QUALPRO DIAGNOSTICS

TECHNICAL SERIES

Hepatitis C Virus

Perspectives

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Foreword

Qualpro Diagnostics is a part of the innovative **TULIP** Group of companies based at Goa, India.

The group's commitment in building products of international standards, through indigenous R&D has accorded the company virtual leadership in most product segments in the Indian marketplace. Its state-of-art manufacturing facility conforms to the strictest FDA (India) and GMP regulations. In its efforts to build world-class Quality products, the group has recently received the ISO 9001(2000) certification from TUV. It is this commitment to Quality, which has given the group international acclaim

.

The products are now exported to over 45 countries globally with an ever-increasing user base. With decades of experience in *in-vitro* diagnostics (IVD), **TULIP** has created a strong knowledge base. **TULIP** believes that in the knowledge-based society of the 21st century, regular upgradation of knowledge is essential not only for better diagnosis and patient care, but also to improve the overall quality of life

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Publishing of **Technical Series** is one such initiative to make available to the Laboratory professionals and clinicians updated knowledge that is vital for them to set trends in their day-to-day practice.

INTRODUCTION

Primary viral infection of liver cells is caused by several hepatotropic viruses transmitted by oral and parenteral routes. While the clinical, epidemiological, pathological and immunological aspects of viral hepatitis have many common, yet subtle differences, the etiologies of liver disorders cannot be established on the basis of clinical signs and symptoms or on the basis of abnormal liver function tests alone.

The virological and immunological properties of these five well established agents of viral hepatitis are summarised in the Table 1.

BACKGROUND

Hepatitis C is an acute or chronic necroinflammatory disease of the liver that is caused by a unique hepatotropic flavivirus. The disease was first recognized as early as year 1970, when serological tests for Hepatitis A virus (HAV) and Hepatitis B virus (HBV) became generally available. It was noted at that time that most cases of transfusion associated hepatitis were not caused by either HAV or HBV, leading to the term non-A, non-B Hepatitis (NANBH). In the late 1980's, a virally encoded antigen associated with non-A, non-B hepatitis (NANBH) was identified and called the Hepatitis C virus (HCV).This finding rapidly led to the cloning of the viral genome, including the recognition of its proclivity to establish persistent infection, its strong association with chronic hepatitis, cirrhosis and hepatocellular carcinoma.

Interestingly HCV was the first virus discovered by molecular cloning without the use of biological or biophysical methods. This was accomplished by extracting, copying into the cDNA and cloning all the nucleic acid from the plasma of a chimpanzee infected with NANBH by a contaminated factor XIII concentrate.

CLASSIFICATION OF THE HCV VIRUS

The HCV is a spheric, enveloped, positive strand RNA virus approximately 50 nm in diameter. Its structure, genomic organization and replication cycle, are similar to the members of the virus family *Flaviviridae*, yet sufficiently distinct to merit classification into a separate novel genus *Hepacivirus.*

TABLE-1: Hepatitis Viral agents, antigens and antibodies

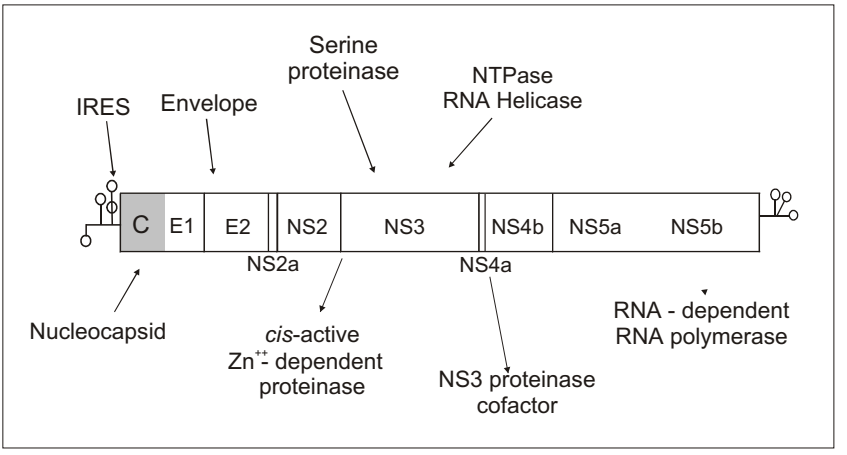
Characteristics	HAV	HBV	HCV	HDV	HEV	HGV
Family	*Picornaviridae*	*Hepadnaviridae*	*Flaviviridae / Togaviridae*	*Viroid*	*Caliciviridae*	*Flaviviridae*
Virion size (nm) & Shape Envelope	27, icosahedral	42, spherical	55, spherical	35,spherical	32, icosahedral	?
Genome	-	+	+	+	-	+
Type	ss RNA	Partially ds DNA	ssRNA	ssRNA	ssRNA	ssRNA
Size (kb)	7.5	3.2	10	1.7	7.5	9.4
Relpication	Positive - strand RNA	Positive - strand RNA intermediate	Positive - strand RNA	Helper HBV	Positive - strand RNA	Positive - strand RNA
Physical shape	Linear	Open circular	Linear	Closed circular	Linear	?
Polyadenylation	+	-	+/-?	-	+	?
Anitgen(s)	HAV antigen	HBsAg, HBeAg, HBcAg	Env/NS1/E1,E2,core/NS3, NS4,NS5	HDV antigen	HEV antigen	NS2 / NS3 / NS5 / E1,E2
Antibody(ies)	Anti - HAV -HAV IgM	Anti-HBs, -HBe -HBc IgM	Anit-core,E1,E2, -NS2, NS3, NS4, NS5	Anti - HDV	Anti - HEV	Anti - HGV
Gene amplification	RT-PCR	PCR	RT-PCR	RT-PCR	RT-PCR	RT-PCR

*ssRNA: single-stranded RNA
dsRNA: double-stranded RNA

ORGANISATION OF THE HCV GENOME

The genome of the HCV is a positive sense, single stranded RNA molecule approximately 9.7 kilobases in length.

