Cytomegalovirus (CMV) detection & quantitation using real-time qPCR (SYBR Green format).

Created: Feb 27, 2019; **Last modified**: Mar 23, 2021, **Version:** 2.0

This protocol describes the real-time quantitative assay for human Cytomegalovirus (CMV) detection and quantitation through a SYBR green based real time assay. CMV is a member of the Herpesviridae family and Betaherpesvirinae subfamily which includes the genera Muromegalovirus and Roseolovirus (HHV-6 and HHV-7) and related to Alphaherpesvirinae subfamily viruses such as herpes simplex viruses (HSV)-1 and -2 and varicella-zoster virus (VZV) as well as to the Gammaherpesvirinae subfamily member, Epstein‑Barr virus (EBV). CMV has a double-stranded DNA (dsDNA) between 230‑240 kbp in size, the largest of the human viruses, encoding for 150 to 192 open reading frames (ORFs) with the potential to encode a protein. CMV does not usually cause disease in healthy individuals but establishes latency or persistency throughout life after primary infection which is reactivated under immunologically suppressed conditions, such as hematopoietic stem cell or solid organ transplantation and HIV infection. In these settings it causes severe, sometimes fatal, diseases. CMV is the major cause of congenital infection occurring in 0.2‑2% of all births. Congenital CMV can lead to neurologic sequelae, including sensorineural hearing loss and developmental delays.

Oligonucleotide primers

Name	Sequence*	Bp	%GC	Tm[b]	Hair	HmD	HtD	Amplicon	Ref
CMVgb-F	5'-ACT-gCA-CgT-ACg-AgC-TgT-Tgg-3'	21	57	60	-0.45	-10.87	-8.25	91 pb	1
CMVgb-R	5'-CCT-TCA-CgT-TCA-TAT-CAC-gCA-g-3'	22	55	56	-1.13	-6.3			

* Reverse oligonucleotide primer sequences given in this table are the reverse-complement of the sequence present in the alignments and as they should be ordered for synthesis. Hairpin (Hair), homodimer (Hm) and heterodimer (Ht) ΔQ's are given in kcal/mol.

qPCR components and conditions

USB® VeriQuest® SYBR® Green qPCR Master Mix (2X) with Fluorescein. Cat. No. 75665 (Affymetrix)

dH_2O	cf	1.7 µL
Master mix	1 x	5 µL
10 µM Forward oligo	100 nM	0.1 µL
10 µM Reverse oligo	200 nM	0.2 µL
Template	10 ng	3 µL
		vf: 10 µl

