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BRIEF OVERVIEW ON HEPATITIS C VIRUS IMMUNOASSAYS

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ABSTRACT

The publication deals with a brief overview of Hepatitis C Virus (HCV) and donor blood screening for HCV by using conventional Rapid, Enzyme Linked Immunosorbent Assay (ELISA) and Chemiluminescence Immunoassay (CLIA) also. The advantages of various generation of HCV tests in terms of sensitivity, specificity and reduction in window period are discussed.

Keywords:
Hepatitis C Virus, Immunoassay, Enzyme Linked Immunosorbent Assay, Chemiluminescence Immunoassay.

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1. INTRODUCTION

Human being has incredible creation of God having glandular important organ called liver. The utility list of this vital organ is so large and some its critical functions are to make some proteins important for blood clotting and other body functions, filtration of blood comes from digestive tract and to keep humans free from toxins and other harmful substances etc. The viral hepatitis is a common problem for public health in India. Different kinds of hepatitis viruses like A, B, C, D and E are the causative agent of the viral hepatitis [1]. The major root cause of viral hepatitis is either through contaminated water or infected blood or body fluids. The water borne viral hepatitis occurs either by Hepatitis A or Hepatitis E Virus. Hepatitis A Virus (HAV) and Hepatitis E Virus (HEV) can be prevented and controlled by maintenance of good hygiene, pure drinking water and fresh food etc. The Hepatitis B virus (HBV) Hepatitis C Virus (HCV) and Hepatitis D Virus (HDV) infection are transmitted through infected blood, plasma derivatives, organ, tissues and contaminated needles etc. and cause both acute as well as chronic disease. The HBV, HDV and HCV are the major causative agent of the liver disease, liver cirrhosis, liver failure and even liver cancer. The infection of more than one type of viral hepatitis (HBV, HDV

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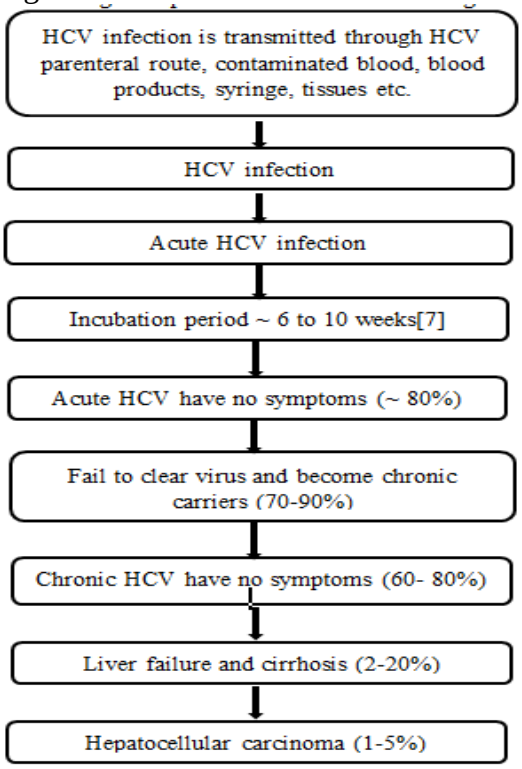
or HCV) is much more severe and fatal for human being than the person infected with single viral hepatitis disease. It is evident from several studies that alcohol intake accelerates progression of viral hepatitis to cirrhosis.

In India, serological identification of HCV infection is generally based on the detection of HCV-Ab by ELISA / CLIA / Rapid tests and confirmed by supplemental assays like Recombinant Immuno-Blotting Assay (RIBA) or Line Immuno Assay (Innolia) and / or Nucleic Acid Test (NAT), which uses Polymerase Chain Reaction (PCR) technology for HCV-RNA detection. NAT detects HCV-RNA at very early stage followed by HCV core Ag or fourth generation HCV Ag-Ab test then HCV-Ab test. The use of highly sensitive HCV-Ab or HCV-Ag assay or HCV Ag-Ab assay or HCV-RNA by NAT reduce the seroconversion window period and increase the opportunity of clinician to start treatment early after exposure of the HCV and prevent the public from HCV infection.

