Overview

Useful For
Detecting variants in the cytomegalovirus genes, *UL97* and *UL54*, which are associated with antiviral resistance

This test is **not useful for** the initial diagnosis of CMV infection

Genetics Test Information
This test uses next-generation sequencing to assess whether variants that are associated with antiviral resistance are present in the genes *UL97* and *UL54*.

Testing Algorithm
This test may be performed following quantitative nucleic acid amplification test results that suggest an increase, or stabilization, of cytomegalovirus (CMV) viral load while the patient is on appropriate antiviral therapy.

Method Name
Next-Generation Sequencing (NGS)

NY State Available
Yes

Specimen

Specimen Type
Plasma EDTA

Ordering Guidance
This test is not intended for the initial diagnosis of cytomegalovirus (CMV) infection. If a diagnostic test is needed, order CMVQN / Cytomegalovirus (CMV) DNA Detection and Quantification by Real-Time PCR, Plasma.

Next-generation sequencing testing may be indicated if a patient with known CMV infection has a rising or stabilizing viral load while on antiviral therapy. This test can assist in determining whether CMV is present that has acquired variants associated with antiviral resistance and should only be performed on patients who have had a recent (ie, within the previous 7 days) CMV quantitative plasma viral load of 500 IU/mL or more.

Testing for CMV resistance should be performed no more frequently than once every 4 weeks.

Changes in CMV viral load greater than 0.5 log are considered significant and may prompt testing for antiviral resistance if the patient is on appropriate therapy.
Additional Testing Requirements
Plasma submitted for next-generation sequencing (NGS) testing must have been collected within 7 days of a viral load assay (CMVQN / Cytomegalovirus [CMV] DNA Detection and Quantification by Real-Time PCR, Plasma or similar test) with a result of 500 IU/mL or more.

Shipping Instructions
1. Freeze plasma specimen immediately, and ship specimen frozen on dry ice.
2. If shipment will be delayed for more than 14 days, freeze plasma specimen at -20 to -80 degrees C (up to 30 days) until shipment on dry ice.

Specimen Required
Supplies: Sarstedt 5 mL Aliquot Tube (T914)
Collection Container/Tube: Lavender top (EDTA)
Submission Container/Tube: Plastic vial
Specimen Volume: 1.2 mL
Collection Instructions: Centrifuge and aliquot plasma.
Additional Information: Plasma submitted for next-generation sequencing testing must have been collected within 7 days of a viral load assay (ie, CMVQN) with a result of > or = 500 IU/mL

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

Specimen Minimum Volume
1 mL

Reject Due To

<table>
<thead>
<tr>
<th>Gross hemolysis</th>
<th>OK</th>
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</thead>
<tbody>
<tr>
<td>Gross lipemia</td>
<td>OK</td>
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Specimen Stability Information

<table>
<thead>
<tr>
<th>Specimen Type</th>
<th>Temperature</th>
<th>Time</th>
<th>Special Container</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma EDTA</td>
<td>Frozen (preferred)</td>
<td>30 days</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Refrigerated</td>
<td>14 days</td>
<td></td>
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</table>

Clinical & Interpretive

Clinical Information
Cytomegalovirus (CMV) is a DNA virus with a seroprevalence of approximately 50% in the United States. Acute infection can be asymptomatic or cause a mononucleosis-like illness in immunocompetent individuals. After acute infection, the virus enters a latent state. Reactivation of the virus can occur, particularly if a patient becomes immunosuppressed. Immunosuppressed patients are also at higher risk of severe acute infection. CMV disease may range from congenital
Test Definition: CMVNG
Cytomegalovirus (CMV) Drug Resistance, Next-Generation Sequencing, Plasma

disease, retinitis, inflammation of the gastrointestinal tract, encephalitis, and pneumonia.

Treatment for CMV typically involves antiviral drugs as well as decreasing the degree of immunosuppression (if applicable and medically advisable). Currently, anti-CMV drugs bind and inhibit either a viral kinase (UL97 gene product) or a viral DNA polymerase (UL54 gene product).

