Confirmatory PCR sequencing of Human betaherpesvirus 5 (cytomegalovirus, HCMV, HHV-5).

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Human betaherpesvirus 5 (HCMV, HHV-5) virus species belongs to the genus *Cytomegalovirus*, *Herpesviridae* family, *Betaherpesvirinae* subfamily. The lifetime risk of acquiring HCMV is close to 100%. HCMV infections often affect the salivary glands, are typically asymptomatic but can be life-threatening for immunocompromised individuals (HIV, organ transplant recipients, and newborns). Congenital infections are the most common cause of congenital infection and a leading cause of neurological and hearing disabilities in childhood including developmental delays, vision problems, microcephaly, seizures, and death. About 10–15% of congenitally infected infants show symptoms at birth. Other clincal features in newborns include jaundice, hepatosplenomegaly, petechiae, and low birth weight. After initial infection, HCMV remains dormant and can reactivate leading to mucoepidermoid carcinoma, prostate, breast, ovarian cancers or glioblastoma. Diagnosis of congenital HCMV relies on the detection of DNA in newborn urine, saliva, or blood within the first 2–3 weeks of life. Post-transplant infections are a major concern after solid organ and hematopoietic stem cell transplant especially when the donor is HCMV+, and the recipient is HCMV-. HCMV infection post-transplant can range from asymptomatic viremia to severe disease including HCMV syndrome (Fever, Malaise, Leukopenia or thrombocytopenia) or tissue-invasive disease leading to colitis, esophagitis, or gastritis causing diarrhea, abdominal pain, or bleeding (GI tract), pneumonitis, dyspnea and hypoxia (Lungs), hepatitis and/or elevated liver enzymes (Liver), retinitis, visual disturbances or blindness (Eyes) and, rarely, encephalitis (CNS). This protocol describes the PCR components and conditions used for the confirmatory amplification and sequencing of HCMV through nested end-point PCR targeting the viral envelope glycoprotein B (gB) gp55 enconding region.

Oligonucleotide primers used

Name	Target	PCR	Sequence	bp	%GC	Tm[b]	Position	Amplicon	Ref
CMV-FO2	gB encoding gp55 region	1	5’-gAA-TTC-gCg-CAT-gAT-CTC-3’	18	50	52	912-929	815	5
CMV-RO2			5’-ggA-AAC-gTg-TCC-gTC-TT-3’	17	53	52	1711-1727		
CMV-FI2		2	5’-gCg-AgT-AAA-gTT-CCA-gTA-3’	18	44	49	968-985	719	4
CMV-RI2			5’-gTT-CTg-gCA-Agg-YA-3’	14	57	49	1675-1687		

NOTE: 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.

Oligonucleotide primer map

>Human Betaherpesvirus 5 envelope glycoprotein B (gB) encoding gene, gp55 region (LY505272.1)
CTTTACCCGCTGCTTGTACGAGTTGAATTCGCGCATGATCTCTTCGAGGTCAAAAACGTTGCTGGAACGCAGCTCTTTCTGCGAGTAAAGTTCCAGT
ACCCTGAAGTCGGTATTTTCCAGCGGGTCGATATCCAGGGCGATCATGCTGTCGACGGTGGAGATACTGCTGAGGTCAATCATGCGTTTGAAGAGGT
AGTCCACGTACTCGTAGGCCGAGTTCCCGGCGATGAAGATCTTGAGACTGGGAAGCTGACATTCCTCAGTGCGGTGGTTGCCCAACAGGATTTCGTT
GTCCTCGCCCAGTTGACCGTACTGCACGTACGAGCTGTTGGCGAAATTAAAGATGACCACGGGTCGTGAGTAGCAGCGTCCTGGCGAATCCTTCACG
TTCATATCACGCAGCACCTTGACGCTGGTTTGGTTGATGGTCACGCAGCTGGCCAGGCCCAAGACATCACCCATGAAACGCGCGGCAATCGGTTTGT
TGTAGATGGCCGAGAGAATGGCTGACGGGTTGATCTTGCTGAGTTCCTTGAAGACCTCTAGGCTGCGCCGTTGATCCACACACCAGGCTTCTGCGAT
TTGCGCCAGCGCCCGGTTGATGTAACCGCGCAATGTGTCATAGGTGAACTGCAGCTGGGCGTAGACCAGATTGTGCACCGATTCCATGCTGGATAAA
TGAGTTGCATTGTTGCCATCTGCACTTCTTTTGGTTCTACTATGAGTAAGATTCAGACTGGAGCGGTTGGCCAAACGTTCGAGTTCCACCAGAGATT
TTTGCTTGATACCTTGCCAGAACACCACCAAACCACCAGTGGTTTCAAACACGGACACGTTTCCATATTTTTCATATG