Genomic organisation of HCV

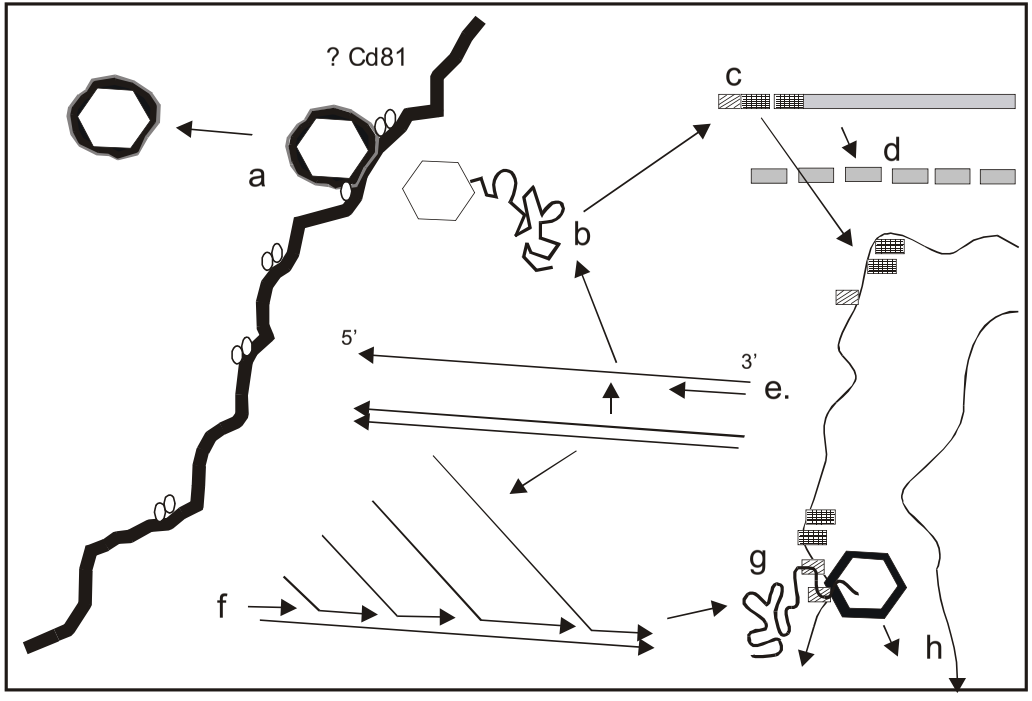


The RNA structure is most similar to that of the family *Flaviviridae*. Consistent with the known functions of the flavivirus proteins, the three N-terminal HCV proteins (C, E1 and E2/ NS2) are probably structural. The four C terminal proteins (NS2, NS3, NS4and NS5) are believed to have a function in viral replication. The 5' UTR (Untranslated Region) is the most highly conserved portion of the genome and is thought to have a role in the translation of the HCV open reading frame. The E1 and E2 regions of the HCV genome demonstrate the highest mutation rate at the nucleotide and amino acid level. The presence of this rapidly changing region may permit a mechanism by which HCV evades host immune surveillance and establishes persistent infections.

REPLICATION CYCLE

Little is known about the details of the HCV replication cycle because there is no permissive cell culture system in which this process can be studied. However, analogies with other positive strand RNA viruses suggest the following scenario which is highly speculative.

Speculative scheme for major events in replication of HCV



a = attachment of virion into hepatocyte.

b = viral RNA released into cytoplasm.

c = cap-independent translation.

d = processing of viral core protein.

e = assembly.

f = synthesis.

g = assembly of virus particles.

h = release from cell.

The liver is usually presumed to be the primary source of the virus present in the blood, however additional data suggest that the virus may replicate within the peripheral mononuclear cells of the lymphoid or the bone marrow.

From the mathematical models of viral kinetics it has been suggested that the half life of a HCV virion in blood is approximately 2.5 hours and upto 1×10^{12} virions may be produced each day in a chronically infected individual. The high level of virion turnover, coupled with the error prone proof reading by NS5B RNA polymerase, results in rapid accumulation of variations within the viral genome. Multiple variants can be recognised from plasma and liver of an individual at any given time.

HCV GENOTYPES, SUBTYPES AND QUASISPECIES

In addition to the impressive heterogeneity that often exists amongst HCV sequences present within an infected individual, (quasispecies variation) there is also a remarkable genetic heterogeneity and divergence amongst the sequences that have been recovered from different individuals (strain and genotype variations). Based on HCV sequences recovered from multiple geographical regions of the world, there are at least six major genotypes of the HCV that have been identified, and many more are in the process of being characterised.

At the second International Conference of HCV and related viruses, a consensus nomenclature system was proposed for the future studies of the HCV genotypes and subtypes.

According to this system the HCV is classified on the basis of their nucleotide sequence into major genetic groups designated as “genotypes” and are assigned a number (arabic numerals) in the order of their discovery. The more closely related HCV strains within same genotypes are designated “subtypes” and are assigned lower case letters (in alphabetical order) in the order of their discovery. The complex of genetic variants found within an infected individual’s isolate are termed as the “quasi species”.It is hypothesized that the distinct viral quasispecies play a role in the pathogenesis and progressive HCV infection. Due to the sequence variability of the quasispecies post infection, HCV is present in patients as a pool of viruses representing different epitopes. Modification of both B and T epitope patterns during HCV infection have been observed and clones contribute to HCV evasion from the immune system.

Terminology used in studies related to the HCV genomic heterogeneity

Terminology	Definition	% Nucleotide* similarity
Genotype	Genetic heterogeneity among different HCV Isolates	65.7-68.9
Subtype	Closely related isolates within each of the major genotypes	76.9 - 80.1
Quasispecies	Complex of genetic variants within individual isolates	90.8 -99.0

* % Nucleotide Similarity refers to the nucleotide sequence identities of the full - length sequences of the HCV genome.

Comparitive sequence analysis amongst HCV subtypes

Comparative sequence analysis among HCV subtypes of a 222-nucleotide segment derived from the viral NS5 region*

