

AperTO - Archivio Istituzionale Open Access dell'Università di Torino

Nation-wide measure of variability in HCMV, EBV and BKV DNA quantification among centers involved in monitoring transplanted patients

This is the author's manuscript

Original Citation:

Availability:

This version is available <http://hdl.handle.net/2318/1633753> since 2017-05-15T08:58:32Z

Published version:

DOI:10.1016/j.jcv.2016.07.001

Terms of use:

Open Access

Anyone can freely access the full text of works made available as "Open Access". Works made available under a Creative Commons license can be used according to the terms and conditions of said license. Use of all other works requires consent of the right holder (author or publisher) if not exempted from copyright protection by the applicable law.

(Article begins on next page)

1 **Nation-wide measure of variability in HCMV, EBV and BKV DNA quantification among**
2 **centres involved in monitoring transplanted patients**

3

4 Isabella Abbate^{a,1}, Antonio Piralla^{b,1}, Agata Calvario^c, Annapaola Callegaro^d, Cristina Giraldi^e,
5 Giovanna Lunghi^f, William Gennari^g, Giuseppe Sodano^h, Paolo Ravaniniⁱ, Pier Giulio Conaldi^j,
6 Marialinda Vatteroni^k, Aurelia Gaeta^l, Pierpaolo Paba^m, Rossana Cavalloⁿ, Fausto Baldanti^{b,o,*},
7 Tiziana Lazzarotto^p and the AMCLI - Infections in Transplant Working Group (GLaIT)²

8

9 ^aLaboratorio di Virologia, INMI L. Spallanzani, Roma

10 ^bSS Virologia Molecolare, SC Microbiologia e Virologia, Fondazione IRCCS Policlinico San Matteo, Pavia

11 ^cUOC Microbiologia e Virologia, Policlinico di Bari, Bari

12 ^dUO di Microbiologia e Virologia, Ospedali Riuniti, Bergamo

13 ^eUO di Microbiologia e Virologia, P.O. Annunziata-A.O., Cosenza

14 ^fLaboratorio di Virologia, IRCCS Ospedale Maggiore Policlinico Mangiagalli e Regina Elena, Milano

15 ^gUO di Microbiologia e Virologia, Azienda Ospedaliero-Universitaria di Modena, Modena

16 ^hUOC Microbiologia e Virologia, AORN Azienda Ospedaliera dei Colli, Ospedali Monaldi-Cotugno-CTO, Napoli

17 ⁱUO di Microbiologia e Virologia, Ospedale Maggiore della Carità, Novara

18 ^jLaboratorio di Patologia Clinica, Microbiologia e Virologia - ISMETT, Palermo

19 ^kUO di Virologia, Azienda Ospedaliera Universitaria Pisana, Pisa

20 ^lUOC Virologia, Policlinico Umberto I Sapienza, Roma

21 ^mUOC Virologia Molecolare, Policlinico Fondazione Tor Vergata, Roma

22 ⁿSC Microbiologia e Virologia, AOU Città della Salute e della Scienza, Università di Torino

23 ^oSezione di Microbiologia, Dipartimento di Scienze Clinico-Chirurgiche, Diagnostiche e Pediatriche, Università degli
24 studi di Pavia, Pavia

25 ^pUO di Microbiologia, DIMES, Policlinico S. Orsola Malpighi, Università di Bologna, Bologna

26

27

28 *Corresponding author at: Department of Clinical, Surgical, Diagnostic and Pediatric Sciences,
29 University of Pavia, Viale Brambilla 74, Pavia, 27100, Italy. Fax: +39 0382 502599.

30 *E-mail addresses:* f.baldanti@smatteo.pv.it, fausto.baldanti@unipv.it (F. Baldanti).

31

32 ¹Contributed equally

33 ²Other participants in the GLaIT group along with their affiliation are listed in Appendix A.

34

35 Word count abstract: 248 (250)

36 Word count text: 1,587 (2,500)

37

38

39

1 ABSTRACT

2 *Background:* Inter-laboratory variability in quantifying pathogens involved in viral disease after
3 transplantation may have a great impact on patient care, especially when pre-emptive strategies are
4 used for prevention.

5 *Objectives:* The aim of this study was to analyze the variability in quantifying CMV, EBV and
6 BKV DNA among 15 virology laboratories of the Italian Infections in Transplant Working Group
7 (GLaIT) involved in monitoring transplanted patients.

