

The Laboratory Diagnosis and Evaluation of Treatment for Hepatitis C Virus Infection

Chronic Hepatitis C Virus (HCV) infection is now a disease that can be treated. The introduction of pegylated interferons in combination with ribavirin cures HCV infection in over 50% of adults. Chronic infection is defined as the presence of HCV RNA in the blood for more than 6 months. Facts about HCV infection include:

- Most common chronic blood borne infection in the USA
- Leading cause of cirrhosis in the USA
- 10,000 to 20,000 deaths/year and this number is expected to triple in the next 10 to 20 years
- Compare to colon cancer at 30,000 deaths/year
- Associated with an increased risk of liver cancer
- Most common reason for liver transplant in the USA

<p>Health care professionals in primary care, specialty, and public health settings should routinely question patients about risk factors for HCV infection</p>
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<p>Persons for whom routine HCV testing is recommended</p>

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| <ul style="list-style-type: none">• Persons who ever injected illegal drugs, including those who injected once or a few times many years ago• Persons who received a blood transfusion or organ transplant before July, 1992• Persons who received clotting factor concentrates before 1987• Persons who were ever on long-term dialysis• Children (after 18 months of age) born to HCV-positive women• Healthcare, emergency medical, and public safety workers after needlesticks, sharps, or mucosal exposures to HCV-positive blood• Persons with evidence of chronic liver disease• HIV positive patients |
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It is estimated that the virus can remain viable on unclean surfaces for up to 4 days and perhaps longer. Therefore sharing household items such as nail clippers, toothbrushes, or razors should be discouraged in family members or others who share a house with somebody with documented hepatitis C infection. Because the risk of infection is low, routine testing for hepatitis is not recommended for individuals living with an infected person or having sex with an infected steady partner. However, these individuals should be tested if they request it. Other risk factors which may lead to blood exposure and to consider when talking to patients are, sex with multiple partners or prostitutes, sexual practices which lead to traumas, intranasal cocaine use, tattoos, body piercing and manicures. However, in many patients infected with Hepatitis C, the source of their infection may never be identified.

Hepatitis C virus is an RNA virus which is transmitted via blood. It has 6 major genotypes and 50 subtypes. Genotype 1 causes 70% to 75% of infections in the USA and is characterized by a lower rate of response to treatment (50% cure rate). Genotype 2 and 3 have a much better response to treatment (80% cure rate). It is estimated that 85% of adults who are infected with Hepatitis C go on to have chronic infection. Chronic infection is promoted by a high rate of viral mutation, lack of a vigorous host T-cell response, and replication in hepatocytes without cytotoxicity.

Approximately 1.8% of the USA population is infected (approximately four million people). The highest prevalence is in the 40 year to 60 year age range. Infection is usually asymptomatic, thus most people are unaware of their disease. Acute infection, though rarely recognized, has an incubation period of 2 to 26 weeks. Viral RNA is detected first and shortly thereafter antibodies develop. Only 15% of those infected go on to spontaneously cure their disease. In practice, most people are diagnosed when routine blood tests reveal abnormal liver chemistries, they donate blood, or a physician notes risk factors and screens for the disease. Many people present for the first time with end-stage liver disease. Patients may present with extra-hepatic manifestations of chronic disease which include:

- Rheumatoid symptoms
- Keratoconjunctivitis sicca
- Lichen planus
- Glomerulonephritis
- Lymphoma
- Essential mixed cryoglobulinemia
- Porphyria cutanea tarda
- Depression

Because testing for the presence of Hepatitis C is complicated and false positive and false negative results occur, an algorithm for the use of laboratory tests to diagnosis patients with Hepatitis C has been developed by the Centers for Disease Control (CDC). This algorithm is designed to be cost effective and produce accurate results. The Pathology Center laboratory is adopting this approach to provide the best service for our patients and physicians (see attached flow chart for algorithm). The tests available for diagnosis of Hepatitis C are:

1. HCV antibody screening test (EIA):

The advantages of this test include:

- Reproducible, inexpensive, FDA-approved for use
- Suitable for screening at risk populations and those with clinical liver disease
- Negative results are sufficient to exclude diagnosis of chronic HCV infection in immuno competent patients.

The disadvantages of this test include:

- Positive results of screening tests must be confirmed by more specific tests
- False negatives may occur in hemodialysis and immunodeficient patients
- Negative results may occur in acute infections as antibodies may take up to 6 weeks to develop after onset of symptoms of acute hepatitis

2. HCV antibody confirmation Recombinant Immuno Blot Assay (RIBA):

Advantages of this test include:

- FDA-approved for confirmatory testing of positive antibody screening test
- High specificity in detection of antibodies
- Can be performed on same serum submitted for initial antibody screening test

Disadvantages of this test include:

- Positive result does not distinguish between past infection or chronic disease.
- Extra test which may not be needed in patient with high likelihood of having disease
- Needed to confirm positive results in infected persons who have spontaneous cure of disease and those with probable false positive results in the antibody screening assay

3. HCV RNA quantitative PCR (viral load). This test detects viral RNA. The lower limit of detection is 200 IU/ml (500 RNA copies per ml). Results are reported as IU/ml.

Advantages of this test include:

- Can be used as supplemental test in patient suspected of having chronic disease with a positive antibody screening test
- Can be used for diagnosis of acute infection and in immunodeficient patients suspected of having disease with a negative antibody screen test
- Used to monitor therapy in patients with chronic disease

Disadvantages of this test include:

- HCV RNA can be transiently negative in persons with acute infection but they can still go on to develop chronic infection
- HCV RNA can be intermittently positive in patients with chronic infection
- Use as supplemental test is valid only when the test is positive, i.e. patient has detectable viral load.
- **Special handling** of serum sample collected for testing is necessary for accurate results; if specimen is not collected or transported properly false negative results may be reported.
- False positive results can occur through contamination.