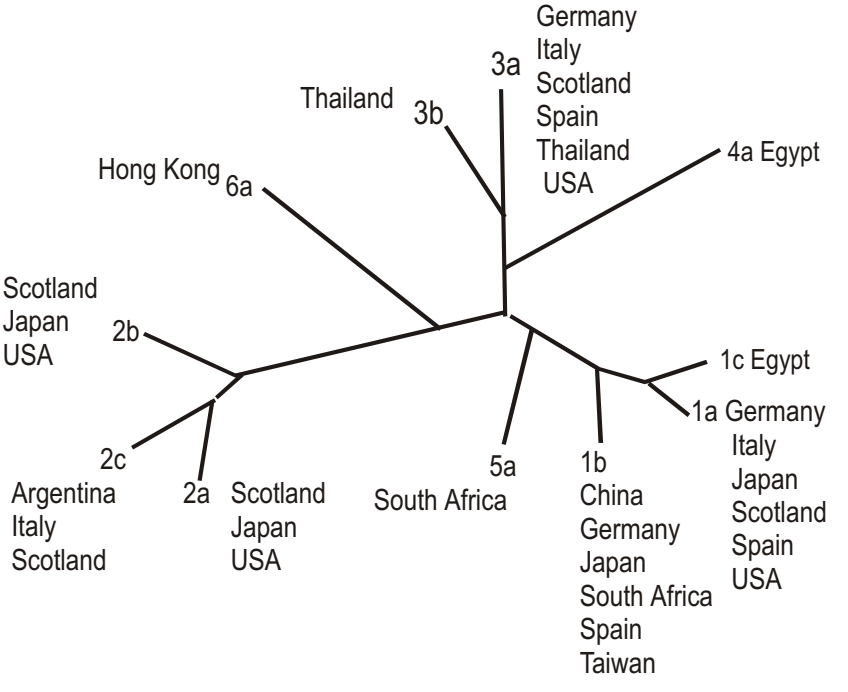
	% Similarity to										
Subtype	**1a**	**1b**	**1c**	**2a**	**2b**	**2c**	**3a**	**3b**	**4a**	**5a**	**6a**
1a	100	81	85	65	66	63	67	66	68	69	64
1b		100	77	64	67	64	67	71	64	70	65
1c			100	68	70	67	65	70	64	61	61
2a				100	82	77	67	67	66	66	68
2b					100	81	64	69	65	67	66
2c						100	64	65	65	66	65
3a							100	79	65	67	64
3b								100	66	68	61
4a									100	66	66
5a										100	68
6a											100

*Nucleotide position 7975 to 8196 of the prototype virus.

The phylogenetic grouping of HCV strain appears to be independent of the segment of genome that is analysed.

Substantial regional differences appear to exist in the distribution of HCV genotypes. Although genotypes 1,2 and 3 appear to have a worldwide distribution, their relative prevalence varies from one geographical area to another.

Worldwide geographic distribution of HCV genotypes and subtypes



HCV subtypes 1a and 1b are most common in USA and also predominant in Europe. Subtype 1b is responsible for upto 73% of the HCV infections in Japan. Subtype 2c is found commonly in north Italy whereas subtype 3a is particularly prevalent in IV drug users in Europe and North America. The genotype 4 is prevalent in North Africa and the Middle East and 5 and 6 seems to be confined to South Asia and Hong Kong respectively. Genotype 7, 8, 9 have been identified in Vietnamese patients and 10 and 11 have been identified in Indonesia. In India, genotype 1 and 3 are common to all parts of India, where as in the south of India, genotype 1 and 4 are responsible for infection.

The presence of numerous genotypes may provide clues about the historical origin of the HCV as well as proof that HCV has been endemic in these areas for a long time.

CLINICAL RELEVANCE OF THE HCV GENOTYPES

The impact of HCV heterogeneity and genotypes on the day to day clinical management of chronic HCV infection has not been well established with regards to its role in progression of liver disease, the outcome of HCV infections and response to interferon therapy. However, the sensitivity and specificity of serologic and virologic assays for the detection of HCV may be influenced by the heterogenity of the HCV.

Potential role of HCV heterogeneity and genotypes has been suggested for mother to infant transmission and sexual transmissions. It is also suggested that genotypes 3a and 1a are closely associated with the IV drug usage and 1b in patients who acquired HCV through blood transfusion. Genotype 1b is reportedly associated with more severe disease and poor response to treatment.

Studies have provided some evidence that viral factors including the genotype may potentially play an important role in the development of chronic infection following exposure to HCV.

EPIDEMIOLOGY OF HCV

HCV is most often transmitted by percutaneous exposure to infected blood. The predominant modes of transmission may change over time and differ between and even within countries.

HCV has been reported in virtually every country and the rough prevalence data estimates that more than 170 million people are infected worldwide.

In developed nations the HCV prevalence is typically 1-2%, except for a few geographical regions such as Egypt, Japan, Taiwan and Italy where a high prevalence rate of infection, of between 10-30% have been reported. In India, the HCV prevalence rate is reported to be in the range of 1.5 - 2%.

Folk remedies such as acupuncture, cutting with unsterilized knives and mass inoculation programs where frequently, unsterilized needles were often reused, have been suggested as likely modes of transmission. IV drug use, use of contaminated needles associated with illicit drugs, also remain one of the major causes of HCV infection worldwide.

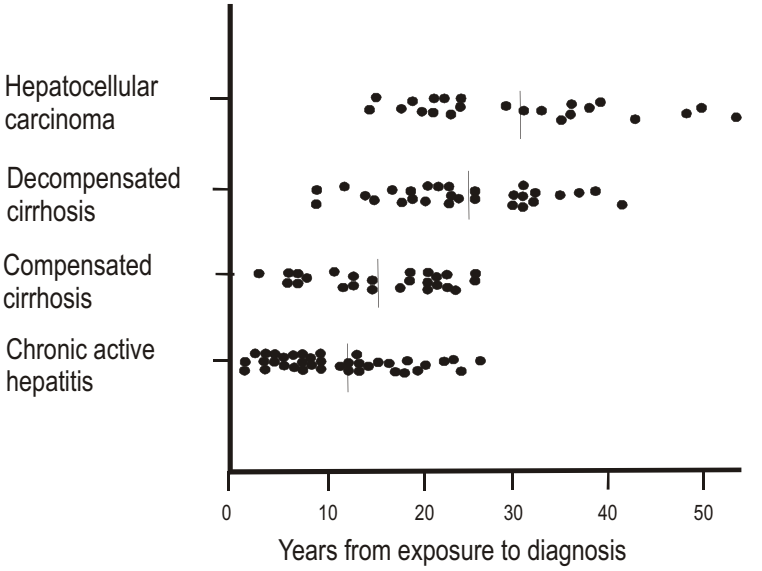
BIOLOGICAL BASIS OF TRANSMISSION OF HCV

By using sensitive techniques, HCV RNA can be detected in blood (including serum and plasma), saliva, tears, synovial fluids, ascitic fluids, CSF and breast milk. However, information regarding potential infectiousness of body fluids is scarce. Without percutaneous exposure into blood stream, it is not clear as to how the virus reaches the liver, its primary site of replication. Ability of the virus to replicate in peripheral mononuclear cells has been also implicated. Mounting circumstantial evidence also suggests that HCV may be transmitted during sexual intercourse, though infrequently. HCV is uncommonly transmitted from mother to infant and the perinatal frequency of infection may vary between 0 - 8%.