8 *Study Design:* Panels from international Quality Control programs for Molecular Diagnostics
9 (QCMD, year 2012), specific for the detection of CMV in plasma, CMV in whole blood (WB),
10 EBV and BKV were used. Intra- and inter-laboratory variability, as well as, deviation from QCMD
11 consensus values were measured.

12 *Results:* 100% specificity was obtained with all panels. A sensitivity of 100% was achieved for
13 EBV and BKV evaluation. Three CMV samples, with concentrations below 3 log₁₀ copies/ml, were
14 not detected by a few centers. Mean intra-laboratory variability (% CV) was 1.6 for CMV plasma
15 and 3.0 for CMV WB. Mean inter-laboratory variability (% CV) was below 15% for all the tested
16 panels. An higher inter-laboratory variability was observed for CMV WB with respect to CMV
17 plasma (3.0 vs 1.6% CV). The percentiles 87.7%, 58.6%, 89.6% and 74.7% fell within ± 0.5 log₁₀
18 difference of the consensus values for CMV plasma, CMV WB, EBV and BKV panels,
19 respectively.

20 *Conclusions:* An acceptable intra- and inter-laboratory variability was observed in this study, in
21 comparison with international standards. However, further harmonization in viral genome
22 quantification is reasonable expectation for the future.

23 **Keywords:** multicenter evaluation; standardization; transplantation; CMV-DNA; EBV-DNA;
24 BKV-DNA

25

26

1 **1. Background**

2 Cytomegalovirus (CMV) and Epstein-Barr virus (EBV) are major causes of post-transplant viral
3 disease both in solid organ and hematopoietic stem cell transplantation, while Poliovirus BK virus
4 reactivation, with virus-associated nephropathy, represents the most frequent cause of graft loss
5 after renal transplantation [1,2]. The reliability and accuracy of viral load determination are
6 therefore critical for the management of transplant patients. In fact, virological monitoring of
7 transplanted patients is based on standardized protocols for genome quantification in order to apply
8 clinical cut-offs in pre-emptive approaches for disease prevention [3-6]. However, measurements of
9 viral load performed with commercially available assays might differ significantly, particularly
10 according to the extraction method used, which is a source of variability with different clinical
11 specimens. Finally, there is a need for an inter-laboratory comparison of results and evaluation of
12 individual assays with standardized panels, particularly with collaborative multicentre networks.

14 **2. Objectives**

15 The aim of this study was to analyze the variability obtained among 15 Italian virology
16 laboratories, belonging to the Working Group for Transplantation (Gruppo di Lavoro Infezioni nel
17 Trapianto, GLaIT) of the Italian Association of Clinical Microbiologists (Associazione
18 Microbiologi Clinici Italiani, AMCLI). Therefore, panels from international Quality Control
19 programs for Molecular Diagnostics (QCMD, year 2012), specific for the detection and
20 quantification of CMV in plasma, CMV in whole blood (WB), EBV and BKV were used.

22 **3. Study design**

23 *3.1. QCMD samples*

24 The QCMD panels used in the study were: QCMD 2012 CMV plasma, QCMD 2012 CMV WB,
25 QCMD 2012 EBV and QCMD 2012 BKV-JCV. A number of samples with various amounts of the

1 different viruses suspended in an appropriate matrix and negative controls were tested in each
2 panel. For a detailed description and composition of the panels see www.qcmd.org.

3

4 *3.2. Extraction and quantitative real-time PCR assays*

5 Each sample was tested by each of the laboratories using commercial and *in house* methods
6 adopted for routinely virologic monitoring of transplanted patients (Table 1). Nucleic acid
7 extraction was performed by the majority of the laboratories using automatic extraction with
8 commercial with sometimes in-house modifications; Real-time PCR amplification was carried out
9 by all the laboratories with commercially available kits, with only one exception. Quantitative
10 results were expressed as log₁₀ copies/ml for all three viruses tested. For positive samples detected
11 below the lowest limit of quantification, when a detected number of copies was not available, an
12 arbitrary value of half of the lowest limit of quantification was used.

