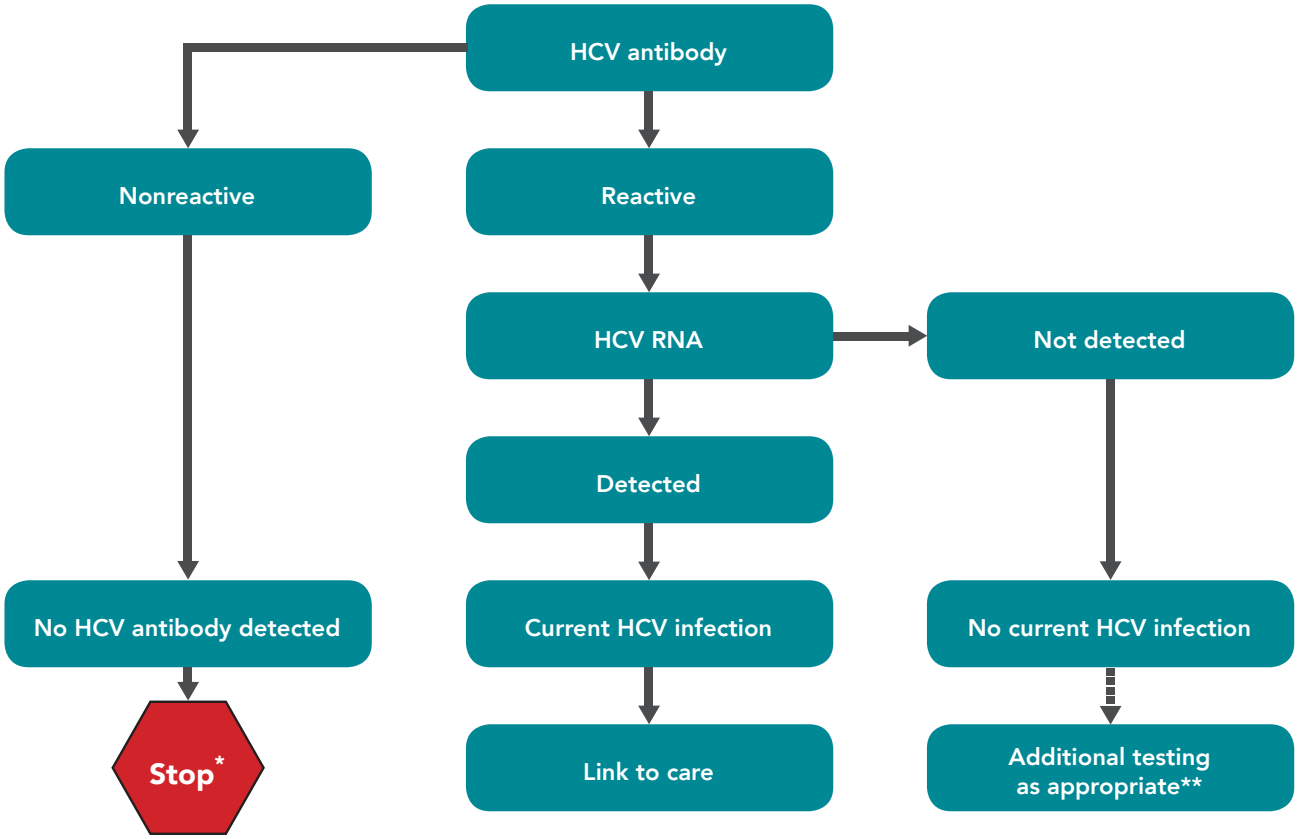
TRICORE REFERENCE LABORATORIES

HEPATITIS C VIRUS (HCV) DIAGNOSTIC AND RESISTANCE TESTING

DIAGNOSTIC TESTING

Q What are TriCore's current recommendations to a provider who wants to order HCV testing (if they already know HCV antibody test is positive)? What is the sequence of testing? What is the most efficient lab testing process for provider and patient?

A The recommended process for HCV testing is generally diagramed below in the CDC algorithm. A positive HCV antibody (screen) should be followed by an HCV quantitative RNA viral load (confirmatory). In the continuum of care, a patient with a positive anti-HCV screen and detectable viral load should be connected to an HCV specialist for additional HCV testing. When ordering an HCV genotype if an HCV viral load has not been performed within 6 months, then a viral load will be done prior to a genotype. Genotype testing is done to guide the most appropriate antiviral regimen. Resistance testing is recommended as a follow up test to a genotype 1a results to determine if the first line drug is an appropriate therapy.