2. HCV INFECTION

HCV infection is a major public health problem not only for India but for all over the world also. HCV is implicated in 28% of cases of liver cirrhosis and 26% of cases of hepatocellular carcinoma worldwide, which accounts for almost 500,000 deaths per year[2,3] Population prevalence of chronic HCV infection in India is around 1 percent[4]. Different generation of anti-HCV assay can affect the prevalence rate in different population and practices between different regions of the country group[5]. Globally ~170-180 million persons are suffering with HCV[6]. Therefore, it is important to reduce the prevalence of HCV by blood collection from voluntary non-paid (non-remunerated) donors, proper screening of blood with advanced and highly sensitive serological and molecular assay. HCV infection resulting to hepatocellular carcinoma through various stages is as follow:



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3. STRUCTURE AND GENERAL CHARACTERISTICS OF HCV

Previously Non-A Non-B Hepatitis (NANBH) finally characterized as a Hepatitis C virus (HCV) in year 1989[8]. It is a single stranded enveloped RNA virus having genome length of ~9.5 kb and belongs to *Hepacivirus* genus of the family *Flaviviridae*. Structural and Non-Structural proteins comes under region encoding polyprotein precursor. The Structural protein contains a nucleocapsid protein (core) and two envelope glycoproteins (E1 and E2), and Non-Structural proteins contains NS1, NS2, NS3, NS4 and NS5 (Figure – 1).

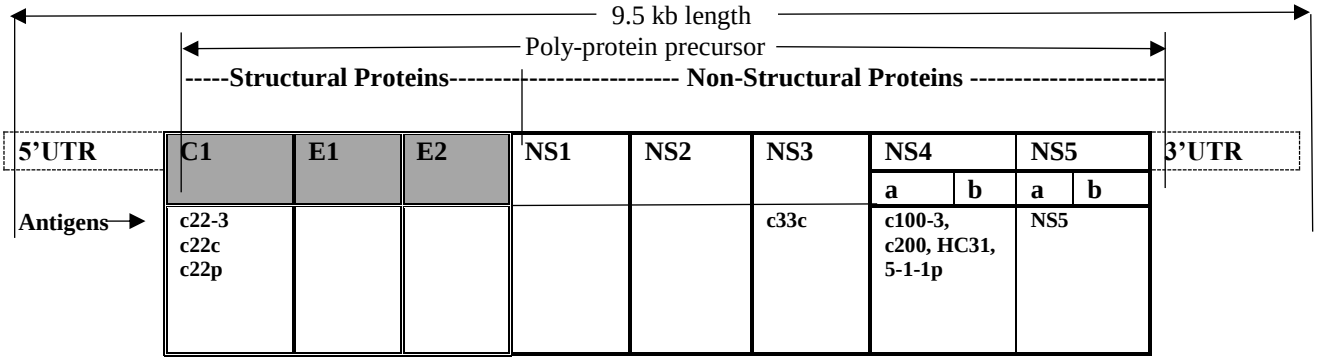

Figure 1: Hepatitis C Virus genome

4. IMMUNOASSAY OF HCV

Since development of Radio Immunoassay (RIA) in 1960 by Nobel prize winner Rosalyn Yelow and Solomon Berson[9] and ELISA by Engvali & Perlmann in 1972[10] immunoassay becomes predominant immunological tool for detection of wide range of analytes specially for transfusion transmitted infection. Now a days detection of HCV infection basically depends on two types of test, one is serological tests which include rapid test, ELISA, CLIA, Supplemental assay (strip based like RIBA / Innolia) etc. and other is molecular diagnostic assay like HCV-RNA detection based on NAT. Serological test detects Antigen(Ag) or Antibody(Ab) or combo of Ag-Ab of HCV and molecular diagnostic test detects HCV RNA. ELISA for detection of HCV has progressed from 1st generation to 4th generation assay and manual test to automation test with enhanced detection capability. The 1st generation ELISA for HCV was introduced in 1989[11] by using recombinant c100-3 derived from NS4 protein [12, 13]. In 2nd generation three end proteins were used and approved for use by US-FDA in 1992[13] with substantial improvement in sensitivity as well as in specificity[14]. For 3rd generation assay NS5 is also incorporated to reduce window period and improve sensitivity and specificity. The fourth generation assay detects HCV Ag & Ab both hence window period reduced further to average 26.8 days than 7-8 weeks required by third generation assay.

Table 1: Different generation of ELISA used for detection of HCV infection

Generation	Coated material	Advantage / Disadvantage
1st	Single epitope of virus from NS4 protein	Poor sensitivity, long window period between infection and detection (4-6 months)[7]
2nd	Structural (core) and nonstructural (NS3 and NS4) proteins	Increased sensitivity than 1st generation also window period between infection and

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		detection (10-24 weeks)[15]
3rd	Structural (core) and nonstructural (NS3 and NS4) proteins + NS5	Better sensitivity than 2nd generation also window period between infection and detection (7-8 weeks)[7]
4th	Structural (core) and nonstructural (NS3, NS4 and NS5) proteins + Antibody (Antigen-antibody combo)	Better sensitivity than 3rd generation also window period between infection and detection (average 26.8 days)[15]

Rapid immunoassays are used as alternative testing strategies in resource poor settings[16] and low testing burden. However these are is more costly than conventional immunoassay and not, therefore, intended for testing of large number of samples [17].