There are a number of assays available to test for the presence of CMV. Quantitative assays report the concentration of CMV DNA present and can be used to monitor the viral load in patients who are at risk of CMV disease as well as assess response to antiviral therapy. A rising CMV viral load (ie, increase of 0.5 log or more between samples) correlates with an increased risk of CMV disease and may indicate treatment failure (ie, due to antiviral resistance) if the patient is on appropriate therapy.

Variants in UL97 and UL54 have been associated with antiviral resistance. This test uses next-generation sequencing (NGS) to analyze the sequence of UL97 and UL54. Identified variants are reported if they have been previously associated with CMV antiviral resistance and are present in at least 15% of the sequences analyzed. This assay uses a database of known resistance-associated variants that is periodically updated with new variants that are reported in the scientific literature.

Reference Values
None Detected/Not Predicted

Interpretation
If a resistance-associated variant is detected, a patient's antiviral regimen may need to be adjusted for optimal response.

If no resistance-associated variants are detected, it is still important to assess the patient's clinical response and quantitative viral load before determining that the infecting virus is susceptible to a treatment regimen (see Cautions).

Predicted drug resistance is reported separately for each antiviral drug.

Predicted resistance to one antiviral may or may not be associated with predicted cross-resistance to other drugs.

Cautions
This assay is designed to detect resistance-associated variants in the genes UL97 and UL54. Only variants that have been reported in the literature to confer antiviral resistance and included in the sequence analysis will be reported. Unrecognized resistance variants may exist, which are not reported in this assay. Furthermore, previously reported resistance-associated variants may not result in a resistant phenotype in all cases.

Supportive Data
Accuracy:
The accuracy of the cytomegalovirus (CMV) drug resistance by next-generation sequencing (NGS) assay was determined by testing a combination of clinical and spiked plasma samples. Clinical samples were compared to the results of Sanger sequencing performed at another large reference laboratory. Spiked samples were compared to the expected results. Among 28 clinical plasma samples tested by Sanger sequencing and the NGS assay, an overall agreement of 96.4% (27/28) was observed at the level of determining overall drug resistance (Table 1).
Table 1. Comparison of NGS and Sanger sequencing for determination of antiviral drug resistance in clinical plasma samples (n=28).

<table>
<thead>
<tr>
<th>Number of interpretations by Sanger sequencing predicting</th>
<th>Susceptible</th>
<th>Ganciclovir resistance</th>
<th>Cidofovir resistance</th>
<th>Foscarnet resistance</th>
</tr>
</thead>
<tbody>
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<td>Susceptible</td>
<td>11</td>
<td>0</td>
<td>0</td>
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<tr>
<td>Ganciclovir resistance</td>
<td>0</td>
<td>16</td>
<td>0</td>
<td>1(*)</td>
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<tr>
<td>Cidofovir resistance</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
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<tr>
<td>Foscarnet resistance</td>
<td>0</td>
<td>0</td>
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<td>2</td>
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</table>

(*) This sample showed an A692S allelic variant (which confers Foscarnet resistance) in UL54 by Sanger sequencing, but M460I and M460V allelic variants (which confer Ganciclovir resistance) in UL97 were detected by NGS.

The data were analyzed at the level of individual resistance-associated variants, and showed the following results:

Table 2.

<table>
<thead>
<tr>
<th>Number of variants detected by Sanger Sequencing</th>
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<tbody>
<tr>
<td>UL97</td>
</tr>
<tr>
<td>DEL 598-603</td>
</tr>
<tr>
<td>A594V</td>
</tr>
<tr>
<td>C603W</td>
</tr>
<tr>
<td>C607Y</td>
</tr>
<tr>
<td>H520Q</td>
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<tr>
<td>L595S</td>
</tr>
<tr>
<td>L595W</td>
</tr>
<tr>
<td>M460V</td>
</tr>
<tr>
<td>M460I</td>
</tr>
<tr>
<td>A692S</td>
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<tr>
<td>A987G</td>
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<tr>
<td>UL54</td>
</tr>
<tr>
<td>A692S</td>
</tr>
<tr>
<td>A987G</td>
</tr>
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</table>

Not detected TNP
(a) This specimen showed a C607Y variant (15.7% prevalence) in UL97 by NGS; however, this variant was not detected during initial testing by Sanger sequencing. Sanger sequencing utilizes a prevalence threshold of 20%. In contrast, NGS analysis utilizes a lower prevalence threshold of 15%. The predicted resistance of this specimen was the same by both assays.

