



UK Clinical Virology Network

Performance of in-house real-time PCR assays for the detection of cytomegalovirus

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Summary

Laboratories across the UK are using a range of different in-house assays for the detection of viral targets. A small-scale assessment of nine such assays in use nationwide for the detection for cytomegalovirus (CMV) infections indicated that whilst the majority of these assays perform at an equivalent level of sensitivity, the data collected here suggest that a few have a sensitivity lower than this main group.

Introduction

Laboratories across the UK are using a range of different in-house assays for the detection of viral targets, but no comparative study on their performance has been performed. To address this, the Clinical Virology Network (CVN) set up an assessment of these in-house PCR assays, and in collaboration with the Health Protection Agency Microbial Evaluations Centre (HPA-MEC) produced a panel of specimens to gain some insight into how the different assays compare to enable adoption of best practice.

Materials and Methods

Laboratories were invited via the CVN to submit to the study SOPs of in-house real-time assays in regular diagnostic use. A summary of these is included in Appendix 1.

A panel of 20µl aliquots of extracted DNA generated from both clinical and tissue-culture grown material was prepared at the West of Scotland Specialist Virology Centre (WOSSVC). This incorporated two different extraction methods to avoid any bias associated with this process: Qiagen BioRobot and BioMérieux easyMAG. The panel composition is shown in Table 1. Two nucleic acid extraction methods were used to act as a control for any variation in extract quality caused by sample extraction methods. The negative clinical sample (a serum) was confirmed as such at WOSSVC by triplicate realtime PCR assays, all of which were negative.

Table 1. Specimen panel composition (for CMV)

Sample type	Extraction method	No. of samples	Details
Virus-positive tissue culture fluid	Qiagen BioRobot 9604	9	Extract diluted to limit of originating lab's assay \pm four fivefold dilution steps
Virus-positive tissue culture fluid	BioMérieux easyMAG	9	Extract diluted to limit of originating lab's assay \pm four fivefold dilution steps
Clinical sample (swab)	Qiagen BioRobot 9604	7	Clinical material from six known CMV-positive and one negative specimens
TOTAL		25	

Appropriate dilutions of the extracted material (prepared using nuclease-free water) were established by the WOSSVC using their CMV realtime PCR diagnostic assay SOP. The panel

was distributed on dry ice to the participating laboratories, to be stored at -70°C and tested within 10 working days of receipt.

Results

Nine laboratories submitted SOPs for inclusion in the study; all returned results. Respondents provided information on C_t (cycle at which the signal crosses the threshold signifying positivity), run validation and interpretation of results. One laboratory (UCL) had their panels mistakenly delivered to them at room temperature. Indeed, one sample was missing from the panel delivered to UCL, for reasons unknown.

Table 2 details the number of positive clinical specimen and serial dilution tests recorded by each participating laboratory. Results from Glasgow were obtained by the diagnostic staff of that laboratory, completely independently of the Scientific Co-ordinator.

Table 2. Number of positive specimens from each laboratory

Laboratory	No. of CMV-positive dilutions ($n=18$)	No. of CMV-positive clinical specimens ($n=7$)
Aberdeen	8	5
Edinburgh	6	5
Glasgow	8	6
Liverpool	7	6
Newcastle	8	4
Plymouth	9	5
Southampton	9	5
UCL	6	4
Mean	7.6	5.0

Liverpool, Plymouth and Southampton provided quantitative results; these have not been shown here.

Three laboratories (Plymouth, Newcastle, Southampton) provided duplicate results. Several results submitted by Plymouth showed one positive and one negative result. Of these, Newcastle stated that results were repeated to add data but the first result is the one reported.

A graphical representation of tissue culture sample data—as C_t values—is given in Figure 1. A breakdown of percentages of positive and negative results reported is shown in Figure 2. No result was classified by any laboratory as 'low positive'. Of the clinical sample material, all laboratories confirmed the negative clinical sample. Only Liverpool and Glasgow reported all six positive and one negative result correctly. Newcastle and UCL detected a lower than average number of positive samples.

Of the tissue culture dilution samples, only Newcastle and Southampton detected CMV DNA in sample CMV23 and Glasgow in CMV34 (the lowest dilutions detected by any laboratory), although Newcastle's result was reported as negative. Glasgow and Liverpool SVC did not detect DNA in samples CMV32 and 33; all other laboratories did. Plymouth's results were the most consistent across the dilution series. Aside from the above, performance of all assays was similar with respect to the diluted tissue culture nucleic acid extract.