13

14 *3.3. Statistical analysis*

15 Intra- and inter-laboratory variability was calculated, as well as, variation with respect to the
16 QCMD consensus as the coefficient of variation (%CV). The Pearson correlation analysis and the
17 Bland-Altman analysis were performed to examine the level of agreement between the 15
18 laboratories' results and the QCMD samples. Results were considered to be quantitatively
19 discordant when the results of the Bland-Altman analysis were discordant by more than $\pm 0.5 \text{ Log}_{10}$
20 of the QCMD consensus values. Statistical analysis was performed using Graph Pad Prism
21 software, version 5.00.288.

22

23 **4. Results**

24 The results obtained by the different GLaIT laboratories were analyzed to obtain a description of
25 intra- and inter-laboratory variability and a quantitative comparison with respect to the consensus
26 values reported by the different QCMD panels.

1 For each of the four panels tested (QCMD CMV plasma, CMV WB, EBV and BKV) no false
2 positive results were obtained by any of the GLaIT laboratories (specificity 100%). A sensitivity of
3 100% was achieved with the EBV and BKV evaluations. Concerning the CMV plasma panel,
4 sample #3 (2.24 log₁₀ copies/ml) was not detected by 1/15 (6.6%) centres and sample #4 (2.08 log₁₀
5 copies/ml) was not detected by 4/15 (26.6%) centres. For the CMV WB panel only sample #8 (2.58
6 log₁₀ copies/ml) was not detected by 4/13 (30.8%) centres. For the BKV/JCV panel, no cross
7 reactivity with the JCV virus was observed (6 samples) and all of the centres detected all the five
8 samples containing BKV.

9 The QCMD CMV plasma and WB panels contained duplicate samples to allow intra-laboratory
10 variability evaluation. The results indicated that the mean intra-laboratory % of the coefficient of
11 variation (CV) was 1.6 for CMV plasma and 3.0 for CMV WB.

12 In Table 2, for each sample the mean, standard deviation (SD), CV (%), median and range of
13 log₁₀ copies/ml are reported. The mean SD for CMV plasma, CMV WB, EBV and BKV were
14 respectively 0.27, 0.49, 0.25 and 0.37. The mean % CV for CMV plasma, CMV WB, EBV and
15 BKV were respectively: 9.4%, 13.7%, 6.71% and 13.3%. The mean Delta log₁₀ for CMV plasma,
16 CMV WB, EBV and BKV were respectively: 0.93, 1.40, 0.97 and 1.29. It should be emphasized
17 that the variability was usually larger when considering samples with a virus concentration lower
18 than 3 log₁₀ copies/ml.

19 In order to compare the results obtained by the different GLaIT laboratories with those of the
20 international quality control study, consensus values for each QCMD panel were extrapolated and
21 used for comparison. Significant correlations were observed in CMV WB, CMV plasma, EBV and
22 BKV panel results with the Spearman coefficient which ranged from 0.82 to 0.96 (data not
23 showed). Bland-Altman plots were used to describe the log₁₀ difference between the GLaIT
24 laboratory results and the consensus values (Figure 1). According to previous reports [7-9], ±0.5
25 log₁₀ was considered an acceptable variability. In CMV the plasma panel, 114/130 (87.7%) of the
26 determinations were within ±0.5 log₁₀ difference, while in the CMV WB panel only 51/87 (58.6%)

1 were within $\pm 0.5 \log_{10}$ difference. In the CMV plasma panel, the majority of the discordant results
2 (14/16, 87.5%) were observed in samples with a $< 3.0 \log_{10}$ DNA copies number (Figure 1A), while
3 in the CMV WB panel discordant results were observed for all sample concentrations (Figure 1B).
4 In the EBV panel (Figure 1C), 120/134 (89.6%) of the measurements were within a $\pm 0.5 \log_{10}$
5 difference, with no evident differences among different sample concentrations. In the BKV panel
6 (Figure 1D), a total of 56/75 (74.7%) determinations fell within $\pm 0.5 \log_{10}$ difference; and for the
7 EBV panel, no differences among the different sample concentrations were detected.

8

9 **5. Discussion**

10 Since significant inter-laboratory variability in quantifying CMV, EBV and BKV genomes may
11 impact the quality of transplanted patient care, especially when pre-emptive strategies are used for
12 prevention, initiatives aimed at harmonizing viral genome quantification among different
13 laboratories should always be encouraged. In fact, transplant centres collect patients from all
14 Italian regions while post-transplant monitoring may be carried out by local laboratories. To the
15 best of our knowledge, this is the first report which simultaneously measures variability in
16 quantifying CMV, EBV and BKV DNA which represent the three major viral pathogens
17 responsible for disease in solid organ transplantation.

