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Results of cytomegalovirus DNA viral loads expressed in copies per millilitre and international units per millilitre are equivalent

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ABSTRACT

Quantification of Cytomegalovirus (CMV) DNA is required for the initiation and monitoring of anti-viral treatment and the detection of viral resistance. However, due to the lack of standardisation of CMV DNA nucleic acid tests, it is difficult to set universal thresholds. In 2010, the 1st WHO International Standard for Human Cytomegalovirus for Nucleic Acid Amplification Techniques was released. Since then CMV DNA viral load assays have been calibrated using this standard. Three external quality assessment (EQA) providers sent the same five samples to their participants and analysed the results to determine the equivalence of reporting CMV DNA results in international units per millilitre (IU/mL), and compared the difference in results reported in IU/mL with those reported in copies per millilitre (c/mL), and to determine the rate of adoption of IU/mL. About 78% of participants continue to report results in c/mL even though six of the 12 commercial assays are calibrated against the standard. The range of the results reported in IU/mL was less than those reported in c/mL indicating that the adoption of the WHO standard successfully improved the reporting of the CMV viral load. The variation in individual sample results reported by different assays, irrespective of whether in IU/mL or c/mL, is still great and therefore more standardisation of the assays is needed to allow the setting of treatment and monitoring thresholds. This study can act as a bench mark to determine rate of future adoption if reporting CMV DNA viral load results in IU/mL.

1. Background

Human cytomegalovirus (CMV) is a double-stranded DNA virus of the Herpesviridae family (Ramanan and Razonable, 2013; Ross et al., 2011) causing mild or asymptomatic infection in healthy humans and becomes latent systemically in the host (Ross et al., 2011). Transmission occurs through various routes, including person-to-person, vertically from mother to child or through blood or solid organ donation (Ramanan and Razonable, 2013; Razonable and Hayden, 2013). CMV is the most common cause of congenital infection (Ross et al., 2011).

CMV can cause serious infection in the immunocompromised (Ramanan and Razonable, 2013; Razonable and Hayden, 2013; Babady et al., 2015). To reduce the risk of transmission to the immunocompromised, recipient are treated with antiviral drugs. The success of treatment is determined by testing for CMV DNA viral load (VL) using nucleic acid tests (NAT) (Ramanan and Razonable, 2013; Razonable and Hayden, 2013). Accurate CMV DNA VL quantification is critical in management and the prevention of CMV infection (Kraft et al., 2012). In 2010 the 1st WHO International Standard for Human Cytomegalovirus for Nucleic Acid Amplification Techniques (Code 09/162) (NIBSC, Potters Bar, UK) (Freyer et al., 2010) was released. Whilst many commercially manufactured assays that measure CMV DNA VL now report test results in international units per millilitre (IU/mL), the transition from reporting results in copies per millilitre (c/mL) to IU/mL has taken a relatively long period of time.

Assessment of the performance of CMV DNA VL testing can be determined through participation in External Quality Assessment (EQA) schemes (Hayden et al., 2012). One drawback with these schemes is

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Table 1
Summary of results, expressed as log_{10} copies per millilitre (log_{10} c/mL) and International Units per millilitre (log_{10} IU/mL), reported by participants testing four samples positive for human cytomegalovirus DNA, distributed by three external quality assessment (EQA) scheme providers.