* For persons who might have been exposed to HCV within the past 6 months, testing for HCV RNA or follow-up testing for HCV antibody should be performed. For persons who are immunocompromised, testing for HCV RNA should be performed.

** To differentiate past, resolved HCV infection from biologic false positivity for HCV antibody, testing with another HCV antibody assay can be considered. Repeat HCV RNA testing if the person tested is suspected to have had HCV exposure within the past 6 months or has clinical evidence of HCV disease, or if there is concern regarding the handling or storage of the test specimen.

Adapted from Centers for Disease Control and Prevention (CDC), 2013.

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HEPATITIS C VIRUS (HCV) DIAGNOSTIC AND RESISTANCE TESTING, continued

DIAGNOSTIC TESTING, continued

Q Does TriCore perform reflex testing to HCV quantitative RNA viral load?

A Currently, TriCore does not perform reflex testing to HCV quantitative RNA viral load from a positive HCV antibody test. Our testing protocols for each method have different specimen requirements.

Q Does TriCore perform reflex testing to HCV genotype?

A Currently, TriCore does not have an order code that offers reflex testing to HCV genotype from a detectable viral load. There are several clinical variables that are being considered to support the build of a new order code.

RESISTANCE TESTING

Q What are HCV drug resistance assays?

A Hepatitis C Virus (HCV) drug resistance assays are genotypic (sequencing) resistance assays that analyze regions of HCV genotypes 1a or 1b and genotype 3 using next generation sequencing (NGS) techniques. Amino acid substitutions in the NS3/4A, NS5A, or NS5B region are identified, and a viral susceptibility calls of "resistance possible" or "none/undetermined" are reported for direct acting agents (DAAs).

Q What is the Q80K polymorphism and how does it impact treatment considerations?

A The Q80K protease polymorphism has been shown to be prevalent (>40%) among patients with HCV genotype 1a viruses in the US.[1] The Q80K polymorphism significantly reduces the efficacy of simeprevir (Olysio™), an NS3/4A protease inhibitor approved for use in combination with pegylated interferon α plus ribavirin (peg-IFN/RBV).[1]

Q Is HCV drug resistance assay testing indicated for use as a baseline assessment tool (prior to initiation of therapy)?

A The role of baseline HCV resistance testing continues to evolve, since it is known that the effect of baseline polymorphisms can impact sustained virologic response rates. Recommendations for baseline resistance testing can be found for both the HCV NS3/4A[1,2] and HCV NS5A[2,3] class of compounds.

Q Are there other situations in which the HCV NS5A Drug Resistance Assay might be indicated?

A Besides testing at baseline with an elbasvir/grazoprevir regimen, current AASLD-IDSA Hepatitis C guidelines recommend RAV testing for patients with genotype 1 with cirrhosis or an urgent need for treatment who were previously treated with an NS5A inhibitor and are being considered for retreatment.[2]

Q Are there other situations in which the HCV NS5B Drug Resistance Assay might be indicated?

A Resistance testing with HCV NS5B may be considered for virologic breakthrough on treatment to establish the presence or absence of resistant variants that may impact future treatment options. Data from clinical trials suggest the possibility that treatment emergent amino acid substitutions in the HCV NS5B region may limit the effectiveness of NS5B DAAs.[4,5,6]

Q How are the HCV drug resistance assays performed?

A Depending on the test selected, either the NS3/4A, NS5A, or NB5B region of HCV from patient plasma samples is amplified by RT-PCR using either genotype 1a or 1b, or genotype 3 specific primers. Nucleic acid sequences are determined using NGS methods and then compared to subtype specific reference sequences. Amino acid differences from the reference sequences are reported. Analysis based on Monogram's HCV genotypic interpretation database yields an assessment of "resistance possible" or "none/undetermined" for each currently available inhibitor.

Q Have the HCV Drug Resistance Assays been validated?

A The HCV Drug Resistance Assays have been validated according to CAP/CLIA specifications in Monogram's CAP/CLIA accredited clinical reference laboratory in South San Francisco, CA.

[1] Olysio™ (Highlights of Prescribing Information). Titusville, NJ: Janssen Therapeutics; 2013. Reference ID: 3412095.