18 Mission of the GLaIT group is to improve standardization of diagnostic procedures for
19 microbiological monitoring of solid organs and stem cell transplant recipients. Two different studies
20 aimed to measure variability in CMV and EBV DNA quantification have already been performed
21 [9,10]. Concerning CMV, the present study, in contrast with the 2009 report [9], takes into account
22 both CMV DNA quantification in plasma and WB. As for the former CMV study, no false positive
23 samples were obtained and a sensitivity of 100% was obtained in samples with a DNA load greater
24 than $3 \log_{10}$ copies/ml. Although in the past a variability of less than $1 \log_{10}$ was obtained only in
25 samples with a viral load greater than $3.7 \log_{10}$ copies/ml, the results reported here ranged from 0.93
26 to $1.40 \log_{10}$ variation for plasma and WB even when considering samples with concentrations

1 below 3.0 log₁₀ copies/ml. A greater variability was observed for CMV in WB with respect to
2 plasma; this is in line with the more complex matrix represented by blood, where nucleic acid
3 extraction is more laborious. At the same time, the CMV blood panel results, although more
4 variable, display a greater linearity. Overall, the % accuracy measured fell within ±0.5 log₁₀ and
5 ranged from 58.6% to 89.6%. This accuracy measured in a multicentre study is considered
6 acceptable and is higher than those observed in a similar study including fewer centres (n=4) [11].

7 It should be underlined that in the present CMV quantification analysis, in contrast with previous
8 studies, all of the laboratories, with only one exception, used a commercial real-time PCR method
9 and only three different real-time methods were used. These real-time methods were however
10 associated with a variety of different manual or automated commercial and in house modified
11 protocols for nucleic acid extraction. It is reasonable to suppose that much of the variability
12 observed among the different quantifications was associated with the extraction procedures, rather
13 than the PCR amplification. This was also the case for EBV and BKV DNA determinations, where
14 as for CMV DNA, no false positive results were obtained for any samples. In the present study, the
15 best results (lower variability) were obtained with the EBV DNA panel. However, no direct
16 comparison can be made with the previous EBV study [10] due to the different composition of the
17 panels used for the evaluation. For BKV DNA, no cross reactivity was observed for samples
18 positive for the other Poliomavirus (JCV), included in the same QCMD panel and, although this
19 represents the first study by our group on quantification of this virus, an acceptable level of
20 variability was achieved. International standards are available since 2011 for CMV DNA and 2012
21 for EBV DNA [12, 13]; this represents an opportunity to improve harmonization in CMV and EBV
22 genome quantification.

23 In conclusion, the results of this multicentre study indicate that CMV, EBV and BKV DNAemia
24 are quantified with acceptable variability using a variety of extraction volumes and protocols with
25 different commercial and in-house molecular protocols.

26

1 **Competing interests**

2 None declared.

3

4 **Funding**

5 This work was supported by the Ministero della Salute, Fondazione IRCCS Policlinico San Matteo
6 Ricerca Corrente grant 80207.

7

8 **Ethical approval**

9 None. All experiments were performed with samples made available by QCMD 2012.

10

11 **Acknowledgements**

12 We thank Daniela Sartori for careful preparation of the manuscript and Laurene Kelly for English
13 revision.

14

15 **Appendix A. AMCLI-Infections in the Transplant Working Group (GLaIT) - list of other
16 participants.**

17

18 **Maria Rosaria Capobianchi** (Laboratorio di Virologia, INMI L. Spallanzani, Roma).

19 **Maria Luisa Scarasciulli** (Laboratorio di Virologia, U.O.C. Microbiologia e Virologia, Policlinico
20 di Bari, Bari).

21 **Francesca Greco** (UO di Microbiologia e Virologia, P.O. Annunziata-A.O., Cosenza).

22 **Erminio Torresani** (Laboratorio di Virologia, IRCCS Ospedale Maggiore Policlinico Mangiagalli
23 e Regina Elena, Milano).

24 **Fabio Rumpianesi** (UO di Microbiologia e Virologia, Azienda Ospedaliero-Universitaria di
25 Modena, Modena).

