Test Definition: QHV6C

Human Herpesvirus-6 A and B DNA Detection and Quantification, PCR, Spinal Fluid

Overview

Useful For

As an adjunct in the rapid diagnosis of human herpesvirus-6 infection using cerebrospinal fluid specimens

This test **should not be used** to screen asymptomatic patients.

Method Name

Real-Time Polymerase Chain Reaction (PCR)

NY State Available

Yes

Specimen

Specimen Type

CSF

Specimen Required

Container/Tube: Sterile vial

Preferred: Aliquot tube

Specimen Volume: 0.5 mL

Collection Instructions:

1. Collect specimen from collection vial 2.
2. **Do not centrifuge.**

Forms

If not ordering electronically, complete, print, and send an Microbiology Test Request (T244) with the specimen.

Specimen Minimum Volume

0.3 mL

Reject Due To

All specimens will be evaluated at Mayo Clinic Laboratories for test suitability.

Specimen Stability Information

Specimen Type	Temperature	Time	Special Container
CSF	Refrigerated (preferred)	7 days	
	Ambient	24 hours	

	Frozen	7 days	

Clinical & Interpretive

Clinical Information

Human herpesvirus-6 (HHV-6) is a member of the Herpesviridae family. These DNA viruses contain a capsid surrounded by a lipid envelope. Among members of this group, this virus is most closely related to cytomegalovirus (CMV) and HHV-7. As with other members of the herpesvirus group (herpes simplex virus [HSV]-1, HSV-2, varicella-zoster virus, CMV, Epstein-Barr virus, HHV-7, HHV-8), HHV-6 may cause primary and reactivated infections.(1) Infection with HHV-6 occurs early in childhood. Most adults (80%-90%) have been infected with this virus.

Human herpesvirus-6 was first linked with exanthem subitum (roseola infantum) in 1998; since then, the virus has been associated with central nervous system disease almost exclusively in patients who are immunocompromised.(1) HHV-6 is commonly detected in patients post transplantation. Clinical symptoms associated with this viral infection include febrile illness, pneumonitis, hepatitis, and encephalitis. However, most HHV-6 infections are asymptomatic.(2)

Human herpesvirus-6 is designated as variant A (HHV-6A) or variant B (HHV-6B) depending on restriction enzyme digestion patterns and its reaction with monoclonal antibodies. Generally, variant B has been associated with exanthem subitum, whereas variant A has been found in many immunosuppressed patients.(3) Infection with HHV-6 is very common, approaching 100% seroprevalence in developed countries.(4) In about 1% of the population, HHV-6 can integrate into the host genome. Often asymptomatic in immunocompetent hosts, reactivation can cause serious disease in immunocompromised individuals, particularly those with AIDS and transplant recipients, which can cause rejection of the transplanted organ and even death.(1)

This assay will be used to assist with diagnosis and monitoring of HHV-6 disease in patients who are suspected of having disease due to HHV-6 infection. It will also be used as an initial indicator of infection versus chromosomally-integrated HHV-6.

Reference Values

Undetected

Interpretation

The quantification range of this assay is 500 to 5,000,000 copies/mL (2.70 log to 6.70 log copies/mL)

An "Undetected" test result indicates the absence of human herpesvirus-6 (HHV-6) DNA in cerebrospinal fluid (CSF).

A test result of "<500 copies/mL (<2.70 log copies/mL)" indicates that HHV-6 DNA is detected in the CSF, but the assay cannot accurately quantify the level of HHV-6 DNA.

A test result of ">5,000,000 copies/mL (>6.70 log copies/mL)" indicates that the HHV-6 DNA level present in CSF is above 5,000,000 copies/mL (6.70 log copies/mL), and the assay cannot accurately quantify the level of HHV-6 DNA. A viral load above 5,000,000 copies/mL should raise suspicion for chromosomally-integrated HHV-6 (ci-HHV-6), and additional testing to rule out ci-HHV-6 may be needed.

A test result that indicates the presence of both HHV-6A and HHV-6B DNA in the CSF specimen will be reported as "See Comment Below" for both subtypes with the following comment: "HHV-6 DNA is detected. Unable to assess subtype (HHV-6A and/or HHV-6B). Unable to quantify viral load." This comment indicates the inability to discriminate between subtypes (HHV6A and/or HHV6B) nor provide a viral load due to cross reactivity between HHV subtypes (see Supportive Data, Analytical Specificity).

