

Hepatitis C: An overview of its laboratory diagnosis

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ABSTRACT

Hepatitis C virus (HCV) is a heterogeneous virus with multiple genotypes. It has a slowly progressive course with a fatal outcome. Chronic infection with HCV is a major cause of liver cancer. Diagnosing the disease early at an early stage is necessary so as to prevent its progression to chronicity. Screening for anti-HCV IgG antibody can be done by a number of immunoassays but at the same time it becomes difficult to differentiate between acute and chronic infection. To overcome this problem nucleic acid testing for detecting HCV ribonucleic acid (RNA) can be used as the mainstay for diagnosing the infection.

Keywords: Anti-HCV antibody, Hepatitis C virus, Nucleic Acid testing

Hepatitis C virus (HCV) has infected an estimated 130 million people worldwide. HCV-infected people are at risk for developing chronic liver disease, cirrhosis and primary hepatocellular carcinoma (HCC). It is estimated that at least 20% of patients chronically infected would develop cirrhosis within 20 years.¹ Chronic hepatitis C has become a silent epidemic with a global impact.

HCV is a single-stranded RNA virus possessing an icosahedral capsid and an envelope. It belongs to the genus *Hepacivirus* and is a member of the family Flaviviridae.² Its genome is 9.6-kb long. It has 2 untranslated regions at its 5' and 3' ends and contains a single open reading frame (ORF) that encodes a polyprotein of approximately 3000 amino acids. A host signal peptidase in the structural region and viral encoded proteases in the nonstructural region cleave this polyprotein into 10 single peptides. The core protein and the envelope glycoproteins E1 and E2 are the structural proteins. These are the major components of the virion and are coded in the 5' region of the ORF. Nonstructural proteins include the p7, NS2, NS3, NS4A, NS4B, NS5A, and NS5B proteins and are present at the 3' region of the ORF. An estimated 10 trillion virion particles can be produced per day during the active phase of infection due to an active viral replication.³ An increased

occurrence of heterogeneity that is seen in the nucleic acid and protein sequence is due to the presence of the envelope glycoprotein E2.^{4,5} This region is known as the hyper-variable region-1 (HVR-1). It is the accumulation of mutations in the HVR-1 due to strong immune pressure that leads to the origin of quasispecies in the infected individuals.⁶

HCV virus has six genotypes numbered 1-6 and each of this group contains many subtypes named a,b,c etc.⁷ 1a and 1b are the most prevalent genotypes in the United States and western Europe, followed by genotypes 2 and 3. Genotype 4 is common in Egypt, genotype 5 in South Africa, and genotype 6 in southeast Asia.⁸

Detection of HCV remains an issue of major concern. Early diagnosis of infection is important as it helps the clinician to commence treatment so as to prevent the progression of the disease to chronic active hepatitis, cirrhosis and hepatocellular carcinoma. At the same time the diagnosis of hepatitis C infection has a personal impact in the form of lifestyle changes like reduced work hours or alcohol intake which indirectly influences economic status and social participation.^{9,10}

The diagnosis of HCV involves the detection of the anti HCV antibody but nucleic acid testing (NAT) remains the gold standard. At the same time, performing these tests in a routine clinical laboratory might pose difficulties due to the requirement of technical expertise, costly equipment, reagents and special procedure areas. Tests used for detecting HCV antibodies fail to differentiate acute infection from past resolved infection and false positivity. This difficulty is overcome by further testing for HCV RNA using nucleic acid testing.