SPECTRUM OF CLINICAL HCV INFECTION

Chronic hepatitis C infection varies greatly in its course and outcome. At one end the spectrum are patients who have no signs or symptoms of liver disease and completely normal levels of serum liver enzymes (ALT). Liver biopsy usually shows some degree of chronic hepatitis, but the degree of injury is usually mild, and the overall prognosis may be good. At the other end of the spectrum are patients with severe hepatitis C infection who have symptoms, HCV RNA in serum, and elevated serum liver enzymes, and who ultimately develop cirrhosis and end-stage liver disease. In the middle of the spectrum are many patients who have few or no symptoms, mild to moderate elevations in liver enzymes, and an uncertain prognosis. Researchers estimate that at least 20% of patients with chronic hepatitis C develop cirrhosis, a process that takes 10 to 20 years post infection. After 20 to 40 years, a smaller percentage of patients with chronic disease may develop liver cancer.

Mean time between exposure to HCV & diagnosis of HCV-related complications



It has been noted that coinfection with HBV accelerates the progression of the disease. HIV infection increases the level of HCV viremia and is associated with more rapid progression of the liver disease. Immunosuppression associated with agammaglobulinemia and organ transplantation also accelerates disease progression. In children asymptomatic infections as well as liver failures may occur. Elderly persons and males appear to be at a relatively higher risk for cirrhosis as well as liver cancer.

CLINICAL SYMPTOMS AND SIGNS

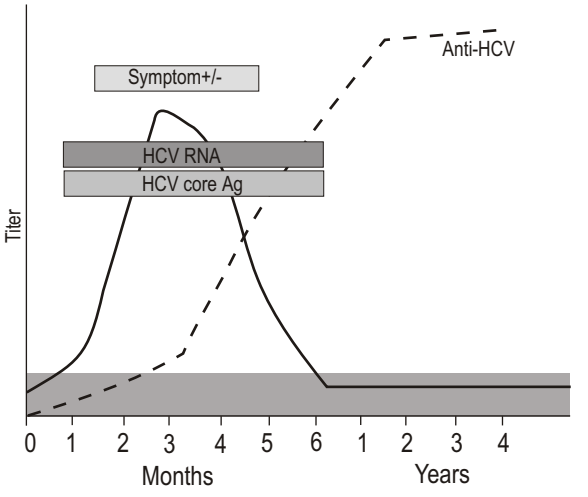
Many people with chronic hepatitis C have no symptoms of liver disease. If symptoms are present, they are usually mild, nonspecific, and intermittent. They may include:

- Fatigue
- Mild right-upper-quadrant discomfort or tenderness
- Nausea
- Poor appetite
- Muscle and joint pains

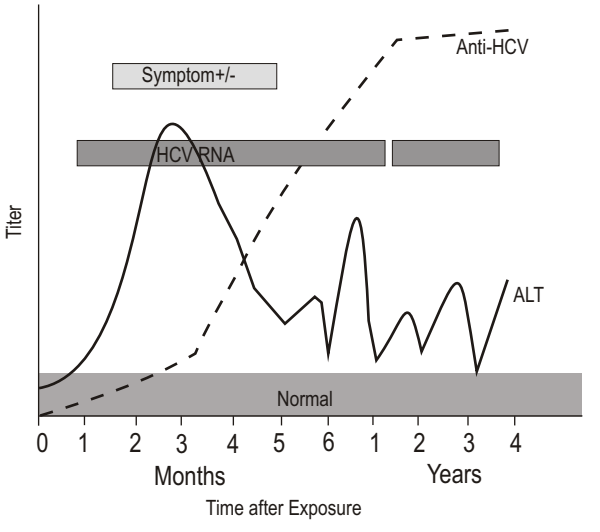
Similarly, the physical examination is likely to be normal or show only mild enlargement of the liver or tenderness. Some patients have vascular spiders or palmar erythema.

SEROLOGICAL PATTERN OF HCV INFECTION

Acute HCV infection with recovery



Acute HCV infection with progression to chronic infection



CLINICAL FEATURES OF CIRRHOSIS

Once a patient develops cirrhosis or if the patient has severe disease, symptoms and signs are more prominent. In addition to fatigue, the patient may complain of muscle weakness, poor appetite, nausea, weight loss, itching, dark urine, fluid retention, and abdominal swelling.

Physical findings of cirrhosis may include:

- Enlarged liver
- Enlarged spleen
- Jaundice
- Muscle wasting
- Excoriations
- Ascites
- Ankle swelling

EXTRAHEPATIC MANIFESTATIONS

Complications that do not involve the liver develop in 1 - 2 % of people with hepatitis C. The most common is cryoglobulinemia, which is marked by

- Skin rashes, such as purpura, vasculitis, or urticaria
- Joint and muscle aches
- Kidney disease
- Neuropathy
- Cryoglobulins, rheumatoid factor, and low complement levels in serum

Other complications of chronic hepatitis C are:

- Glomerulonephritis
- Porphyria cutanea tarda

Diseases that are less well documented to be related to hepatitis C are:

- Seronegative arthritis
- Keratoconjunctivitis sicca (Sjögren's syndrome)
- Non-Hodgkin's type B-cell lymphomas
- Fibromyalgia
- Lichen planus

There is a poor correlation between necroinflammatory liver injury, serum ALT levels, HCV RNA and extent of fibrosis. It is not possible to predict which HCV patient will develop cirrhosis and which will go to clinically decompensated liver disease.

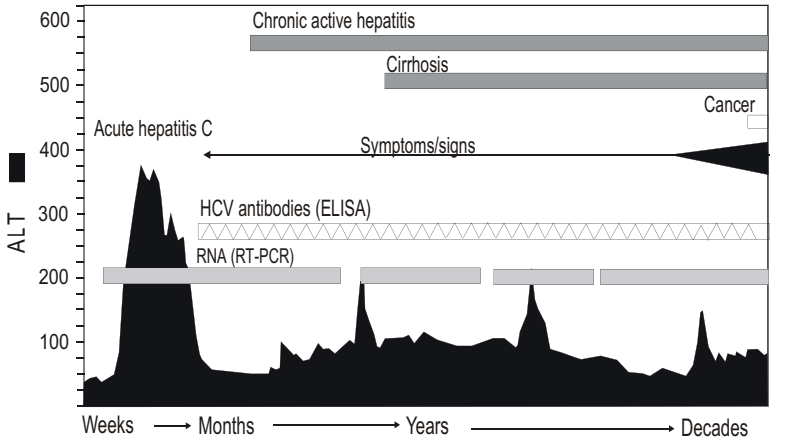
The major complication of HCV infection is the development of hepatic fibrosis progressing to cirrhosis. Primary hepatocelluar carcinoma is typically a late complication of chronic hepatitis C infection.

