Overview

Useful For

Detection and identification of codon substitutions in the hepatitis C virus (HCV) NS3, NS5A, and NS5B genomic regions that confer resistance to current direct-acting antiviral (DAA) drugs used for treatment of chronic hepatitis C infection due to HCV genotype 1a, 1b, or 3 (any subtype)

Guiding initiation or change of antiviral drug combinations for the treatment of chronic HCV infection

This assay should **not** be used as a screening test for HCV infection.

This test should **not** be ordered for HCV infection due to genotype 2, 4, 5, or 6.

Highlights

This assay uses next-generation sequencing to detect and identify hepatitis C virus (HCV) antiviral drug resistance in patients with chronic hepatitis C and those being considered for direct-acting antiviral (DAA) drug combination therapy.

This test can be used to predict the likelihood of a curative response to current FDA-approved DAA drug combinations used for treatment of chronic hepatitis C.

Testing Algorithm

See Chronic Hepatitis C Treatment and Monitoring Algorithm: Direct Antiviral Agent (DAA) Combination in Special Instructions.

Special Instructions

- Chronic Hepatitis C Treatment and Monitoring Algorithm: Direct Antiviral Agent (DAA) Combination

Method Name

Polymerase Chain Reaction followed by Next-Generation Sequencing (sequencing by synthesis method)

NY State Available

No

Specimen

Specimen Type

Serum SST

Advisory Information

This test is intended for detection of preexisting antiviral drug resistance-associated substitutions (RAS) in individuals known to be infected with hepatitis C virus (HCV) genotype 1a, 1b, or 3 (any subtype) and being considered for HCV NS3, NS5A, and NS5B inhibitor combination therapy.

Additional Testing Requirements

Prior to requesting this test, patients must have a confirmed serum or plasma hepatitis C virus (HCV) RNA level of 50,000 IU/mL or higher within the preceding 30 days and a known HCV genotype result of 1a, 1b, or 3 (any subtype). The following tests are available to provide these prerequisite results:

-HCVQG / Hepatitis C Virus RNA Quantification with Reflex to HCV Genotype, Serum

-HCVQN / Hepatitis C Virus (HCV) RNA Detection and Quantification by Real-Time Reverse Transcription-PCR (RT-PCR), Serum

-HCVG / Hepatitis C Virus Genotype, Serum

Shipping Instructions

1. Freeze aliquoted serum immediately, and ship specimen frozen on dry ice only.

2. If shipment will be delayed for more 24 hours, freeze serum at -20 to -80 degrees C (for up to 60 days) prior to shipment on dry ice.

Necessary Information

The following 2 ask-at-order entry questions must be answered at the time of test ordering:

1. Does the patient have a hepatitis C quantification test result (viral load) within the last 30 days that is greater than 50,000 IU/mL? Answer "Yes" or "No".

2. Does the patient have a known hepatitis C genotype of 1a, 1b, or 3 (any subtype)? Answer "Yes" or "No".

Note: Test orders for submitted specimens with a "No" answer to either ask-at-order entry question above will be canceled.

Specimen Required

Supplies: Aliquot Tube, 5 mL (T465)

Collection Container/Tube: Serum gel

Submission Container/Tube: Polypropylene vial

Specimen Volume: 2.5 mL

Collection Instructions:

1. Centrifuge blood collection tube per collection tube manufacturer's instructions (eg, centrifuge within 2 hours of collection for BD Vacutainer tubes).

2. Transfer serum into aliquot tube.

Specimen Minimum Volume

0.8 mL

Reject Due To

Hemolysis	Mild OK; Gross OK
Lipemia	Mild OK; Gross OK
Icterus	Mild OK; Gross OK
Other	Red-top tube or tube with any anticoagulant

Specimen Stability Information

Specimen Type	Temperature	Time
Serum SST	Frozen (preferred)	60 days
	Refrigerated	7 days

Clinical and Interpretive

Clinical Information

Interferon-free, direct-acting antiviral (DAA) drug combination therapy is now a standard of care for patients with chronic hepatitis C virus (HCV) infection. However, poor compliance with therapy and the existence of pretreatment antiviral drug resistance may compromise efficacy of such drug therapy. Naturally occurring (preexisting) or treatment-induced mutations in the viral genomic sequences that are targets of such antiviral agents can lead to antiviral resistance and therapeutic failure. Clinical trials and postmarketing studies of DAA therapy indicated that preexisting, resistance-associated substitutions (RAS) in the relevant HCV genomic regions of certain genotypes or emergence of certain RAS during DAA therapy can lead to treatment failure. Per current recommendations from the FDA and professional society practice guidelines (see table below and Clinical Reference section), use of certain FDA-approved DAA drugs for treating chronic HCV due to genotypes 1a, 1b, and 3 (any subtype) requires pretreatment testing for RAS in the relevant HCV genomic regions to guide selection of optimal DAA combination therapy.