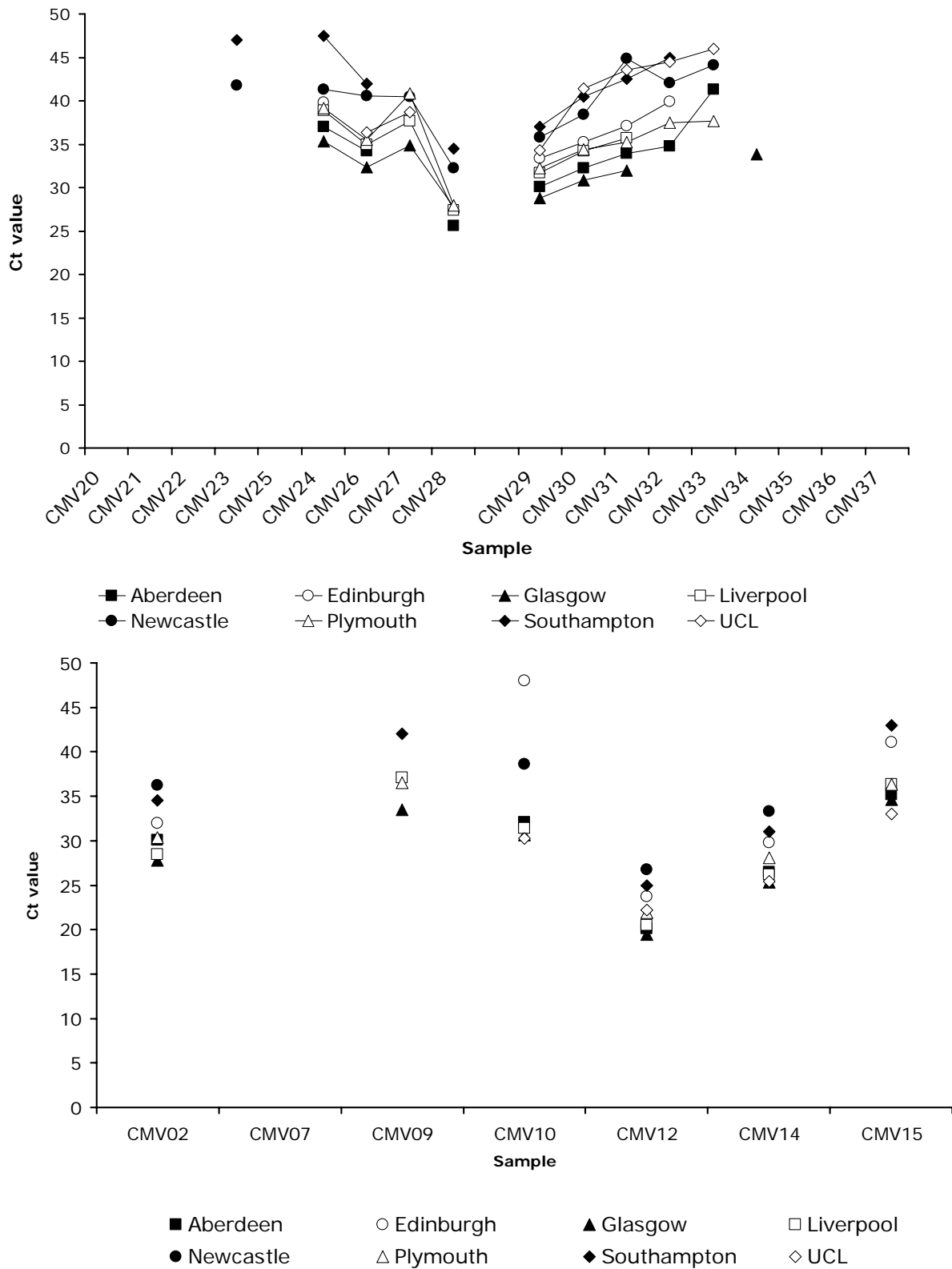


Figure 1. Graphical representation of C_t results from dilutions of nucleic acid extracted from (top) tissue culture-grown virus and (bottom) clinical samples