DIFFERENTIAL DIAGNOSIS

The major conditions that can be confused clinically with chronic hepatitis C include:

- Autoimmune hepatitis
- Chronic hepatitis B and D
- Alcoholic hepatitis
- Nonalcoholic steatohepatitis (fatty liver)
- Sclerosing cholangitis
- Wilson's disease
- Alpha-1-antitrypsin-deficiency-related liver disease
- Drug-induced liver disease

Natural History of HCV infections



BASIS FOR DIAGNOSIS OF HCV INFECTIONS

Acute HCV infection is typically mild and often not diagnosed till it becomes chronic. The two basic methods for HCV laboratory diagnosis are based on:

(a) Serological identification of anti-HCV antibodies from the plasma / serum of infected patients

(b) Detection of HCV RNA in the serum of infected patients

Most of the current diagnostic tests for detection of anti-HCV antibodies cannot differentiate between chronic and acute HCV infections because anti-HCV IgM occurs variably in acute infection and is also detected at high rates in patients with chronic HCV infection. However, double antigen sandwich based fourth generation immunoassays that detects IgG & IgM simultaneously, provides clinical decision makers an advanced tool to diagnose HCV infection at all stages of the disease.

Anti-core and anti-NS3 may be the first antibodies to appear during acute phase (defined by increased ALT levels and symptoms). Anti-NS5 appears somewhat later while anti-NS4 is the last antibody to be detected in an acute self limited infection.

HCV RNA in serum or liver appears to be the earliest detectable marker of acute HCV infection preceeding the appearance of anti-HCV by several weeks. HCV viremia may persist despite normalization of serum ALT levels. In acute self limited HCV infection the HCV RNA in serum usually last for fewer than four months.

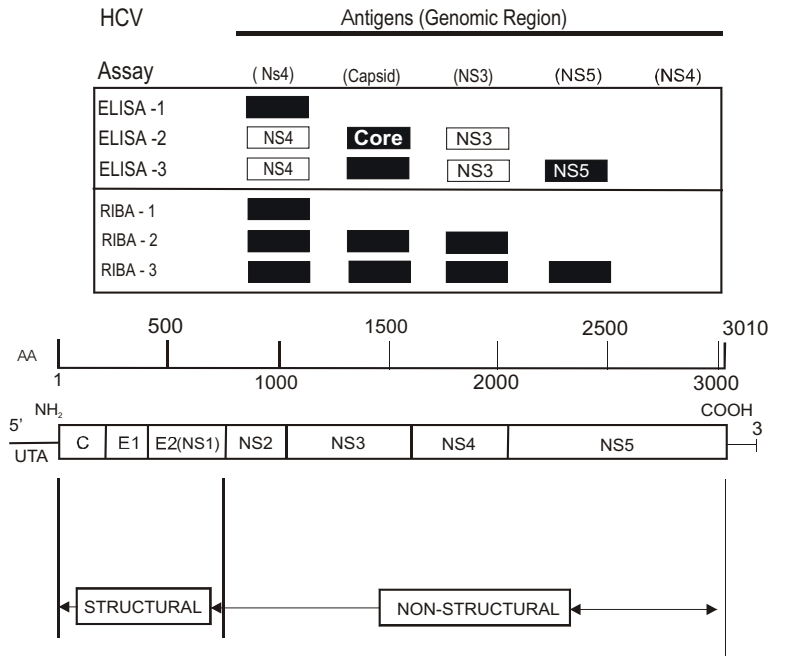
DEVELOPMENT OF SEROLOGICAL ASSAYS

As the HCV genome and the sero-response to viral proteins has been understood over time, the development of immunoassays based on the HCV recombinant proteins have been progressing in sensitivity and specificity and ability to detect early seroconversions in HCV infected individuals. The first generation immunoassays used a fusion protein from the NS4 region. The first generation assays were able to detect antibodies to HCV 4-6 months after transfusion in a majority of recipients and often the anti-HCV was undetectable by these assays. Subsequently second generation immunoassays were developed and were based on use of three immunodominant regions of the HCV antigen, namely capsid, NS3 and NS4. The second generation immunoassays shortened the time of detection of anti-HCV antibodies by 3-12 weeks as compared to the first generation assays, and have picked up more patients as positives who were reported negative with first generation methods. Third generation immunoassays which incorporate an additional NS5 antigen to the second generation assays have demonstrated greater sensitivity as compared to the second generation assays. This increase in sensitivity is due to the increased detectability of anti-NS5.Third generation assays have also shown greater correlation with supplemental assays such as RIBA 3.0 with fewer indeterminates as compared to the assays of the earlier generation.

The first, second and third generation HCV antibody assays still lack sensitivity in seroconversions or show inexplicable discrepancies with confirmatory assays. This is primarily due to poor cross-reactivity with the current HCV genotype 1 antigen-based assays.To solve this problem fourth generation assays that uses antigens having highly conserved epitopes from multiple HCV genotypes that includes genotypes 2 & 3 apart from genotype 1 are being developed and evaluated. Also attempts are being made to develop 4th generation assays based on Double antigen sandwich technology where all antibody isotypes (IgM, IgG, IgA etc.)of the active HCV seroresponse can be captured on the testing platforms.

Development of double antigen based sandwich assays using the antigen on the capture as well as tracer componets of the immunoassay systems have further improved sensitivity and specificity with results nearing comparability to HCV-RNA based assays. The 4th generation immunoassays currently are available as EIA and rapid tests based on Lateral Flow Technology.