PCR components

First PCR		1 rx (µl)
dH_2O	C*f*	2.15
10x Buffer PCR	1X	1.25
$MgCl_2$ 50 mM	3.0 mM	0.75
4x dNTPs 10 mM	200 µM	0.25
CMV-FO2 10 µM	800 nM	1
CMV-RO2 10 µM	800 nM	1
Taq 5 UI/µL	0.04 IU/µL	0.1
DNA	-	6
	Vf	12.5
	↓	
	Run CMV Conf 1	

Second (nested) PCR		1 rx (µl)
dH_2O	C*f*	14.8
10x Buffer	1X	2.5
$MgCl_2$ 50 mM	2.0mM	1.0
4x dNTPs 10 mM	200 µM	0.5
CMV-FI2 10 µM	800 nM	2
CMV-RI2 10 µM	800 nM	2
Taq 5 UI/µL	0.04 IU/µL	0.2
1:10 diluted 1st PCR product	-	2.0
	Vf	25.0
	↓	
	Run CMV Conf 2	

PCR conditions

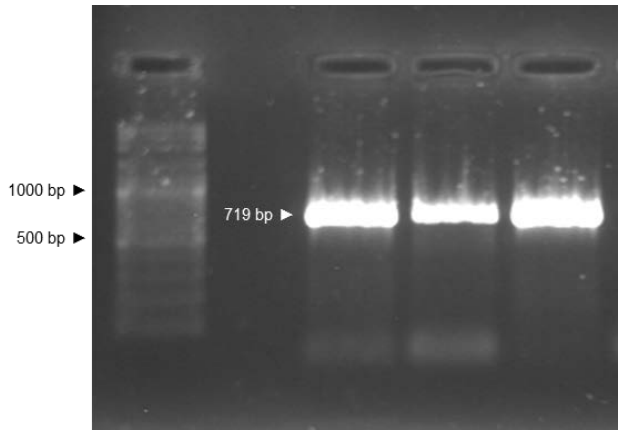
Total time*: 1 h 17 m		Denaturing		Annealing	Extension		
CMV Conf 1	Temperature	94°	94°	62°	72°	72°	4°
	Time	5 min	30 sec	30 sec	30 sec	5 min	5 min
			x30 cycles				

Total time*: 1 h 17 m		Denaturing		Annealing	Extension		
CMV Conf 2	Temperature	94°	94°	54°	72°	72°	4°
	Time	5 min	30 sec	30 sec	30 sec	5 min	5 min
			x30 cycles				

* Times shown correspond to MinAmp Plus thermal cyclers

Electrophoresis conditions

Add 5 µL of orange loading buffer to 10 uL of the PCR product and load these 15 uL into a 1.5% agarose gel with at least 1.5 inch comb separation for 55 minutes at 130 VDC (5.2 V/cm).



Notes

1. Clean work area with 70% Ethanol before and after preparing PCR master mix.

2. Prepare all PCRs in ice or cold-pack microtube racks to lower static evaporative rate and volume losses.

3. Prepare 1st and 2nd PCR mastermix in PCR enclosures. After 1st PCR open tubes in post-PCR area **ONLY** and add 125 µL of dH_2O to dilute PCR product 1:10 for use in 2^{nd} (nested) PCR.

References

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Revision history

1. Original document.
2. Version 2 incorporates synthesis legend below oligonucleotide table.
3. Version 3, corrected oligo concentrations and volumes, modified cycling times.