- 1 **Daniele Di Carlo** (Laboratorio di Patologia Clinica, Microbiologia e Virologia - ISMETT,
- 2 Palermo).
- 3 **Carlo Federico Perno** (UOC Virologia Molecolare, Policlinico Fondazione Tor Vergata, Roma).
- 4 **Cristina Costa** (SC Microbiologia e Virologia, A.O. Città della Salute e della Scienza, Università
- 5 di Torino).
- 6 **Liliana Gabrielli, Giulia Piccirilli** (UO di Microbiologia, Policlinico S. Orsola Malpighi,
- 7 Bologna).
- 8

1 **References**

- 2 [1] J.A. Fishman. infection in solid-organ transplant recipients. *N. Engl. J. Med.* 357 (2007) 2601-
3 2614.
- 4 [2] H. H. Hirsch, P. Randhawa and the AST Infectious Diseases Community of Practice. BK
5 Polyomavirus in solid organ transplantation. *Am. J. Transplant.* 13(S4) (2013) 179-188.
- 6 [3] R. Raymund. R.R. Razonable, R.T. Hayden. Clinical utility of viral load in management of
7 cytomegalovirus infection after solid organ transplantation. *Clin. Microbiol. Rev.* 26 (2013)
8 703-727.
- 9 [4] M.L. Gulley and W. Tang. Using Epstein-Barr viral load assays to diagnose, monitor, and
10 prevent posttransplant lymphoproliferative disorder. *Clin. Microbiol. Rev.* 23 (2010) 350-366.
- 11 [5] B.H. Chung, Y.A. Hong, H.G. Kim, I.O. Sun, S.R. Choi, H.S. Park, S.H. Lee, B.S. Choi, C.W.
12 Park, Y.J. Choi, Y.S. Kim, C.W. Yang. Clinical usefulness of BK virus plasma quantitative
13 PCR to prevent BK virus associated nephropathy. *Transpl. Int.* 25 (2012) 687-695.
- 14 [6] S. Hassan, C. Mittal, S. Amer, F. Khalid, A. Patel, R. Delbusto, L. Samuel, G. Alangaden, M.
15 Ramesh. Currently recommended BK virus (BKV) plasma viral load cutoff of ≥ 4 log₁₀/mL
16 underestimates the diagnosis of BKV-associated nephropathy: a single transplant center
17 experience. *Transpl. Infect. Dis.* 16 (2014) 55-60.
- 18 [7] X.L. Pang, J.D. Fox, J.M. Fenton, G.G. Miller, A.M. Caliendo, J.K. Preiksaitis for the
19 American Society of Transplantation Infectious Diseases Community of Practice; Canadian
20 Society of Transplantation. Interlaboratory comparison of cytomegalovirus viral load assays.
21 *Am. J. Transplant.* 9 (2009) 258-268.
- 22 [8] J.K. Preiksaitis, X.L. Pang, J.D. Fox, J.M. Fenton, A.M. Caliendo, G.G. Miller for the
23 American Society of Transplantation Infectious Diseases Community of Practice.
24 Interlaboratory comparison of Epstein-Barr virus viral load assays. *Am. J. Transplant.* 9 (2009)
25 269-279.