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Earlier, US Centers for Disease Control and Prevention (CDC) recommended that a person is considered to have current HCV infection if a positive result of anti-HCV screening is confirmed by positive results of a further test, using either a recombinant immunoblot antibody assay (RIBA) or NAT to detect HCV RNA. The CDC issued another set of guidelines that expand earlier recommendations by including an option to use signal-to-cutoff ratios to limit the number of samples needing supplemental testing.¹¹

A number of immunoassays are available to screen anti-HCV IgG in serum or plasma specimens. Unlike hepatitis B, IgM antibody detection cannot be done because it has variable levels in both acute and chronic stage of the infection. Presently, a third-generation ELISA that uses multiple antigens including antigens from the core, NS3, NS4 and the NS5 proteins of HCV which represent about 60% of the total amino acid profile of HCV polyprotein, is available. It has a good sensitivity but it fails to differentiate between active and post-infection cases. Another drawback is that third-generation ELISA is not very good in detecting genotypic variations.¹²⁻¹⁵

RIBAs such as RIBA-3 (Chiron) and strip immunoblot assays are intended for use as additional, more specific tests for detection of anti-HCV IgG in serum or plasma specimens that have been found reactive in anti-HCV screening assays. RIBA is based on immobilization of HCV recombinant antigens and synthetic peptides from core, NS3 and NS5 proteins as individual bands onto a membrane.⁸ But samples positive with RIBA still require confirmation by NAT.

To overcome the time factor of EIAs, further developments were made in the form of rapid assays. These tests do not require any technical expertise and costly equipment. These assays are based on the principle of immunochromatography. The OraQuick HCV Rapid Antibody Test was recently approved by the FDA for anti-HCV IgG detection. It can be tested on fingerprick, whole-blood and venous blood specimens from individuals aged >15 years and at risk for infection with HCV and from persons with signs and symptoms of hepatitis. But a positive test again needs confirmation by detecting HCV RNA.⁸

Recently HCV core antigen (HCVc Ag) has emerged as a potential marker for diagnosis of HCV infection. HCVcAg can detect the HCV antigens either

prior to HCV antibody formation or in the presence of HCV-Ab. Recently, a new ELISA has been developed that is capable of detecting both HCV antibody and core antigen in a single assay.² Though its sensitivity has been reported to be 80% to 99% but it is still lower than NAT.⁸

So the final confirmation of acute HCV infection stays with HCV RNA detection using polymerase chain reaction or other molecular techniques. All NATs exhibit high specificities of up to 99% across all 6 genotypes of HCV.⁸ So NAT remains the gold standard for diagnosis of active HCV infection.

Recently CDC has recommended two amendments in the guidelines for HCV testing. First is the use of HCV antibody detection by using OraQuick rapid test kit over a wider spectrum to access to persons at risk for HCV infection. Another recommendation is the discontinuation of RIBA HCV.¹⁶

In our department, we are detecting HCV antibodies by the third generation ELISA. There were a total of 7874 samples received during January 2011 to December 2012. Out of these samples those which tested positive for anti HCV antibodies were 711 (9.02%). In a study done by Suri *et al*¹⁷ in a tertiary care centre of north India an overall prevalence of 13.25% and 14.27% was observed in 2010 and 2011 respectively. In another study by Panigrahi AK *et al*¹⁸ at AIIMS, New Delhi 13.83% patients with acute hepatitis and chronic liver diseases were positive for anti-HCV antibody. Seroprevalence rate of anti-HCV antibody was found to be 1.7%¹⁹ in Jaipur and 1.57% in a study in Cuttack, Orissa.²⁰

In ideal situations proper detection of HCV should be done by using both screening and confirmatory tests so as to avoid unnecessary stigma to the patients.

REFERENCES

1. Ono-Nita SK, Nita ME, Carrilho FJ. Hepatitis C – facts in numbers. *Arq Gastroenterol* 2006;43(2):71-2.
2. Hosseini-Moghaddam SM, Iran-Pour E, Rotstein C, Husain S, Lilly L, Renner E, *et al*. Hepatitis C core Ag and its clinical applicability: potential advantages and disadvantages for diagnosis and follow-up? *Rev Med Virol* 2012;22(3):156–65.
3. Neumann AU, Lam NP, Dahari H, Gretch DR, Wiley TE, Layden TJ, *et al*. Hepatitis C viral dynamics in vivo and the antiviral efficacy of interferon-alpha therapy. *Science* 1998; 282 (5386): 103–7.
4. Simmonds P. Viral heterogeneity of the hepatitis C virus. *J Hepatol* 1999; 31:54–60.
5. Farci P, Purcell RH. Clinical significance of hepatitis C virus genotypes and quasispecies. *Semin Liver Dis* 2000;20:103–26.