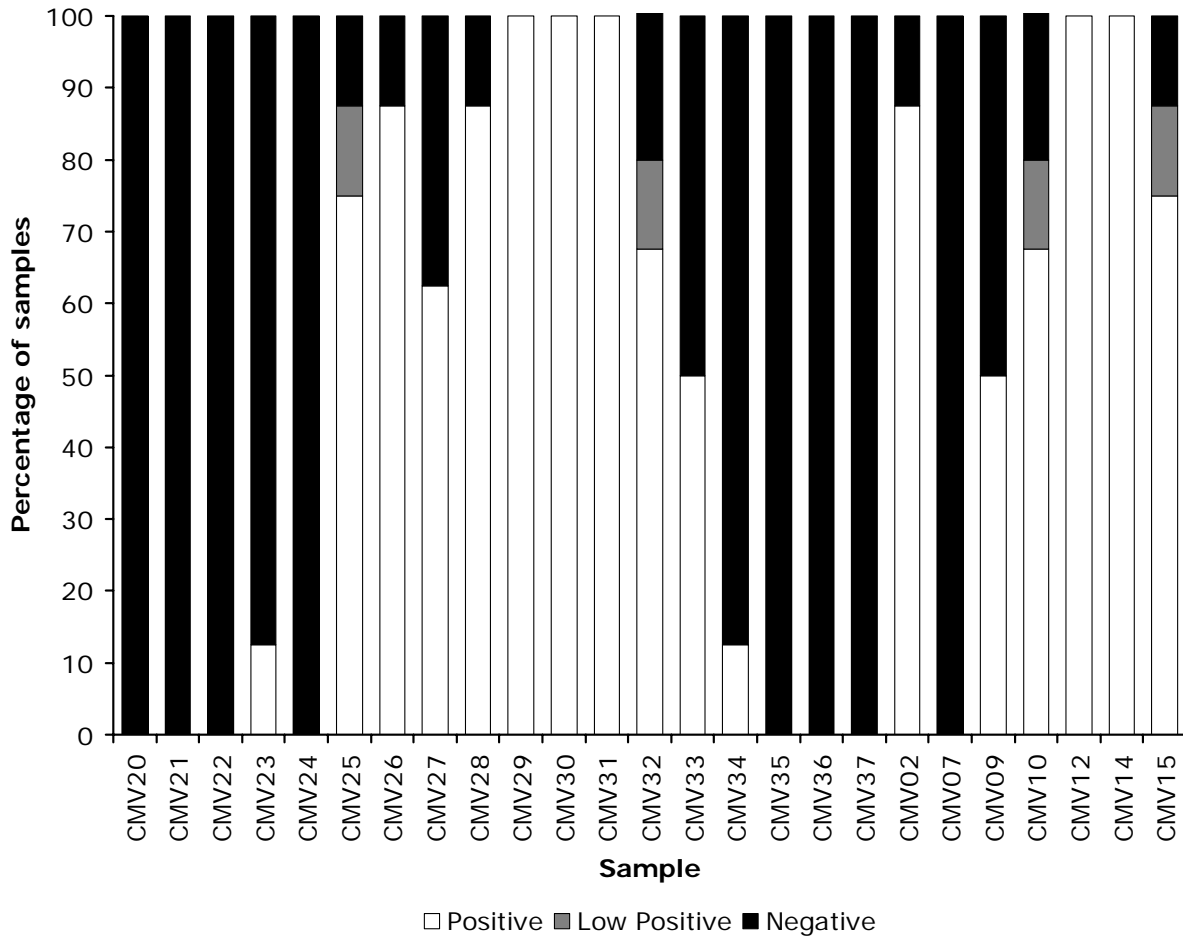


Figure 2. Distribution of final results from the panel specimens expressed as percentages

Conclusion

A range of in-house assays for the detection of CMV are in use across the United Kingdom. (Appendix 1a). The results from the assays were generally comparable on the samples consisting of viral nucleic acid extracted from tissue culture – Newcastle, Southampton and Glasgow detected DNA in a lower dilution than other laboratories.

Marked differences in sensitivity were observed in the clinical sample part of the panel. Here the results generated by Glasgow and Liverpool were best, being the only laboratories to detect all six positive samples. Overall, there was more variation in the clinical sample results submitted by the participating laboratories than would be expected.

Appendix 1

Appendix 1a. Details of SOPs for the detection of CMV submitted by participating laboratories

Source	Reference	Platform	Detection method*	Gene target	Sample volume
Plymouth	Guiver, M., Fox, A.J. <i>et al.</i> (2001). <i>Transplantation</i> 71 1609 - 1615	ABI 7000	DLP	UL55	5µl
Liverpool	Boeck, H., Huang, M. <i>et al.</i> (2004). <i>J Clin Virol</i> 42 1142-1148	Smartcycler	DLP	UL55/UL123	5µl
Aberdeen	Najioullah, F., Thouvenot, D. <i>et al.</i> (2001). <i>J Virol Methods</i> 92 55-64	ABI 7500	DLP	HXFL4	10µl
King's	Kearns, A.M., Guiver, V. <i>et al.</i> (2001). <i>J Virol Methods</i> 95 121-131	Lightcycler	FRET	UL55	5µl
UCL		ABI 7000	DLP		20µl
Glasgow	Najioullah, F., Thouvenot, D. <i>et al.</i> (2001). <i>J Virol Methods</i> 92 55-64	ABI 7500	DLP	HXFL4	10µl
S'thampton	Guiver, M., Fox, A.J. <i>et al.</i> (2001). <i>Transplantation</i> 71 1609 - 1615	ABI 7700/7900	DLP	UL55	5µl
Edinburgh	Guiver, M., Fox, A.J. <i>et al.</i> (2001). <i>Transplantation</i> 71 1609 - 1615	ABI 7500	DLP	UL55	10µl
Newcastle	Kearns, A.M., Guiver, V. <i>et al.</i> (2001). <i>J Virol Methods</i> 95 121-131	Lightcycler	FRET	UL55	5µl