- 1 [9] D. Lilleri ,T. Lazzarotto, V. Ghisetti ,P. Ravanini,M.R. Capobianchi, F. Baldanti, G. Gerna
2 SIV-AMCLI Transplant Surveillance Group. Multicenter quality control study for human
3 cytomegalovirus DNAemia quantification. *New Microbiol.* 32 (2009) 245-253.
- 4 [10]I. Abbate, M. Zanchetta, M. Gatti, L. Gabrielli, S. Zanussi, M.G. Milia, T. Lazzarotto, R.
5 Tedeschi, V. Ghisetti, M. Clementi, A. De Rossi, F. Baldanti, M.R. Capobianchi. Multicenter
6 comparative study of Epstein-Barr virus DNA quantification for virological monitoring in
7 transplanted patients. *J. Clin. Virol.* 50 (2011) 224-229.
- 8 [11]J. Rychert, L. Danziger-Isakov, B. Yen-Lieberman, G. Storch, R. Buller, S.C. Sweet, A.K.
9 Mehta, J.A. Cheeseman, P. Heeger, E.S. Rosenberg, J.A. Fishman. Multicenter comparison of
10 laboratory performance in cytomegalovirus and Epstein-Barr virus viral load testing using
11 international standards. *Clin. Transplant.* 28 (2014) 1416-1423.
- 12 [12]J.F. Fryer, A.B. Heath, R. Anderson, P.D. Minor and the collaborative study group.
13 Collaborative study to evaluate the proposed 1st WHO International Standard for human
14 cytomegalovirus (HCMV) for nucleic acid amplification (NAT)-based assays. WHO ECBS
15 Report (2010); WHO/BS/10.2138.
- 16 [13]J.F. Fryer, A.B. Heath, D.E. Wilkinson, P.D. Minor and the collaborative study group.
17 Collaborative study to evaluate the proposed 1st WHO International Standard for Epstein-Barr
18 virus (EBV) for nucleic acid amplification (NAT)- based assays. WHO ECBS Report (2011);
19 WHO/BS/11.2172.
- 20 [14]M. Furione, V. Rognoni, E. Cabano, F. Baldanti. Kinetics of human cytomegalovirus (HCMV)
21 DNAemia in transplanted patients expressed in international units as determined with the
22 Abbott RealTime CMV assay and an in-house assay. *J. Clin. Virol.* 55 (2012) 317-322.
- 23 [15]F. Baldanti, M. Gatti, M. Furione, S. Paolucci, C. Tinelli, P. Comoli, P. Merli, F. Locatelli.
24 Kinetics of Epstein-Barr virus DNA load in different blood compartments of pediatric
25 recipients of T-cell depleted HLA-haploidentical stem cell transplantation. *J. Clin. Microbiol.*
26 46 (2008) 3672-3677.

1 [16] B.K. Saundh, S. Tibble, R. Baker, K. Sasnauskas, M. Harris, A. Hale. Different patterns of BK
2 and JC polyomavirus reactivation following renal transplantation. *J. Clin. Pathol.* 63 (2010)
3 714-718.

4

1 **Figure Legend**

2 **Figure 1.** Bland-Altman plots are used to describe the Log difference between the GLaIT
3 laboratory results and QCMD consensus values. A (CMV plasma panel), B (CMV WB panel),
4 C (EBV panel) and D (BKV panel).

5

Table 1. Methods for viral DNA extraction and quantification.