Evolution of serological assays



Generations of serological assays for diagnosis of HCV

Assay Generation	Antigen(s)	Diagnostic Window (weeks)	Analytical Data
1 (1989)	NS4	16	Sensitivity:80% Specificity: 30%
2 (1992)	Core, NS3, NS4	10	Sensitivity:90% 2-5% false neg. in immunosuppressed patients
3 (1995)	Core, NS3, NS4, NS5	7-8	Sensitivity:>99% Specificity: >99%

Clinical features associated with HCV infection

Incubation period - Average - Range	 6-7 weeks 2-26 weeks
Clinical illness	30-40%
Jaundice	20-30%
Chronic hepatitis	70%
Persistent infection	85-100%
Immunity	No protective antibody response identified

IgM CLASS ANTI-HCV ANTIBODIES

The primary IgM class anti-HCV response is usually against the core polypeptide and is detectable in patients with acute HCV infections and is still the first active antibody response. The IgM anti-HCV is rapidly followed by the IgG response. IgM anti-HCV is however not limited to the acute phase of the disease and is also found for protracted periods in long term HCV chronic patients. However, there is a strong association of the

presence of anti-HCV IgM with HCV RNA positivity, patients progressing to ESRD, ESLD, in HCV disease reactivation patients and non responders to therapy.

Thus anti-HCV IgM activity is useful as a serological marker for ongoing, persistent and active HCV replication and HCV infection.

Serological assays that can detect anti-HCV IgM along with anti-HCV IgG class of antibodies may more consistently detect and indicate acute as well as chronic HCV seroresponse during the spectrum of the HCV infection. Since, during early phase of acute infection, patients are usually asymptomatic, from transfusion point of view, assays detecting an evolving IgM response may add value to the detection of infected blood.

SUPPLEMENTARY ASSAYS

Supplementary assays such as recombinant immunoblot assays and HCV RNA based assays are used to reconfirm the positive results of EIAs and other first line rapid immunoassays.

RECOMBINANT IMMUNOBLOT ASSAYS

Immunoblot assays are used to confirm anti-HCV reactivity with screening tests. These tests are also called “Western blots”. Serum is incubated on nitrocellulose strips on which recombinant viral proteins are blotted /sprayed. Color changes brought about by reaction of Enzyme conjugated to Anti-human IgG with the substrate indicate that antibodies are adhering to the blotted proteins on the solid phase. An immunoblot is considered positive if two or more proteins react and is considered indeterminate if only one positive band is detected. In some clinical situations, confirmatory testing by immunoblotting is helpful, such as for the patients with anti-HCV detected by EIAs who test negative for HCV RNA. The EIA anti-HCV reactivity in such cases could represent a false-positive reaction, or recovery from hepatitis C, or continued virus infection with levels of virus too low to be detected (the last occurs only rarely when sensitive PCR assays are used). If theImmunoblot test for anti-HCV is positive, the patient has most likely recovered from hepatitis C and has a persistent antibody to the virus. If the immunoblot test is negative, the EIA result was probably a false positive.

Immunoblot tests are routine in blood banks when anti-HCV-positive sample is found positive by EIA. Immunoblot assays are highly specific and valuable in verifying anti-

HCV reactivity. Indeterminate tests require further follow-up testing, including attempts to confirm the specificity by repeat testing for HCV RNA.

HCV RNA BASED ASSAYS

HCV RNA can be detected in plasma and serum by RT PCR's and branched DNA (bDNA) based assays. These assays vary in their ability to detect HCV RNA but RT PCR's are generally considered to be more sensitive than bDNA based assays.

Detection of HCV RNA indicates ongoing infection whereas clearance from serum of HCV RNA spontaneously or after treatment correlates with ALT normalisation and improvement in liver histological findings. Testing for HCV RNA is particularly useful for diagnosis of HCV when ALT is normal or when the values are slightly elevated, or when anti HCV is negative or when several other causes of liver diseases are not implied. HCV RNA estimation is especially helpful in diagnosis of infections in immunosuppressed, immunocompromised, organ transplant or patients having chronic renal failure.

Since the maternal antibodies are passively transferred to infants, the diagnosis of HCV infection in infants must be based on viral DNA based tests. The anti HCV antibodies persist in the infants even after 18 months of age. Since viremia can be intermittent in the first year of life and some HCV RNA positive infants never develop anti-HCV antibodies, infection in infants can be excluded only based on repeat HCV RNA test findings.

It must however be understood that HCV RNA assays have an intrinsic variation. Additionally there is a lack of a quantitative gold standard for HCV RNA based assays and the reporting units differ. Serial measurement of viremia must be performed using the same tests and preferably using the same laboratory setup.

SEROLOGICAL DIAGNOSIS OF HCV: OPEN ISSUES

In spite of the various technological developments in HCV serodiagnosis, the following issues will require continued resolution:

- Improvement of analytical sensitivity and specificity of antibody and antigen assays
- Detection of low antibody titers in immunosupperssed patients
- Earlier detection of seroconversion
- Serological assays to unequivocally distinguish between acute/chronic and resolved infections
- Serological marker for immunity

APPROACH TO LABORATORY DIAGNOSIS OF HCV INFECTIONS

The approach to laboratory diagnosis for HCV infections needs to be looked at based on the local resources and the prevalent infection rates. Predictive values of the assay results would depend upon the prevalent rates and the type of setting. Diagnostic algorithms have been developed to suit these conditions.

BIOCHEMICAL INDICATORS OF HCV INFECTION

- In chronic hepatitis C, increases in the alanine and aspartate aminotransferases range from 0 to 20 times (but usually less than 5 times) the upper limit of normal.
- Alanine aminotransferase levels are usually higher than aspartate aminotransferase levels, but this finding may be reversed in patients who have cirrhosis.
- Alkaline phosphatase and gamma glutamyl transpeptidase are usually normal. If elevated, they may indicate cirrhosis.
- Rheumatoid factor, low platelet counts and white blood cell counts are frequent in patients with cirrhosis, providing clues to the presence of an advanced disease.
- The enzymes lactate dehydrogenase and creatine kinase are usually normal.
- Albumin levels and prothrombin time are normal until advanced stage of the disease.
- Iron and ferritin levels may be slightly elevated.