↓

Run generic program in Applied Biosystems 7500 →

§ Data acquisition

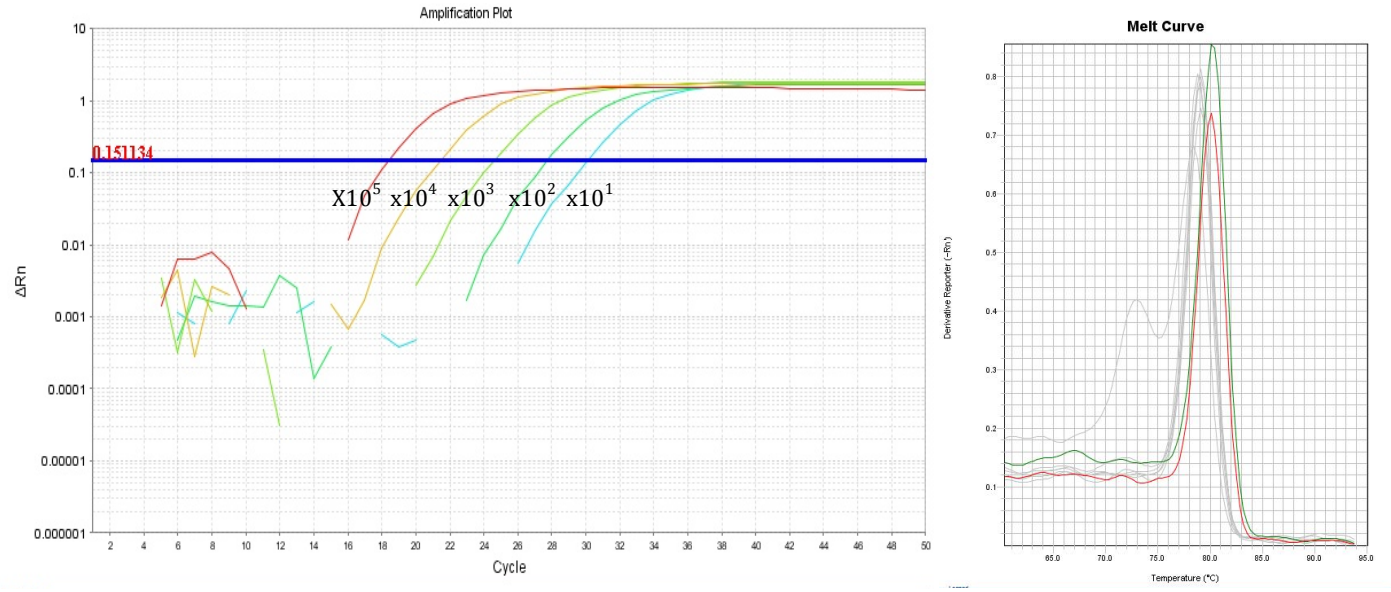
Total time: 2:30 hrs		
95 ºC	3 min	
94 ºC	15 sec	50 cycles
63 ºC §	60 sec	
95 ºC	15 sec	
60 ºC	20 sec	
Ramp §	60 min	
95 ºC	15 sec	

Titration curve preparation for quantitative analysis of viral titres

		Plasmid stock	dH_20 vol	Ct
Working	$2x10^6$ cp/µL	-	-	-
1st	$2x10^5$ cp/µL	10 µL	90 µL	18.39
2nd	$2x10^4$ cp/µL	10 µL	90 µL	21.42
3rd	$2x10^3$ cp/µL	10 µL	90 µL	24.64
4th	$2x10^2$ cp/µL	10 µL	90 µL	27.77
5th	$2x10^1$ cp/µL	10 µL	90 µL	30.12
6th	$2x10^0$ cp/µL	10 µL	90 µL	30.21

Note: Add 90 µL to each of the 6 PCR 0.2 mL tubes. Take 10 µL of initial working stock (at $2x10^6$ cp/µL) and dispense into 1st PCR tube, wash tip 30 times, cap, vortex for 10 seconds and spin down for 10 seconds. Retrieve 10 µL from volumetric centre of PCR tube and dispense into 2nd PCR tube repeating the same procedure for further dilutions.

Performance summary



Standard curve: ***m* = -2.98, Y = 34.3, R^2 = 0.997**
Target amplicon Tm: **79ºC ± 0.2 SD (78.8-79.2 ºC)**
Limit of detection (LODet): **$2x10^0$ cp/µL**
Limit of discrimination (LODis): **$2x10^1$ cp/µL**

Notes

1. Clean workbench with 0.1% NaOCl 0.1% followed by 70% Ethanol before and after work.
2. Preparation of all mastermixes should only be performed in the pre-PCR workbench.
3. Addition of sample DNA should only be performed in the pre-PCR workbench.
4. Addition of positive template DNA should be performed on instrument (post-PCR) area.
5. All mastermixes should be prepared on ice to prevent excess evaporation.
6. Vortex and spin all mastermixes before and after aliquoting to PCR tubes.

References

1. Guiver, Malcolm; Fox, Andrew J.; Mutton, Ken; Mogulkoc, Nesrin; Egan, Jim**.** Evaluation of CMV viral load using TaqMan™ CMV quantitative PCR and comparison with CMV Antigenemia in heart and lung transplant recipients**.** *Transplantation.* 2001 Jun 15;71(11):1609-15.

Revision history

1.0 Original document.

2.0 Changes to document format only.