6. Forns X, Purcell RH and Bukh J. Quasispecies in viral persistence and pathogenesis of hepatitis C virus. *Trends Microbiol* 1999;7(10):402–10.
7. Simmonds P, Alberti A, Alter HJ, Bonino F, Bradley DW, Brechot C, *et al.* A proposed system for the nomenclature of Hepatitis C viral genotypes. *Hepatology* 1994;19(5):1321–4.
8. Kamili S, Drobeniuc J, Araujo AC, Hayden TM. Laboratory Diagnostics for Hepatitis C Virus Infection. *Clin Infect Dis* 2012;55(1):S43–8.
9. Flamm SL. Chronic hepatitis C virus infection. *JAMA* 2003;289(18):2413–7.
10. Su J, Brook RA, Kleinman NL, Corey-Lisle P. The impact of hepatitis C virus infection on work absence, productivity, and healthcare benefit costs. *Hepatology* 2010;52(2):436–42.
11. Guidelines for laboratory testing and result reporting of antibody to hepatitis C virus. Centers for Disease Control and Prevention. *MMWR Recomm Rep* 2003;52:1–13.
12. Das MR, Ali N, Aruna B, Rama D, Nambiar A, Rehana Z *et al.* Indian strains of hepatitis C: Prevalence and detection. *Curr Sci* 1993;65:477–83.
13. Panigrahi AK, Nanda SK, Dixit RK, Acharya SK, Zuckerman AJ, Panda SK. Diagnosis of hepatitis C virus- associated chronic liver disease in India: comparison of HCV antibody assay with a polymerase chain reaction for the 5' noncoding region. *J Med Virol* 1994;44:176–9.
14. Poduri CD, Khanna A, Sugunan VS, Das MR. A conserved motif at the end of hypervariable region (HVR 1) of hepatitis C virus: potential tool in diagnosis. *Immunologist* 1998;6(1):429.
15. Poduri CD, Khanna A, Khundmiri SJ, Khaja MN, Kumar KS, Sugunan VS, *et al.* Predominance of antibodies to hepatitis C virus envelope proteins in various disease statuses of hepatitis C. *Acta Virol* 2001;45(1):1–6.
16. Testing for HCV Infection: An Update of Guidance for Clinicians and Laboratorians. Centers for Disease Control and Prevention. *Morb Mortal Wkly Rep* 2013;62(18):362–5.
17. Suri P, Chhina D, Gupta V, Singh J, Chopra P, Singh R, *et al.* Seroprevalence of parenterally and enterally transmitted hepatitis viruses in a tertiary care hospital of North India – A two year study. *J of Gastro Infs* 2012;2:46–51.
18. Panigrahi AK, Panda SK, Dixit RK, Rao KV, Acharya SK, Dasarathy S, *et al.* Magnitude of hepatitis C virus infection in India: prevalence in healthy blood donors, acute and chronic liver diseases. *J Med Virol* 1997;51(3):167–74.
19. Sharma R, Sinha P, Bachiwal R, Rishi S. Seroprevalence of Anti-hepatitis C Virus Antibody in a Hospital- based population of Jaipur, Rajasthan. *Indian J Community Med* 2007;32(2):158–9.
20. Mishra S, Chayani N, Sarangi G, Mallick B, Pati SB. Seroprevalence of anti HCV antibody in and around Cuttack, Orissa. *Indian J Med Microbiol* 2002;20(1):40–1.