(b) This specimen showed a M460V variant (19.3% prevalence) in UL97 by NGS; however, this variant was not detected during initial testing by Sanger sequencing. Sanger sequencing utilizes a prevalence threshold of 20%. In contrast, NGS analysis utilizes a lower prevalence threshold of 15%. The predicted resistance of this specimen was the same by both assays.

(c) This specimen showed an A692S variant in UL54 (conferring resistance to Foscarnet) by Sanger; however, no UL54 variants were detected by NGS. The NGS assay detected two variants in UL97 (M460V, M460I), but Sanger did not generate a result for UL97 due to poor sequence. Quantity not sufficient for testing by a third method.

(d) This specimen showed a C607Y variant in UL97 by Sanger sequencing. This variant was detected by NGS, but at a prevalence of 9.02%. The predicted resistance of these specimens was the same by both assays.

To supplement the number of positive samples achieved by clinical plasma samples, spiking studies were performed. Sixteen analyte-negative plasma samples were spiked with a plasmid containing variants known to confer resistance and were then analyzed by the NGS assay. The NGS assay exhibited an overall percent agreement of 87.5% (14/16) with the expected result (at the variant level) upon initial testing. After discordant analysis, the NGS assay showed an overall agreement of 100% (16/16).

Limit of Detection:
The limit of detection for this assay was established as 500 IU/mL.

Analytical Specificity:
Specificity was determined using a panel of viruses commonly found in blood along with CMV-positive plasma samples that did not contain resistance variants. In addition, basic local alignment search tool (BLAST) analysis for the UL97 and UL54 primer sequences was performed. The UL97 and UL54 primers were specific for specimens that contained CMV nucleic acid. No variants were detected in wild-type CMV plasma samples. No cross-reacting organisms were identified by BLAST analysis of the primer sequences.

Clinical Reference
Test Definition: CMVNG
Cytomegalovirus (CMV) Drug Resistance, Next-Generation Sequencing, Plasma

2018 Aug;23(4):388-394

Performance

Method Description
This test involves amplification of cytomegalovirus (CMV) UL97 and UL54 genes by polymerase chain reaction (PCR), followed by next-generation sequencing (NGS) of the amplified PCR products using the MiSeq (Illumina). Sequencing results are compared to a reference database of gene variants previously reported to be associated with antiviral drug resistance. Only the UL97 and UL54 genes are sequenced. The use of NGS allows this assay to detect resistance-associated variants even if they are present in a subset of the sequences analyzed. Variants are reported if they are present in 15% or greater of the sequence reads analyzed.(Unpublished Mayo method)

PDF Report
No

Day(s) Performed
Monday through Friday

Report Available
10 to 14 days

Specimen Retention Time
14 days

Performing Laboratory Location
Rochester

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 Regional Manager. 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. This test has not been cleared or approved by the US Food and Drug Administration.
**Test Definition: CMVNG**
Cytomegalovirus (CMV) Drug Resistance, Next-Generation Sequencing, Plasma

### CPT Code Information
87910

### LOINC® Information

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<td>CMVNG</td>
<td>CMV Antiviral Resistance by NGS, P</td>
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<table>
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<td>CMV UL97 mutations</td>
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<td>604561</td>
<td>CMV UL54 mutations</td>
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<td>604562</td>
<td>Ganciclovir Resistance</td>
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<td>604563</td>
<td>Cidofovir Resistance</td>
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<td>604564</td>
<td>Foscarnet Resistance</td>
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<td>CMVQ1</td>
<td>CMV Quant &gt;= 500 IU/mL last 7 days?</td>
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