CLIA is an automated version of ELISA having high throughput which are useful to test and report large testing load blood banks / testing laboratories. Many CLIA based immunoassay are available in Indian market like M/s Abbott, M/s Bio-kit, M/s Ortho, M/s Roche & Siemens etc. The details of five CLIA based assays are given in Table 2.

Table 2: Details of CLIA based assay for HCV Serology

S. No.	Criteria	M/s Roche	M/s Siemens	M/s Abbott	M/s Ortho	M/s Biokit
1	Analyzer	Cobas e411	Advia CentaurCP Advia CentaurXP	Architech i1000SR Architech i2000SR	Vitros ECi Vitros 5600	Bio-Flash
2	Assay pack	100 T / 200 T	200 T	100 T / 500 T	100 T	100 T
3	Type of assay	Sandwich immunoassay	Sandwich immunoassay	Chemiluminescent microparticle immunoassay	Sandwich immunomrtric	Chemiluminescent microparticle immunoassay
4	Principle	ECLIA	CLIA	CLIA	CLIA	CLIA
5	Duration	18 min	40 min	40 min	55min	30 min
6	Type of Ag	Peptide & recombinant Ag representing core, NS3 & NS4 proteins	c200 & NS5	HCr43, c200 & NS5	cC22-3, c200 & NS5	Core, NS3, NS4 & NS5
7	Reagents / coating	Streptavidin coated microparticle	Anti-human IgG monoclonal Ab labeled with ester in buffer with bovine serum albumin, sodium azide and surfactant	HCV Ag coated paramagnetic microparticles	Horse Radish Peroxidase (HRP) labelled Ab conjugate	HCV Ag coated para magnetic microparticles
		Biotinylated HCV specific Ag	Streptavidin coated paramagnetic microparticle Biotinylated recombinant c200 HCV Ag & c22p Ag	Anti-human acridinium labelled conjugate	Human IgG captured on well	Mouse monoclonal Anti-human IgG labelled with isoluminal
		HCV specific	Biotinylated	Tris buffer with	Luminol	Isoluminal

		Ag labelled with ruthenium complex	recombined NS5 Ag in buffer with sodium azide	protein stabilizer	derivative & peracidsat	labelled monoclonal antibody
8	Clinical sensitivity	100%	100%	99.10%	99.54%	100%
9	Clinical Specificity	99.84%	99.90%	99.60%	98.22%	99.70%

Source: Leaflets of CLIA of each manufacturer

5. DISCUSSION

Once the hepatitis C virus entered in an individual the virus starts multiplication and develops HCV RNA followed by HCV-Ag and HCV-Ab but screening of blood is usually done for HCV-Ab test and may be followed by supplemental test {Recombinant Immuno-Blotting Assay (RIBA) or Line Immuno Assay (Innolia)} / HCV-Ag or HCV-RNA. The HCV RNA is detectable by PCR as early as 1 week after exposure and HCV Ab develops in 2-6 weeks in acute infection but in some cases HCV Ab may not be positive for 6-9 months [7]. The cost of the machine, reagents and requirement of highly expertise technical person are the biggest challenges for using NAT. Hence, window period of HCV is increased till development and detection of HCV-Ab. The currently available HCV-Ab assay detects antibody at 6-8 weeks after the onset of infection[5] whereas HCV Ag appears within 2-8 days of HCV RNA detection[15]. Generally, screening assay is used for detection of HCV-Ab in the blood banks / diagnostic center but it does not discriminate between cleared infection and chronic infection [18,19,20]. Therefore, it would be better if blood banks / diagnostic center use either HCV core Ag detection assay or HCV Ag-Ab detection assay for screening because HCV-Ag is developed before the HCV-Ab and / or HCV RNA by Nucleic acid testing (NAT). CLIA for HCV is also a choice for those blood banks and laboratories which has tremendous work load of screening of blood which is an automation of immunoassay, therefore, it reduces the human error(s) if occurs during testing. Overall, the use of HCV fourth generation screening assays based on ELISA or CLIA is worth for HCV detection as viral window period is reduces from 4-6 months (1st generation assay) to about a month (4th generation assay).

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