Sample	EQAS provider	Units	Count	Mean	Median	SD*	Minimum	Maximum	Range
1	Instand	log_{10} c/mL	54	4.98	4.99	0.40	3.56	6.08	2.52
		log_{10} IU/mL	27	5.07	5.09	0.24	4.34	5.34	1.00
	NRL	log_{10} c/mL	9	4.94	4.94	0.42	4.24	5.93	1.68
		log_{10} IU/mL	3	5.07	4.95	0.34	4.73	5.53	0.80
	UKNEQAS	log_{10} c/mL	84	5.03	5.00	0.46	2.83	6.53	3.70
		log_{10} IU/mL	11	4.94	4.95	0.20	4.62	5.28	0.67
	All Data	log_{10} c/mL	147	5.00	4.99	0.44	2.83	6.53	3.70
		log_{10} IU/mL	41	5.03	5.03	0.24	4.34	5.53	1.19
2	Instand	log_{10} c/mL	53	3.97	3.95	0.42	0.00	5.11	5.11
		log_{10} IU/mL	27	4.14	4.13	0.25	3.44	4.68	1.24
	NRL	log_{10} c/mL	9	4.00	4.00	0.39	3.36	4.85	1.48
		log_{10} IU/mL	3	3.95	3.95	0.11	3.82	4.09	0.27
	UKNEQAS	log_{10} c/mL	84	4.03	4.07	0.41	2.94	5.28	2.34
		log_{10} IU/mL	12	3.84	3.97	0.51	2.34	4.48	2.13
	All Data	log_{10} c/mL	146	4.03	4.01	0.41	2.30	5.28	2.98
		log_{10} IU/mL	42	4.04	4.09	0.37	2.34	4.68	2.34
4	Instand	log_{10} c/mL	54	3.97	3.95	0.40	2.30	4.99	2.69
		log_{10} IU/mL	27	4.10	4.15	0.23	3.55	4.52	0.97
	NRL	log_{10} c/mL	9	4.00	3.99	0.44	3.20	4.95	1.76
		log_{10} IU/mL	3	4.00	4.07	0.13	3.82	4.12	0.30
	UKNEQAS	log_{10} c/mL	84	4.10	4.05	0.42	2.49	5.41	2.92
		log_{10} IU/mL	12	3.95	3.99	0.36	3.08	4.41	1.34
	All Data	log_{10} c/mL	147	4.05	4.03	0.42	2.30	5.41	3.12
		log_{10} IU/mL	42	4.05	4.09	0.28	3.08	4.52	1.44
5	Instand	log_{10} c/mL	54	5.04	5.07	0.49	3.41	6.18	2.76
		log_{10} IU/mL	27	5.15	5.25	0.30	4.00	5.72	1.72
	NRL	log_{10} c/mL	9	5.08	5.06	0.40	4.45	6.00	1.55
		log_{10} IU/mL	3	5.46	5.26	0.36	5.16	5.97	0.81
	UKNEQAS	log_{10} c/mL	84	5.12	5.12	0.46	3.38	6.46	3.08
		log_{10} IU/mL	11	4.90	4.93	0.37	4.15	5.45	1.30
	All Data	log_{10} c/mL	147	5.09	5.11	0.47	3.38	6.46	3.08
		log_{10} IU/mL	41	5.11	5.16	0.36	4.00	5.97	1.97

*SD Standard deviation.

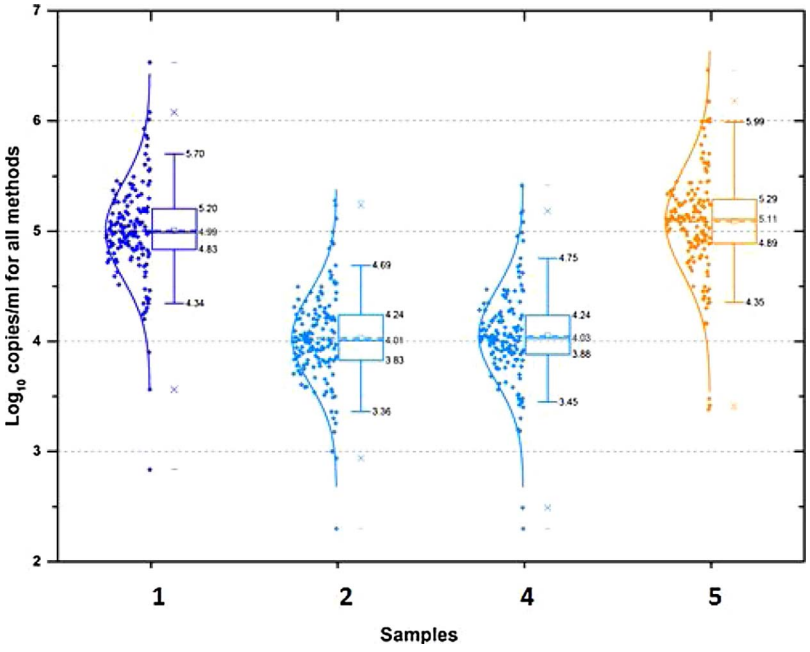

Fig. 1. Accumulated results for quantitative CMV DNA testing for all four CMV DNA positive samples (n = 147 for each sample) from the participants of three EQA providers, INSTAND, NRL and UKNEQAS: results of all applied methods expressed in Log_{10} copies/ml.

that different sample sets are distributed by different scheme providers. Three of scheme providers, INSTAND Gesellschaft zur Foerderung der Qualitaetssicherung in medizinischen Laboratorien e.V. (INSTAND e.V., Duesseldorf, Germany), UK National External Quality Assurance Scheme (UK NEQAS) for Microbiology (London, UK) and the National Serology Reference Laboratory, Australia (NRL, Melbourne, Australia) collaborated by providing the same set of samples to all participants in their respective CMV DNA VL schemes, represents the first and largest global assessment of variation in CMV DNA VL test results reported internationally.

2. Objectives

To compare results of CMV DNA VL assays reporting in IU/mL and