Virus target	Center #	Nucleic acid extraction		Input volume (µl)	Output volume (µl)	Amplification method	Real-time PCR instrument
		Instrument	Protocol				
CMV	1	QIA Symphony	DSP virus/pathogen (modified)	400 (200 for WB)	90 (90 for WB)	CMV Trender Affigene	Stratagene xp3000
	2	NucliSENS EasyMag	generic 2.0.1	250 (100 for WB)	25 (25 for WB)	CMV Alert Real-Time, ELITechGroup	ABI Prism 7300
	3	QIA Symphony	DSP virus/pathogen (modified)	1000	110	CMV ELITe MGB Kit, ELITechGroup	ABI Prism 7300
	4	NucliSENS EasyMag	generic 2.0.1 and specific 2.0 for WB	500 (200 for WB)	55 (55 for WB)	in house PCR (target US8)[14]	ABI Prism 7300
	5	NucliSENS EasyMag	generic 2.0.1	100	50	CMV ELITe MGB Kit, ELITechGroup	ABI Prism 7300
	6	x-tractor gene UV light	Helix DNA Corbet	400 200 for WB	60 150 for WB	CMV Alert Real-Time, ELITechGroup CMV ELITe MGB Kit, ELITechGroup	ABI Prism 7300
	7	NucliSENS EasyMag	generic 2.0.1	500	50	CMV ELITe MGB Kit, ELITechGroup	ABI Prism 7500
	8	QIA Symphony	blood 200V6 for WB	200 for WB	200 for WB		
	9	NucliSENS EasyMag	generic 2.0.1	400 (200 for WB)	60 (85 for WB)	CMV Alert Real-Time, ELITechGroup	ABI Prism 7300
	10	NucliSENS EasyMag	generic 2.0.1	1000 (200 for WB)	25 (55 for WB)	CMV Alert Real-Time, ELITechGroup	ABI Prism 7300
	11	QIA Symphony	DSP virus/pathogen and DSP DNA for WB	500 (200 for WB)	90 (90 for WB)	CMV ELITe MGB Kit, ELITechGroup	ABI Prism 7300
	12	QIA Symphony	DSP virus/pathogen and DSP DNA for WB	500 (200 for WB)	140 (90 for WB)	CMV ELITe MGB Kit, ELITechGroup	ABI Prism 7300
	13	NucliSENS EasyMag	specific 2.0 (modified for WB)	500 (100 for WB)	100 (50 for WB)	CMV ELITe MGB Kit, ELITechGroup	ABI Prism 7300
	14	NucliSENS EasyMag	generic 2.0.1	500 (100 for WB)	55 (55 for WB)	CMV Alert Real-Time, ELITechGroup	ABI Prism 7300
	15	Manual extraction	QIAamp blood mini kit	500 (200 for WB) 200	55 (55 for WB) 100	CMV Alert Real-Time, ELITechGroup CMV r-gene Argene-Biomerieux	ABI Prism 7300 ABI Prism 7500
EBV	1	QIA Symphony	DSP virus/pathogen	400	90	EBV Trender Affigene	Stratagene xp3000
	2	NucliSENS EasyMag	generic 2.0.1	250	25	EBV Alert Real-Time, ELITechgroup	ABI Prism 7300
	3	NucliSENS EasyMag	generic 2.0.1	500	55	EBV ELITe MGB Kit, ELITechgroup	ABI Prism 7300
	4	NucliSENS EasyMag	generic 2.0.1	500	55	in house PCR (target EBNA-1)[15]	ABI Prism 7300
	5	NucliSENS EasyMag	generic 2.0.1	100	100	EBV ELITe MGB Kit, ELITechgroup	ABI Prism 7300
	6	x-tractor gene UV light	Helix DNA Corbet	400	60	EBV Alert Real-Time, ELITechgroup	ABI Prism 7300
	7	NucliSENS EasyMag	generic 2.0.1	500	50	EBV ELITe MGB Kit, ELITechgroup	ABI Prism 7500
	8	NucliSENS EasyMag	generic 2.0.1	400	60	EBV Alert Real-Time, ELITechgroup	ABI Prism 7300
	9	NucliSENS EasyMag	generic 2.0.1	1000	25	EBV Alert Real-Time, ELITechgroup	ABI Prism 7300
	10	NucliSENS EasyMag	generic 2.0.1	500	55	EBV ELITe MGB Kit, ELITechgroup	ABI Prism 7300
	11	NucliSENS EasyMag	generic 2.0.1	1000	60	EBV Alert Real-Time, ELITechgroup	ABI Prism 7300
	12	NucliSENS EasyMag	specific 2.0	500	100	EBV ELITe MGB Kit, ELITechgroup	ABI Prism 7300
	13	NucliSENS EasyMag	generic 2.0.1	500	55	EBV Alert Real-Time, ELITechgroup	ABI Prism 7300
	14	NucliSENS EasyMag	generic 2.0.1	500	55	EBV Alert Real-Time, ELITechgroup	ABI Prism 7300
	15	Manual extraction	QIAamp blood mini kit	200	100	EBV R-gene Argene-Biomerieux	ABI Prism 7500
BKV	1	QIA Symphony	DSP virus/pathogen (modified)	400	90	BKV Trender Affigene	Stratagene xp3000
	2	NucliSENS EasyMag	generic 2.0.1	250	25	BKV Alert Real-Time, ELITechgroup	ABI Prism 7300
	3	QIA Symphony	DSP virus/pathogen (modified)	1000	110	BKV ELITe MGB KIT, ELITechGroup	ABI Prism 7300
	4	NucliSENS EasyMag	generic 2.0.1	500	55	in house PCR (target large T region)[16]	ABI Prism 7300
	5	NucliSENS EasyMag	generic 2.0.1	1000	100	BKV ELITe MGB KIT, ELITechGroup	ABI Prism 7300
	6	x-tractor gene UV light	Helix DNA Corbet	400	60	BKV ELITe MGB KIT, ELITechGroup	ABI Prism 7300
	7	NucliSENS EasyMag	generic 2.0.1	500	50	BKV ELITe MGB KIT, ELITechGroup	ABI Prism 7500
	8	NucliSENS EasyMag	generic 2.0.1	400	60	Light mix Polyomaviruses JC and BK (TibMolBiol)	Lightcycler 2.0
	9	NucliSENS EasyMag	generic 2.0.1	1000	25	BKV Q-PCR Alert Kit, ELITechGroup	ABI Prism 7300
	10	NucliSENS EasyMag	generic 2.0.1	500	55	BKV ELITe MGB KIT, ELITechGroup	ABI Prism 7300
	11	NucliSENS EasyMag	generic 2.0.1	1000	60	BKV Alert Real-Time, ELITechgroup	ABI Prism 7300
	12	NucliSENS EasyMag	specific 2.0	500	100	BKV ELITe MGB KIT, ELITechGroup	ABI Prism 7300
	13	NucliSENS EasyMag	generic 2.0.1	500	55	BKV ELITe MGB KIT, ELITechGroup	ABI Prism 7300
	14	NucliSENS EasyMag	generic 2.0.1	500	55	BKV ELITe MGB KIT, ELITechGroup	ABI Prism 7300
	15	Manual extraction	QIAamp blood mini kit	200	100	JC primers - probe + BKV R-gene, Argene-Biomerieux	ABI Prism 7500