NORMAL SERUM ALT LEVELS

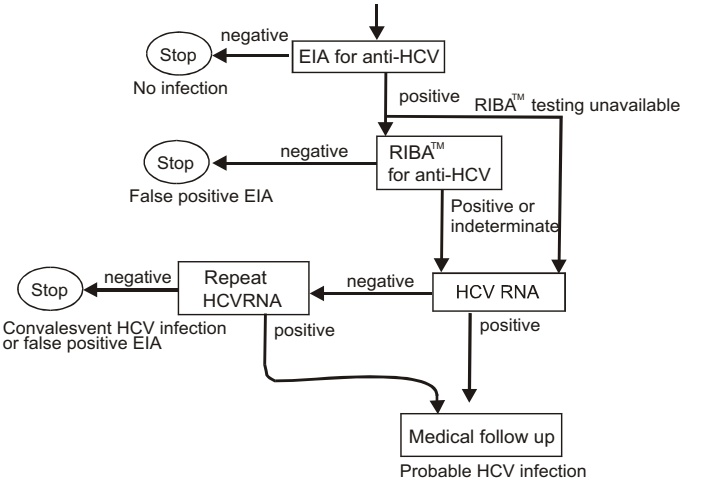
Some patients with chronic hepatitis C have normal serum alanine aminotransferase (ALT) levels, even when tested on multiple occasions. In this and other situations in which the diagnosis of chronic hepatitis C may be questioned, the diagnosis should be confirmed by testing for HCV RNA. The presence of HCV RNA indicates that the patient has ongoing viral infection despite normal ALT levels.

LIVER BIOPSY

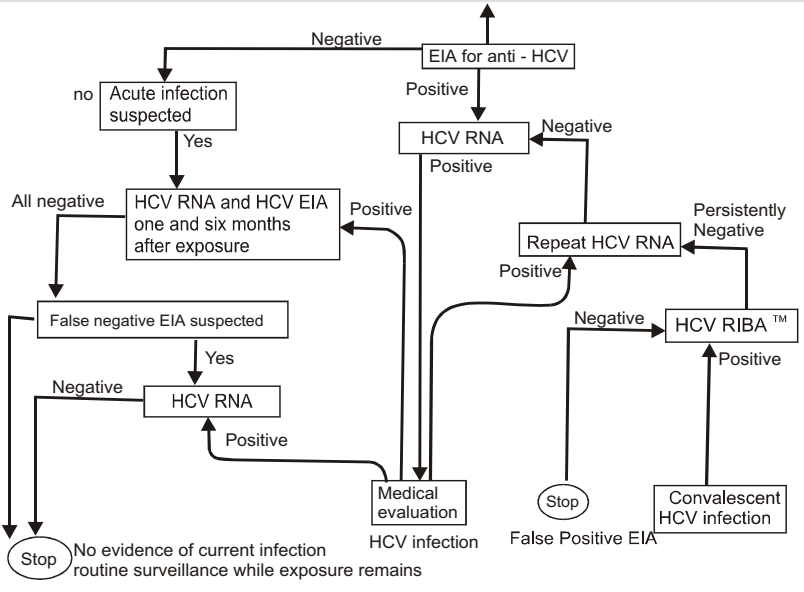
Liver biopsy is not necessary for diagnosis but is helpful for grading the severity of disease and staging the degree of fibrosis and permanent architectural damage of the liver. Hematoxylin stains, Eosin stains and Masson's trichrome stain are used to grade the amount of necrosis and inflammation and to stage the degree of fibrosis. Specific immunohistochemical stains for HCV have not been developed for routine use. Liver biopsy is also helpful in ruling out other causes of liver disease, such as alcoholic liver injury or iron overload.

ALGORITHMS FOR DIAGNOSIS OF HCV INFECTIONS

Low Prevalence Hepatitis C Screening



High Prevalence Hepatitis C Screening



HCV causes the following changes in liver tissue:

- Necrosis and inflammation around the portal areas, so-called “piece meal necrosis” or “interface hepatitis.”
- Necrosis of hepatocytes and focal inflammation in the liver parenchyma.
- Inflammatory cells in the portal areas (“portal inflammation”).
- Fibrosis, with early stages being confined to the portal tracts; in the intermediate stages being the expansion of the portal tracts and bridging between portal areas or to the central area; and in the late stages being frank cirrhosis characterized by architectural disruption of the liver with fibrosis and degeneration.

Grading and staging of hepatitis by assigning scores for severity are helpful in managing patients with chronic hepatitis. The degree of inflammation and necrosis can be assessed as none, minimal, mild, moderate, or severe. The degree of fibrosis can be similarly assessed. Scoring systems are particularly helpful for clinical studies on chronic hepatitis.

PREVENTION OF HCV INFECTION

The key to reducing the incidence of HCV is by decreasing exposure to contaminated blood and reducing post transfusion HCV infection rates. Nosocomial HCV transmission can be controlled through adherence to universal precautions and infection control protocols diligentlly.

TREATMENT OF HCV INFECTION

The therapy of chronic hepatitis C has evolved steadily since alpha interferon was first approved for use in this disease more than ten years ago.

Recombinant forms of alpha interferon are available as therapy of hepatitis C infections. These standard forms of interferon, however, are now being replaced by pegylated interferons (peginterferons). Peginterferon is alpha interferon that has been modified chemically by the addition of a large inert molecule of polyethylene glycol. Pegylation changes the uptake, distribution and excretion of interferon prolonging its half-life. Peginterferon can be given once weekly and provides a constant level of interferon in the blood, whereas standard interferon must be given several times weekly. Peginterferon is more active than standard interferon in inhibiting HCV and yields higher sustained response rates although with similar side effects.

Persons who should be screened for HCV infections

High prevalence	Post exposure testing
Persons who ever inject illegal drugs.	Persons with precutaneous or heavy mucosal exposure to HCV-positive blood.
Persons with elevated aminotransferase levels.	Children born to HCV-infected women.
Persons on hemodialysis.	Sexual partners of HCV-infected persons, who should consider HCV screening, though the risk of transmission through intercourse is low.
Persons who received transfusions or organ transplants including clotting factor concentrates produced before 1987 or either a transfusion before July 1992.	
Persons in settings with demonstrated high HCV prevalence and where risk factor ascertainment may be poor, e.g. inmates, patients attending inner-City clinics for sexually transmitted disease and patients attending some	

Ribavirin is an oral antiviral agent that has an activity against a broad range of viruses. By itself, Ribavirin has little effect on HCV, but its addition to Interferon therapy increases the sustained response rate by two- to three-folds. For these reasons, combination therapy is now recommended for hepatitis C and Interferon monotherapy is applied only when there are specific reasons not to use Ribavirin.

Combination therapy leads to rapid improvements in serum ALT levels and disappearance of detectable HCV RNA in up to 70 percent of patients. However, long-term improvement in hepatitis C occurs only if HCV RNA disappears during therapy and stays undetectable once therapy is stopped. Among patients who become HCV RNA negative during treatment, a proportion of them relapse when therapy is stopped. The relapse rate is lower in patients treated with combination therapy compared with monotherapy. A response is considered “sustained” if HCV RNA remains undetectable for six months or more after stopping therapy.