*DLP – dual-labelled probe; FRET – fluorescence resonance energy transfer

Appendix 1b. Panel details and C_t results by laboratory for diluted tissue culture material

Panel No.	Extract method	Dilution	Laboratory*								
			WOS [†]	Abe	Edi	Gla	Liv	New	Ply	Sou	UCL
CMV20	B [‡]	1:10000	neg	neg	neg	neg	neg	neg	neg	neg	neg
CMV21	B	1:5000	neg	neg	neg	neg	neg	neg	neg	neg	neg
CMV22	B	1:1000	neg	neg	neg	neg	neg	neg	neg	neg	neg
CMV23	B	1:500	neg	neg	neg	neg	neg	neg	neg	neg	neg
CMV25	B	1:100	neg	neg	neg	neg	neg	neg	neg	neg	neg
CMV24	B	1:50	35.5	37.0	39.8	35.33	38.9	41.35	39.2	47.5	neg
CMV26	B	1:10	31.35	34.19	neg	32.31	35.0	40.54	35.5	42.0	36.36
CMV27	B	1:5	30.1	neg	neg	34.9	37.7	40.47	40.8	neg	38.73
CMV28	B	1	27.51	25.64	28.6	27.73	27.4	32.22	27.9	34.5	neg
CMV29	Q ^{††}	1	29.18	30.09	33.4	28.74	31.7	35.8	32.2	37.0	34.33
CMV30	Q	1:5	31.08	32.22	35.2	30.85	34.2	38.42	34.4	40.5	41.41
CMV31	Q	1:10	32.44	33.94	37.1	31.97	35.7	44.88	35.25	42.5	43.51
CMV32	Q	1:50	34.2	34.74	39.9	neg	neg	42.07	37.5	45.0	44.44
CMV33	Q	1:100	35.57	41.3	neg	neg	neg	neg	37.7	46.0	neg
CMV34	Q	1:500	neg	neg	neg	33.85	neg	neg	neg	neg	neg
CMV35	Q	1:1000	neg	neg	neg	neg	neg	neg	neg	neg	neg
CMV36	Q	1:5000	neg	neg	neg	neg	neg	neg	neg	neg	neg
CMV37	Q	1:10000	neg	neg	neg	neg	neg	neg	neg	neg	neg

*Abe – Aberdeen; Edi - Edinburgh; Gla – Glasgow; Kin- King's; Liv – Liverpool; New – Newcastle; Ply – Plymouth; Sou – Southampton

[†]original result determined in WOSSVC

[‡]BioMérieux easyMAG

^{††}Qiagen BioRobot 9604

Appendix 1c. Panel details and C_t results by laboratory for clinical samples

Panel No.	Extract method	Laboratory*								
		WOS [†]	Abe	Edi	Gla	Liv	New	Ply	Sou	UCL
CMV02	Q [‡]	28.97	30.05	32.0	27.79	28.5	36.2	30.35	34.5	N/A
CMV07	Q	neg	neg	neg	neg	neg	neg	neg	neg	neg
CMV09	Q	32.23	neg	neg	33.45	37.1	neg	36.5	42.0	neg
CMV10	Q	28.13	32.06	48.0	30.61	31.4	38.61	neg	neg	30.24
CMV12	Q	16.96	20.08	23.7	19.42	20.5	26.78	21.95	25.0	22.17
CMV14	Q	26.04	26.44	29.8	25.36	26.2	33.29	28.05	31.0	25.4
CMV15	Q	30.42	35.19	41.1	34.65	36.3	neg	36.3	43.0	33.04

*Abe – Aberdeen; Edi - Edinburgh; Gla – Glasgow; Kin- King's; Liv – Liverpool; New – Newcastle; Ply – Plymouth; Sou – Southampton

[†]original result determined in WOSSVC

[‡]Qiagen BioRobot 9604