4. HCV genotype - This test categorizes type of viral RNA present. (performed via PCR and subsequent nucleic acid sequencing)

Advantages of this test include:

- **Essential** test to perform once to evaluate patient prior to start of therapy
- Results determine what dose and type of medication and length of treatment will be used
- May help identify source of infection
- Assess likelihood of response to therapy

Disadvantages for this test include:

- Results may not be obtainable in patients with low viral loads (less than 1,000 RNA copies per ml) or in cases where there are mixed genotypes causing infection.
- **Special handling** of serum sample collected is necessary for accurate results; if specimen is not collected or transported properly false negative results may be reported.

5. HCV RNA qualitative PCR. This test detects viral RNA. This test has a lower limit of detection of 50 IU/ml (100 RNA copies/ml). Results are reported as negative or positive for HCV RNA.

Advantages for this test include:

- Most sensitive PCR test available
- Used clinically at end of treatment and at end of follow-up; to assess treatment response
- Used as confirmation of initial negative quantitative PCR test

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Disadvantages include:

- **Special handling** of serum sample collected is necessary for accurate results; if specimen is not collected or transported properly may have false negative results.
- False positive results can occur through contamination

6. Other tests

Liver function tests and liver biopsies are not sensitive or specific enough to be used for screening; they may be the initial test which triggers testing for Hepatitis C and they are used as adjunct tests by specialists in treatment of Hepatitis C who are caring for patients with the disease.

Please refer to Flow Chart for Reflex Hep C Testing Protocol

The HCV antibody screen which we offer gives us the ability to separate probable false positive reactions from true positive reactions by means of a signal to cutoff ratio.

- Positive screens which have a low signal to cutoff ratio (<3.8) will be automatically reflexed to a RIBA confirmatory test prior to reporting the antibody screen. If the RIBA is negative, the screen will be reported, the patient will be considered not infected with Hepatitis C and the screen to be a false positive.
- If the RIBA is positive, which should happen rarely with a low signal to cutoff ratio, the physician will be called and blood obtained for a Hepatitis C virus quantitative PCR and if positive reflex to genotype if possible and clinically appropriate.
- If the PCR quantitative test is positive, the genotype will be done and the patient considered to have active infection with Hepatitis C and recommended for evaluation by a specialist in Hepatitis C disease.
- If the PCR is negative, then the patient may be one of the 15% who has spontaneous cure of the disease, in which case they will be positive for Hepatitis C antibodies but negative for active disease by the PCR results (No viral RNA detected). A comment will be in the report that a single negative PCR result does not rule out active infection, and a second PCR (qualitative test) is recommended within a month.
- However, if the HCV antibody screen has a high signal to cutoff ratio (≥ 3.8), this indicates the patient has a greater than 95% chance of truly being infected. The physician will be called and blood obtained for the supplemental tests. The supplemental test to confirm the antibody screen in this case will be a Hepatitis C virus quantitative PCR.

- If this is positive, the specimen will automatically have a genotype done and the patient will be considered to have active infection with Hepatitis C and recommended for evaluation by a specialist in Hepatitis C disease.
- If the PCR quantitative is negative, the specimen will automatically be reflexed to a RIBA confirmatory test. If negative, the screen result would be reported and the patient considered not infected with Hepatitis C, (false positive antibody screen). If the RIBA is positive, then the patient is positive for Hepatitis C antibodies and a single negative PCR result does not rule out active infection. The recommendation is a second PCR (qualitative) in a month to confirm spontaneous cure.

CHILDREN

Infection in children is not as common as in adults with a prevalence of 0.2% in children under 12 years of age and 0.4% in children 12 to 19 years of age.

Almost half of the children infected spontaneously cure their disease. Of those chronically infected, less than 10% go on to have chronic hepatitis and less than 5% progress to cirrhosis.

Infants born to Hepatitis C infected mothers have passively acquired maternal antibody for up to 18 months after birth and therefore, should only be screened using a HCV antibody screening test after 18 months of age.

If earlier diagnosis is required, a qualitative PCR can be performed at 1 to 2 months of age, however, a positive result doesn't mean the child will be chronically infected and false positive results occur.

There are no FDA licensed therapies for children younger than 18 years of age. However, therapy may be indicated in select cases. Consultation with a pediatric specialist with experience in the treatment of HCV infections in children is warranted.

Counseling of Patients with HCV Infection

All people with HCV infection should be considered infectious, informed of the possibility of transmission to others, and should refrain from donating blood, organs, tissues or semen. They should not share toothbrushes, nail clippers or razors. They should be counseled to avoid hepatotoxic agents such as medications and alcohol. If they are susceptible, they should be vaccinated against Hepatitis A and B viruses.

REFERENCES:

1. Guidelines for Laboratory Testing and Result Reporting of Antibody to Hepatitis C Virus, MMWR February 7, 2003/Vol. 52/No. RR-3. (Free CME credit at end of report).
2. National Institutes of Health, Consensus Statement of Management of Hepatitis C: 2002, Vol. 19, Number 3 June 10 – 12.
3. American Academy of Pediatrics, Red Book 26th Ed., 2003.

On-line references:

1. National Institutes of Health Website; www.health.nih.gov Search under Hepatitis C
2. CDC Website; www.cdc.gov Search under Hepatitis C

On-line free CME credit can be obtained at:

<http://www2a.cdc.gov/ce/availableactivities.asp>

Toll free number for additional clinical help is: 1-888-4HEPCDC.

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