DAA target region	**HCV genotype**		
	1a	**1b**	**3 (any subtype)**
HCV NS3	Grazoprevir(b) Voxilaprevir(c) Glecaprevir(a)	Grazoprevir(b) Voxilaprevir(c) Glecaprevir(a)	Voxilaprevir(c) Glecaprevir(a)
HCV NS5A	Elbasvir(b) Ledipasvir(e) Pibrentasvir(a) Velpatasvir(c,f) Daclatasvir(d)	Elbasvir(b) Ledipasvir(e) Pibrentasvir(a) Velpatasvir(c,e) Daclatasvir(d)	Pibrentasvir(a) Velpatasvir(c,e) Daclatasvir(d)
HCV NS5B	Sofosbuvir(c,e,f,g)	Sofosbuvir(c,e,f,g)	Sofosbuvir(c,f,g)

Trade names of DAA:

(a) Mavyret = Glecaprevir + Pibrentasvir

(b) Zepatier = Elbasvir + Grazoprevir

(c) Vosevi = Sofosbuvir + Velpatasvir + Voxilaprevir

(d) Daklinza = Daclatasvir

(e) Harvoni = Ledipasvir + Sofosbuvir

(f) Epclusa = Sofosbuvir + Velpatasvir

(g) Sovaldi = Sofosbuvir

Antiviral drug RAS in the relevant HCV genomic regions can be detected and identified genotypically using either Sanger sequencing or next-generation sequencing (NGS) methods. Amino acid changes deemed as RAS are predicted by the NS3, NS5A, and NS5B sequences of the patient's HCV strain by comparing them to the expected amino acid at relevant codon positions within a wild-type HCV reference sequence. DAA drug resistance may be predicted for each drug based on the relevant RAS present in the HCV sequences found in the patient's serum. Prediction of HCV antiviral drug resistance in this NGS assay is based on a combination of FDA-approved prescribing information for the drug and professional society practice guidelines (see table above and Clinical Reference section).

Reference Values

Interpretive report

Interpretation

Interpretation of antiviral drug resistance in this assay is based on a detection threshold of 10% of resistance-associated hepatitis C virus (HCV) variants present in the patient's serum specimen (ie, minimum 10% frequency of such variants).

This assay will confirm the patient's HCV genotype, with possible genotype results generated as 1a; 1b; 1, no subtype; 2a; 2b; 2, no subtype; 3a; 3, no subtype; 4a; 4, no subtype; 5a; 6a; 6, no subtype. However, analysis of resistance-associated substitutions (RAS) and prediction of antiviral drug resistance are restricted only to HCV genotype test results of 1a, 1b, 3a, or 3 no subtype.

Inconclusive result indicates that testing failed, likely due to presence of inhibitory substances in the submitted serum specimen. A new serum specimen should be collected and submitted for retesting if clinically indicated.

Indeterminate result is due to presence of atypical HCV genomic sequences, such as a recombinant HCV strain comprised of genomic sequences from multiple genotypes, preventing definitive determination of the HCV genotype.

Unable to genotype indicates that the assay is unable to reliably determine antiviral resistance because of either low HCV viral load (ie, <50,000 IU/mL) or ambiguous or incomplete HCV target sequences generated with the assay.

Predicted resistance means that the RAS detected have been reported to be associated with reduction in susceptibility to the specific direct-acting antiviral (DAA) drug.

Possible resistance means that the RAS detected may be associated with a reduction in susceptibility to the specific DAA drug due to possible cross-resistance within the same drug class. Current peer-reviewed, published reports do

not have sufficient data to definitively rule out antiviral resistance to the drug.

Not predicted means that no RAS were detected and no resistance to the specific DAA drug is predicted for patient's HCV strain.

Cautions

A patient's response to antiviral therapy depends on multiple factors, including the patient's hepatitis C virus (HCV) genotype, characteristics of the infecting viral strain, patient compliance with the prescribed drug therapy, patient's access to adequate care, drug pharmacokinetics, and drug-drug interactions. Drug-resistance test results should be interpreted only in conjunction with clinical presentation and other laboratory markers when making therapeutic decisions.

Absence of resistance to a drug does not rule out the presence of reservoirs of direct-acting antiviral (DAA)-resistant HCV in the infected patient.

An HCV genotypic drug resistance test is not a direct measure of antiviral drug resistance. Although genotypic testing can detect resistance-associated substitutions (RAS) in the relevant HCV genomic regions, the clinical significance of these RAS requires careful interpretation to predict drug susceptibility. This assay's ability to amplify the HCV target sequences and detect RAS may be limited when the serum HCV viral load is less than 50,000 IU/mL due to HCV strain diversity. Serum specimens submitted for this test should contain a minimum of 50,000 IU/mL of HCV RNA.