CLASS OF PATIENTS WHO SHOULD BE TREATED

Patients with anti-HCV, HCV RNA, elevated serum aminotransferase levels, and evidence of chronic hepatitis on liver biopsy, and with no contraindications, should be offered therapy with the combination of alpha Interferon and Ribavirin. The National Institutes of Health Consensus Development Conference Panel, USA, recommends that therapy for hepatitis C be limited to those patients who have histological evidence of progressive liver disease. The panel recommends that all patients with fibrosis or moderate to severe degrees of inflammation and necrosis on liver biopsy should be treated and that patients with less severe histological disease be managed on an individual basis. Patient selection should not be based on the presence or absence of symptoms, the mode of acquisition, the genotype of HCV RNA, or serum HCV RNA levels.

Patients with cirrhosis found through liver biopsy can be offered therapy if they do not have signs of decompensation, such as ascites, persistent jaundice, wasting, variceal hemorrhage, or hepatic encephalopathy. However, interferon and combination therapy have not been shown to improve survival or the ultimate outcome in patients with preexisting cirrhosis.

Patients older than 60 years also should be managed on an individual basis, since the benefit of treatment in these patients has not been well documented and side effects appear to be worse in older patients.

The role of Interferon therapy in children with Hepatitis C infection remains uncertain. Ribavirin has yet to be evaluated adequately in children, and pediatric doses and safety have not been established. Thus, if children with hepatitis C are treated, monotherapy is recommended, and Ribavirin should not be used outside of controlled clinical trials.

In people with both HCV and HIV infection, benefits of therapy for hepatitis C have only recently been evaluated. The decision to treat people co-infected with HIV must take into consideration the concurrent medications and medical conditions. If CD4 counts are normal or minimally abnormal (» 400/mL), responses are similar in frequency to those in patients who are not infected with HIV. The efficacy of combination therapy in HIV-infected people has been tested in only a small number of patients. Ribavirin may still have significant undocumented interactions with other antiretroviral drugs.

In many of these uncertain situations, the indications for therapy should be reassessed at regular intervals. In view of the rapid developments in hepatitis C today, better therapies may become available within the next few years, at which point expanded indications for therapy would be appropriate.

In patients with clinically significant extrahepatic manifestations, such as cryoglobulinemia and glomerulonephritis, therapy with alpha interferon can result in remission of the clinical symptoms and signs. However, relapse after stopping therapy is common. In some patients, continual, long-term alpha interferon therapy can be used despite persistence of HCV RNA in serum if clinical symptoms and signs resolve on therapy.

CLASS OF PATIENTS WHO SHOULD NOT BE TREATED

Therapy is inadvisable outside of controlled trials for patients who have:

- Clinically decompensated cirrhosis because of hepatitis C
- Normal aminotransferase levels
- A kidney, liver, heart, or other solid-organ transplant
- Specific contraindications to either monotherapy or combination therapy

Contraindications to alpha interferon therapy include severe depression or other neuropsychiatric syndromes, active substance or alcohol abuse, autoimmune disease (such as rheumatoid arthritis, lupus erythematosus, or psoriasis) that is not well controlled, bone marrow compromise, and inability to practice birth control. Contraindications to ribavirin and thus combination therapy include marked anemia, renal dysfunction, and coronary artery or cerebrovascular disease, and, again, inability to practice birth control.

APPROACH TO TREATMENT

Before beginning of treatment preexisting diseases/ disorders in the patient must be assessed and counseling regarding side effects and possible outcomes of treatment must be discussed. The Standard algorithms for pretreatment, during treatment and post treatment, for required dose adjustment and management of side effects must be diligently applied.

FUTURE OF THERAPY FOR HCV INFECTIONS

New medications based on novel approaches for the treatment of HCV infected patients are being developed. The most promising being the use of other cytokines and antivirals such as RNA polymerase inhibitors or protease inhibitors. With increased awareness, better diagnosis and therapeutics, mankind can hope to control the worldwide epidemic of HCV.

REFERENCES AND SUGGESTED READING

- Pieter Leyssen et al; Perspectives for the treatment of Infectious with *Flaviviridae*;Clin. Microbiol. Rev; Vol.13, No. 1, Jan 2000, p 67 -82.
- Gang Yang and Girish N. Vyas; Minireview; Immunodiagnosis of Viral Hepatitides A to E and Non-A to -E; Clin. Diag. Lab. Immunol. Vol.3 No.3, May 1996, p 247-256.
- N.N. Zein, Clinical Significance of Hepatitis C Virus Genotypes; Clin. Microbiol. Rev; Vol.13, No. 2, Apr 2000, p 223 -235.
- Principles and Practice of Infectious Diseases, Edited by G.L. Mandell et al; 5th Edition; Vol 2. Part 1.
- Indian Journal of Hematology and Blood Transfusion; Vol. XIX, No.I, March 2001.
- P. Chen et al; Transient Immunoglobulin M antibody response to Hepatitis C Virus Capsid Antigen in Posttransfusion Hepatitis C: Putative Serological Marker forAcute Viral Infection; Proceedings of the National Academy of Sciences; Vol. 89, 5971-5975.
- Carmela Casino et al; Diagnostic Value of Anti-Hepatitis C Virus (HCV) Core Immunoglobulin M in recurrence of HCV infection after Orthotopic Liver transplantation; Journal of Clinical Microbiology;Vol.:37, No.:8, Aug. 1999, 2726-2728.
- John M. Clemens et al; IgM antibody response in acute Hepatitis C viral infection; Blood; Vol.:79, No.: 1, 1992, 169-172.
- F. Fabrizi et al; IgM antibody response in Hepatitis C virus in end - stage renal disease;Nephrology Dialysis Transplantation; Vol.:11, Issue: 2, 1996, 314-318.
- Jonathan F. Zmuda et al; Recognition of Multiple Classes of Hepatitis Cantibodies increases detection sensitivity in oral fluids; Clinical and Diagnostic Laboratory Immunology, Vol.:8, No.:6, Nov. 2001, 1267-1270.
- Maertens G et al. A fourth generation assay for the screening of HCV Antibodies. Poster presented at the European Association for the Study of the Liver, 34th Annual Meeting, Naples(Italy), April 8 -12, 1999.

NOTES

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