

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