Clinical Reference

1. Pawlotsky JM: Hepatitis C virus resistance to direct-acting antiviral drugs in interferon-free regimens. Gastroenterology 2016;151:70-86

2. Wyles DL, Luetkemeyer AF: Understanding hepatitis C virus drug resistance: clinical implications for current and future regimens. Top Antivir Med 2017;25(3):103-109

3. Sorbo MC, Cento V, Di Maio VC, et al: Hepatitis C virus drug resistance associated substitutions and their clinical relevance: update 2018. Drug Resist Updat 2018;37:17-39

4. Wyles DL: Resistance to DAAs: When to look and when it matters. Curr HIV/AIDS Rep 2017;14:229-237

5. European Association for the Study of the Liver: EASL recommendations on treatment of hepatitis C 2018. J Hepatol 2018;69:461-511

6. American Association for the Study of Liver Diseases and the Infectious Diseases Society of America: HCV guidance: recommendations for testing, managing, and treating hepatitis C. Accessed November 2018. Available at: www.hcvguidelines.org/evaluate/resistance

Performance

Method Description

This test utilizes the commercially available Sentosa SQ HCV Genotyping Assay, v 2.0, which is a next-generation sequencing assay based on a "sequencing by synthesis" method. The assay is designed to specifically amplify 4 sequences ranging from 600 to 1,100 bp in length, located in 3 different non-structural (NS) regions of the hepatitis C virus (HCV) genome: NS3 (67 RAS at 15 codon positions), NS5A (52 RAS at 13 codon positions), and NS5B (7 RAS at 4 codon positions). Clinical serum specimens are subjected to automated HCV RNA extraction and purification, followed by reverse-transcription (RT)-PCR of HCV target sequences, with both a system control and a positive control included in each assay run for quality control purposes. Automated DNA library preparation is performed

using the amplified products, including enzymatic shearing, adapter ligation, purification, and normalization, prior to DNA template preparation and sequencing. Sequencing reactions are conducted with the Ion Personal Genome Machine (PGM) sequencer, and the assembled sequence data are analyzed using proprietary analysis and interpretive software applications. HCV genotype-specific antiviral drug-resistance interpretations are based on a combination of FDA-approved prescribing information for the drug and professional society practice guidelines using a 10% variant detection cutoff threshold.(Instruction manual: Vela Operations Singapore Pte Ltd. Sentosa SQ HCV Genotyping Assay v2.0 (4x16), Version 0.1; no. PS103375A; 06/2017)

PDF Report

No

Day(s) and Time(s) Test Performed

Monday through Friday, 7 a.m.-11 p.m.

Analytic Time

4 days

Maximum Laboratory Time

8 days

Specimen Retention Time

60 Days

Performing Laboratory Location

Rochester

Fees and 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 U.S. Food and Drug Administration.

CPT Code Information

87900

87902

LOINC® Information

Test ID	Test Order Name	Order LOINC Value
HCVDR	HCV Genotypic Drug Resistance, S	82525-7

Result ID	Test Result Name	Result LOINC Value
604410	HCV Genotypic Drug Resistance, S	77202-0
604376	HCV Genotype	92731-9
604377	HCV NS3 RAS	73654-6
604378	Sequence failure at codons:	92732-7
604379	Glecaprevir resistance	92733-5
604380	Grazoprevir resistance	82523-2
604382	Voxilaprevir resistance	92734-3
604383	HCV NS5A RAS	73655-3
604384	Sequence failure at codons:	92732-7
604385	Daclatasvir resistance	82379-9
604386	Elbasvir resistance	82376-5
604387	Ledipasvir resistance	82377-3
604388	Pibrentasvir resistance	92735-0
604389	Velpatasvir resistance	82520-8
604390	HCV NS5B RAS	73655-3
604391	Sequence failure at codons:	92732-7
604392	Sofosbuvir resistance	82382-3
604393	HCV Genotype	92731-9
604394	HCV NS3 RAS	73654-6
604395	Sequence failure at codons:	92732-7
604396	Glecaprevir resistance	92733-5
604397	Grazoprevir resistance	82523-2
604399	Voxilaprevir resistance	92734-3
604400	HCV NS5A RAS	73655-3
604401	Sequence failure at codons:	92732-7
604402	Daclatasvir resistance	82379-9
604403	Elbasvir resistance	82376-5
604404	Ledipasvir resistance	82377-3
604405	Pibrentasvir resistance	92735-0
604406	Velpatasvir resistance	82520-8
604407	HCV NS5B RAS	73655-3
604408	Sequence failure at codons:	92732-7
604409	Sofosbuvir resistance	82382-3
HCRVL	Recent HCV quant over 50,000 IU/mL?	86955-2
HCVKG	Is known HCV genotype 1a, 1b, or 3?	86955-2