[2] American Association for the Study of Liver Diseases (AASLD); Infectious Disease Society of American (IDSA). Recommendations for Testing, Managing, and Treating Hepatitis C. Available at http://www.hcvguidelines.org. Updated February 24, 2016. Accessed March 2, 2016.

[3] Zepatier™ (elbasvir and grazoprevir) tablets, for oral use (package insert). Initial US Approval: 2016. Whitehouse Station, NJ: Merck and Co Inc; 2016.

[4] Viekira PAK (Highlights of Prescribing Information), North Chicago, IL: AbbVie Inc, 2014.

[5] Harvonii (Highlights of Prescribing Information), Foster City, CA: Gilead Sciences; 2014.

[6] Whitcomb J. Data Analysis Review of DeepLogic Files [standard operating procedure]. South San Francisco, Calif: Monogram BioSciences Inc; March 12, 2015.

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HEPATITIS C VIRUS (HCV) TEST MENU GUIDE

TEST NAME METHODOLOGY	TEST CODE	CPT CODE	CLINICAL UTILITY	REPORTED
DIAGNOSTIC TESTING				
Hepatitis C Virus Antibodies Chemiluminescent Immunoassay	HCVAB	86803	Screen for hepatitis C virus antibody	Within 24 hours
Hepatitis C Quantitative Real-Time PCR	HCVQT	87522	Determine the viral load in number of international unites (IU) of hepatitis C virus per milliliter. Quantifiable range of the assay is 15 IU/mL to 100,000,000 IU/mL.	Within 3 days
Hepatitis C Virus Genotype Real-Time PCR	HCGENO	87902	Genotyping for types 1-5 and subtypes 1a and 1b.	Within 9 days
RESISTANCE TESTING				
HCV NS3/4A Drug Resistance GenoSure Real-Time PCR and Next Generation Sequencing	NS34A (550540)	87522	Assessment of drug susceptibility to NS3/4A inhibitors including grazoprivir (Zepatier), paritaprevir (Viekira Pak), simeprevir (Olysio). Detects Q80K polymorphism.	Within 8-13 days
Hepatitis C Virus NS5A Drug Resistance Assay Amplified Real-Time PCR and Next Generation Sequencing	NS5A (C6200)	87900; 87902	Assessment of drug susceptibility to NS5A inhibitors including daclatasvir (Daklinza), elbasvir (Zepatier), ledipasvir (Harvoni), ombitasvir (Viekira Pak) and velpatasvir (Epclusa).	Within 8-13 days
Hepatitis C Virus NS5B Drug Resistance Assay Amplified Real-Time PCR and Next Generation Sequencing	NS5B (C6300)	87900; 87902	Assessment of drug susceptibility to NS5B inhibitors including sofosbuvir (Sovaldi/Harvoni); dasabuvir (Viekira Pak)	Within 8-13 days

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TESTING FOR HCV RESISTANCE ASSAY

The HCV resistance assay used has been validated and approved for genotypes 1a, 1b and 3 ONLY.

TriCore Referral Testing has asked that the following information be available prior to accepting the order and sending it to Monogram Biosciences:

1) An HCV RNA and genotype must be in the system (within 6 months)
   - a. HCV RNA must be:
     - I. ≥ 2000 IU/mL for NS3/4A
     - II. ≥ 500 IU/mL for NS5A
     - III. ≥ 1000 IU/mL for NS5B
   - b. Genotype must be 1a or 1b (or 3 for NS5A testing)
   - c. It is preferred that an HCV RNA and genotype be done within 2 weeks of requesting resistance testing. This is to determine appropriateness for testing.

2) Only one test (i.e., region) can be ordered per sample
   - a. HCV NS3/4A, NS5A and NS5B drug resistance testing is now an orderable code through TriCore (by electronic ordering or requisition)
     - I. NS3/4A
       1. Test code: NS34A
       2. CPT code: 87902
       3. Test order #: 550540
     - II. NS5A
       1. Test code: NS5A
       2. CPT code: 87900; 87902
       3. Test order #: C6200
     - III. NS5B
       1. Test code: NS5B
       2. CPT code: 87900; 87902 (same as NS5A)
       3. Test order #: C6300
   - b. For HCV NS5A genotype 3 resistance testing:
     - I. Order the same way as NS5A testing AND include the comment:
       - a. "Genotype 3 resistance testing"

3) Collection, processing and transport
   - a. Refer to the TriCore Test Directory at www.tricore.org