MAYO CLINIC LABORATORIES

Test Definition: HCVDR

Hepatitis C Virus Genotypic Antiviral Drug Resistance, Serum

Overview

Useful For

Detecting and identifying codon substitutions in the hepatitis C virus (HCV) NS3, NS5A, and NS5B genomic regions that confer resistance to current direct-acting antiviral 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 genotypes 2, 4, 5, or 6.

Testing Algorithm

Testing is successful only if there is sufficient hepatitis C viral (HCV) RNA present in the serum specimen (ie, 5000 IU/mL or above) in the preceding 30 days. If the HCV RNA level within the last 30 days is unknown or not provided, then this test will be canceled.

For more information see Chronic Hepatitis C Treatment and Monitoring Algorithm: Direct Antiviral Agent (DAA) Combination.

Special Instructions

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

Highlights

This assay uses next-generation sequencing to detect and identify hepatitis C virus 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 US Food and Drug Administration-approved DAA drug combinations used for treatment of chronic hepatitis C.

Method Name

Polymerase Chain Reaction (PCR) followed by Next-Generation Sequencing

NY State Available

Yes

Specimen

Specimen Type

Serum SST

Ordering Guidance

This test is intended for detection of preexisting antiviral drug resistance-associated substitutions 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 5000 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 (HCV) RNA Quantification with Reflex to HCV Genotype, Serum
-HCVQN / Hepatitis C Virus (HCV) RNA Detection and Quantification by Real-Time Reverse Transcription-PCR, Serum
-HCVG / Hepatitis C Virus Genotype, Serum

Shipping Instructions

1. Ship specimen frozen on dry ice only.
2. If shipment will be delayed for more than 24 hours, freeze serum at -20 to -80 degrees C before shipment, and transport on dry ice.

Necessary Information

The following 2 questions must be answered at the time of test ordering (if not ordering electronically, note the answers on the test request):

1. What Is the Hepatitis C Virus (HCV) RNA level in IU/mL within the last 30 days? Provide an answer using the following ranges:
- <5000
- 5000 to 1,000,000
- 1,000,001 to 10,000,000
- 10,000,001 to 100,000,000
- >100,000,000

Note: If the answer to this question is not answered or is “Unknown," testing will be canceled.

2. Does the patient have a known hepatitis C genotype of 1a, 1b, or 3 (any subtype)? Yes or No.
Note: If the answer to this question is "No," testing will be canceled.

Specimen Required

Supplies: Sarstedt Aliquot Tube, 5 mL (T914)
Collection Container/Tube: Serum gel (red-top tubes are **not acceptable**)
Submission Container/Tube: Plastic vial
Specimen Volume: 2.5 mL serum
Collection Instructions:
1. Centrifuge blood collection tube per manufacturer's instructions (eg, centrifuge and aliquot within 2 hours of collection for BD Vacutainer tubes).
2. Aliquot serum into a plastic vial. **Specimens sent in serum gel tubes will be canceled**.

Forms

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

Specimen Minimum Volume

Serum: 1.6 mL

Reject Due To

Gross hemolysis	Reject
Gross lipemia	OK
Gross icterus	OK

Specimen Stability Information

Specimen Type	Temperature	Time	Special Container
Serum SST	Frozen (preferred)	60 days	ALIQUOT TUBE
	Refrigerated	7 days	ALIQUOT TUBE

Clinical & Interpretive

Clinical Information

Interferon-free, direct antiviral agent (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 indicate 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 US Food and Drug Administration (FDA) and professional society practice guidelines (see Table 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.

Table.

HCV genomic target of DAA drug	HCV genotype		
	1a	1b	3 (any subtype)
NS3/4	Glecaprevir(a) Grazoprevir(b) Voxilaprevir(c)	Glecaprevir(a) Grazoprevir(b) Voxilaprevir(c)	Glecaprevir(a) Voxilaprevir(c)
NS5A	Daclatasvir(d)	Daclatasvir(d)	Daclatasvir(d)

	Elbasvir(b) Ledipasvir(e) Pibrentasvir(a) Velpatasvir(c,f)	Elbasvir(b) Ledipasvir(e) Pibrentasvir(a) Velpatasvir(c,e)	Pibrentasvir(a) Velpatasvir(c,e)
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 and www.hcvguidelines.org/evaluate/resistance).

Reference Values

An interpretive report will be provided.

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 results indicate 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 results are due to the 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, <5000 IU/mL) or ambiguous or incomplete HCV target sequences generated with the assay.

"Predicted resistance" means that the RAS detected has 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 RASs 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-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 5000 IU/mL due to HCV strain diversity. Serum specimens submitted for this test should contain a minimum of 5000 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(1):70-86. doi:10.1053/j.gastro.2016.04.003
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. doi:10.1016/j.drup.2018.01.004
4. Wyles DL. Resistance to DAAs: When to look and when it matters. Curr HIV/AIDS Rep. 2017;14(6):229-237. doi:10.1007/s11904-017-0369-5
5. European Association for the Study of the Liver: EASL recommendations on treatment of hepatitis C 2018. J Hepatol. 2018;69(2):461-511. doi:10.1016/j.jhep.2018.03.026
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. HCV resistance primer. Updated October 24, 2022. Accessed September 15, 2025. Available at www.hcvguidelines.org/evaluate/resistance

Performance

Method Description

This test utilizes the commercially available ViroKey SQ FLEX Genotyping Assay, 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 436 to 1112 base pairs in length, located in 3 different non-structural (NS) regions of the hepatitis C virus (HCV) genome: NS3 (79 resistance-associated substitutions [RAS], RAS at 17 codon positions), NS5A (65 RAS at 15 codon positions), and NS5B (13 RAS at 6 codon positions). Clinical serum specimens are subjected to automated HCV RNA extraction and purification, followed by reverse-transcription polymerase chain reaction amplification of HCV target sequences, with both system and positive controls included in each assay run for quality control purposes. Automated DNA library preparation is performed using 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 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 US Food and Drug Administration-approved prescribing information for the drug and professional society practice guidelines using a 10% variant detection cutoff threshold. (Instruction manual: ViroKey SQ FLEX Genotyping Assay (4x16). Vela Diagnostics; V3.0, 2023)

PDF Report

No

Day(s) Performed

Once per week

Report Available

4 to 14 days

Specimen Retention Time

60 days

Performing Laboratory Location

Mayo Clinic Laboratories - Rochester Superior Drive

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.

CPT Code Information

87900
87902
87999 (if appropriate for government payers)

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

HCVKG	Is known HCV genotype 1a, 1b, or 3?	86955-2
HCVLR	HCV RNA level IU/mL last 30 days =	86955-2