An "Inconclusive" result indicates that the presence or absence of HHV-6 DNA in the CSF specimen could not be determined with certainty after repeat testing in the laboratory, possibly due to inhibition or the presence of an interfering substance. If clinically indicated, submission of a new specimen for testing is recommended.

Cautions

The sensitivity of the assay is dependent upon the quality of the specimen submitted.

A negative result does not exclude human herpesvirus-6 virus (HHV-6) infection. Therefore, the results obtained should be used in conjunction with clinical findings to make an accurate diagnosis.

This assay detects nucleic acid and, therefore, cannot distinguish between replicating and nonreplicating virus (ie, remnant viral nucleic acid). Test performance depends on the viral load in the specimen and may not correlate with cell culture performed on the same specimen.

Although this assay may detect dual infections of HHV-6A and HHV-6B, low level infections may be masked when in the presence of a higher concentration of an HHV-6 subtype.

Viral loads may vary between laboratory-developed assays. When monitoring a patient’s HHV-6 viral load, the same method and sample type should be used.

This test cannot be converted to IU/mL. It is recommended to utilize the same test for the duration of the clinical care for consistency in reporting units (copies/mL).

Supportive Data

Analytical Sensitivity/Limit of Detection:
Spiked specimens in cerebrospinal fluid (CSF) with human herpesvirus-6 (HHV-6) DNA subtypes A/B (40 for each subtype) for a total of 80 positive specimens were used for establishment of the limit of detection (LOD). The lower LOD of this assay was determined to be 500 copies/mL for HHV-6A and 250 copies/mL for HHV-6B.

Analytical Specificity:
DNA from a panel of 10 organisms from culture collections along with well characterized laboratory strains that cause similar disease or organisms commonly found in CSF were tested to determine if there is any cross-reactivity against the Altona RealStar HHV-6 PCR (polymerase chain reaction) Kit 1.0. No cross-reactivity was observed with the specificity panel.

Analytical specificity was determined by the kit manufacturer (Altona Diagnostics) and did not exhibit cross-reactivity with any of the following targets: BK virus, cytomegalovirus, Epstein-Barr virus, hepatitis A virus, hepatitis B virus,

hepatitis C virus, herpes simplex virus 1, herpes simplex virus 2, human herpesvirus 7, human herpesvirus 8, human parvovirus B19, JC virus, varicella-zoster virus.

Due to new sequence data, cross reactivity of the HHV6-B detection systems (Cy5/Cy5.5) with some strains of HHV-6A cannot be ruled out. A signal in the HHV-6A detection channel (FAM) and HHV-6B (Cy5/Cy5.5) can be observed. This is a known limitation of the assay and will be incorporated in analysis and reporting processes.

Reference Range:
A total of 20 CSF samples collected for noninfectious disease testing (eg, CSF chemistry) were analyzed by the Altona RealStar HHV-6 PCR assay. Of these, 19 were negative. One sample did have a low level of HHV-6 DNA. Given that HHV-6 undergoes latency following primary infection, it is possible to detect viral DNA that may not be associated with active infection or disease. Also, in approximately 1% of patients, HHV-6 may become chromosomally integrated, yielding a positive result by molecular tests.

Although the reference range is typically "negative" for this assay, this assay may detect viral DNA in asymptomatic individuals. However, this assay is only to be used for patients with a clinical history and symptoms consistent with HHV-6 infection and must be interpreted in the context of the clinical picture. This test should not be used to screen asymptomatic patients.

Reportable Range:
The reportable range for this assay is 500 copies/mL to 5,000,000 copies/mL.