WB, whole blood

Table 2. Summary of quantitative performance for CMV plasma, CMV WB, EBV and BKV panels.

QCMV 2012 panel	Sample	No. of values	Mean \pm SD (Log copies/ml)	Inter-lab. CV (%)	Median (Log copies/ml)	Range (Log copies/ml)
CMV plasma	CMV12-01	15	4.43 \pm 0.17	3.93	4.43	4.12-4.65
	CMV12-02	15	3.82 \pm 0.29	7.56	3.89	3.29-4.36
	CMV12-03	14	2.19 \pm 0.33	12.65	2.17	1.56-2.53
	CMV12-04	11	2.05 \pm 0.46	19.56	2.00	1.28-2.35
	CMV12-05	15	3.24 \pm 0.48	14.84	3.40	2.04-3.66
	CMV12-06	15	3.43 \pm 0.21	6.19	3.47	2.98-3.80
	CMV12-07	15	3.46 \pm 0.18	5.15	3.50	3.16-3.70
	CMV12-09	15	3.80 \pm 0.21	5.60	3.83	3.29-4.14
	CMV12-10	15	2.74 \pm 0.26	9.30	2.72	2.18-3.14
	CMV WB	CMV12-01	13	3.82 \pm 0.59	15.33	3.78
CMV12-02		13	4.83 \pm 0.57	11.90	4.96	3.89-5.50
CMV12-03		13	3.01 \pm 0.43	13.95	3.09	2.32-3.65
CMV12-04		13	3.85 \pm 0.54	14.06	3.82	3.15-4.51
CMV12-05		13	3.03 \pm 0.41	13.03	3.08	2.38-3.54
CMV12-07		13	4.55 \pm 0.50	10.91	4.44	3.84-5.17
CMV12-08		9	2.63 \pm 0.44	16.57	2.73	1.81-3.05
EBV		EBV12-01	15	2.52 \pm 0.30	11.92	2.56
	EBV12-02	15	3.57 \pm 0.24	6.89	3.59	3.22-4.20
	EBV12-03	15	4.93 \pm 0.25	5.12	4.87	4.59-5.53
	EBV12-04	15	4.55 \pm 0.23	5.17	4.53	4.26-5.12
	EBV12-05	15	4.12 \pm 0.23	5.61	4.11	3.80-4.68
	EBV12-06	15	4.26 \pm 0.21	5.10	4.20	3.98-4.77
	EBV12-07	15	4.58 \pm 0.23	5.19	4.49	4.32-5.21
	EBV12-08	15	3.26 \pm 0.23	7.02	3.23	2.81-3.81
	EBV12-09	15	3.09 \pm 0.26	8.33	3.10	2.52-3.72
BKV	BK12-02	15	3.58 \pm 0.38	10.59	3.64	2.92-4.19
	BK12-03	15	2.43 \pm 0.34	14.54	2.43	1.81-2.90
	BK12-07	15	1.72 \pm 0.48	26.25	1.76	0.90-2.35
	BK12-08	15	4.64 \pm 0.39	8.51	4.66	3.81-5.26
	BK12-12	15	5.14 \pm 0.34	6.58	5.15	4.35-5.56