Clinical Reference

1. Agut H, Bonnafous P, Gautheret-Dejean A. Laboratory and clinical aspects of human herpesvirus 6 infections. Clin Microbiol Rev. 2015;28(2):313-335
2. De Bolle L, Naesens L, De Clercq E. Update on human herpesvirus 6 biology, clinical features, and therapy. Clin Microbiol Rev. 2005;18(1):217-245
3. Dockrell DH, Paya CV. Human herpesvirus-6 and -7 in transplantation. Rev Med Virol. 2001;11(1):23-36
4. Campadelli-Fiume G, Mirandola P, Menotti L. Human herpesvirus 6: An emerging pathogen. Emerg Infect Dis. 1999;5(3):353-366. doi:10.3201/eid0503.990306
5. Abdel-Haq NM, Asmar BI. Human herpesvirus 6 (HHV6) infection. Indian J Pediatr. 2004;71(1):89-96
6. Dockrell DH, Smith TF, Paya CV. Human herpesvirus 6. Mayo Clin Proc. 1999;74(2):163-170
7. Pellett Madan R, Hand J; AST Infectious Diseases Community of Practice. Human herpesvirus 6, 7, and 8 in solid organ transplantation: Guidelines from the American Society of Transplantation Infectious Diseases Community of Practice. Clin Transplant. 2019;33(9):e13518. doi:10.1111/ctr.13518
8. Pawlowska AB, Karras NA, Liu H, et al. Reactivation of human herpesvirus 6 in pediatric allogeneic hematopoietic stem cell transplant recipients. Transpl Infect Dis. 2021;23(1):e13453. doi:10.1111/tid.13453
9. Wang H, Tomatis-Souverbielle C, Everhart K, Oyeniran SJ, Leber AL. Detection of human herpesvirus 6 in pediatric CSF samples: causing disease or incidental distraction?. Diagn Microbiol Infect Dis. 2023;107(2):116029. doi:10.1016/j.diagmicrobio.2023.116029
10. Yip CCY, Sridhar S, Cheng AKW, et al. Comparative evaluation of a laboratory developed real-time PCR assay and the RealStar HHV-6 PCR Kit for quantitative detection of human herpesvirus 6. J Virol Methods. 2017;246:112-116. doi:10.1016/j.jviromet.2017.05.001

Performance

Method Description

The RealStar HHV-6 (human herpesvirus-6 virus) PCR (polymerase chain reaction) Kit 1.0 (Altona Diagnostics) and the Exact Diagnostics HHV6-A/B Verification Panels and controls (Bio-Rad) are utilized for this assay. The assay employs TaqMan hydrolysis probe chemistry, with production of visible amplification curves and associated crossing point (Cp) values; no melting temperature curve is produced. A probe specific for HHV-6A DNA is labeled with the fluorophore FAM and is detected on the 465-510 channel. A probe specific for HHV-6B DNA is labeled with the fluorophore Cy5 and is detected on the 533-580 channel. The probe specific for the Internal Control (IC) is labeled with the fluorophore JOE, is detected on the 618-660 channel, and will be used within the mastermix. Using probes linked to distinguishable dyes enables the parallel detection and differentiation of HHV-6A and HHV-6B DNA as well as the detection of the IC in corresponding detector channels of the LC480 II instrument. The MagNA Pure 96 instrument (Roche Diagnostics) using the MP96 DNA and Viral NA Small Volume Kit with an input of 200 mcL and a final elution volume of 100 mcL is utilized for viral nucleic acid extraction. The Pathogen Universal 200 protocol is used.(Package insert: RealStar HHV-6 Virus PCR Kit 1.0. Altona Diagnostics; 09/2018)

PDF Report

No

Day(s) Performed

Monday through Friday

Report Available

4 to 6 days

Specimen Retention Time

7 days

Performing Laboratory Location

Mayo Clinic Laboratories - Rochester Main Campus

Fees & Codes

Fees

- Authorized users can sign in to Test Prices for detailed fee information.
- Clients without access to Test Prices can contact Customer Service 24 hours a day, seven days a week.
- Prospective clients should contact their account representative. For assistance, contact Customer Service.

Test Classification

This test was developed and its performance characteristics determined by Mayo Clinic in a manner consistent with CLIA requirements. It has not been cleared or approved by the US Food and Drug Administration.

Test Definition: QHV6C

Human Herpesvirus-6 A and B DNA Detection and Quantification, PCR, Spinal Fluid

CPT Code Information

87533

LOINC® Information

Test ID	Test Order Name	Order LOINC® Value
QHV6C	HHV-6 A and B DNA Quant PCR, CSF	49388-2

Result ID	Test Result Name	Result LOINC® Value
622171	HHV6 A DNA Detect/Quant, CSF	49388-2
622172	HHV6 B DNA Detect/Quant, CSF	49388-2