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An Overview of the Laboratory Assay Systems and Reactives Used in the Diagnosis of Hepatitis C Virus (HCV) Infections

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1. Introduction

In the mid-1970s, a new disease entity termed 'non-A, non-B' (NANB) hepatitis was first described and, in the following years, led to discovery of the causative virus, post-transfusion, and to community-acquired NANB hepatitis increasingly becoming recognized as a potentially serious disease that results in liver cirrhosis and/or hepatocellular carcinoma.[16, 26] Hepatitis C virus (HCV) was first identified in 1989 using molecular methods at the Chiron Corporation, but to date, the virus has never been visualized or grown in cell culture.[37] Hepatitis C virus (HCV) is a single-stranded RNA virus with a genome of about 10 000 nucleotides containing a single large, continuous open reading frame and with organization most closely resembling the *Flaviviridae*.[11] HCV is a global healthcare problem and the World Health Organization (WHO) estimates that at least 170 million people (3 % of the world's population) are infected with HCV worldwide and most of the patients are concentrated in developing countries. [48]

HCV Proteins. HCV proteins may be divided in two groups: Structural proteins and nonstructural proteins. Structural Proteins: The nucleocapsid proteins (core), two envelope glycoproteins (E1 and E2), and a small transmembrane protein p7. E2 likely mediates cell entry by binding to one ore more specific cellular receptors or coreceptors, and has also been suggested to interact with and inhibit interferon-inducible protein kinase R (PKR).[26] P7 may function as a viroporin. Non-structural proteins (NS): NS2, NS3, NS4 (A, B), NS 5 (A, B). NS2 may exist an E2p7NS2 processing intermediate due to inefficient signal peptidase cleavages at the E2-p7 and p7-NS2 junctions. NS2 has also been reported to coimmunoprecipitate. Other functions of NS2 are uncertain. NS3 has serine protease/helicase activity and a multifunctional protein. NS4A is associated with membranes. NS4B is important for RNA replication. It has a GTPase acitivity that is important RNA in replication. NS5B has RNA-depndent RNA polymerase activity resulting in initiating in-vitro RNA synthesis both primer dependent and independent. Anti-HCV reactives manufactured from these group of proteins. [4, 15, 35]

Organization of the HCV genome and polyprotein processing presented in **Figure 1**. [26]

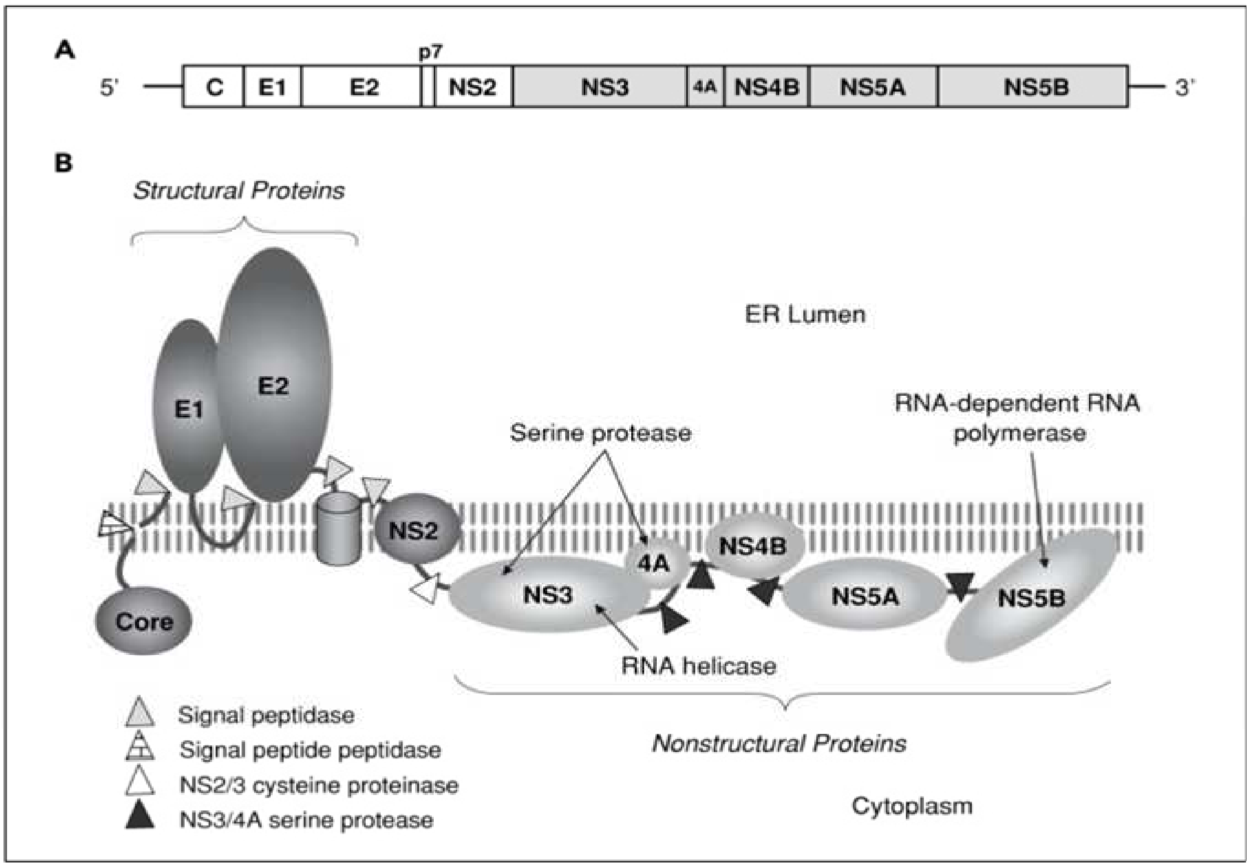


Fig. 1. Organization of the HCV genome and polyprotein.[26]

2. Classification of laboratory assay systems and reactives used in the diagnosis of hepatitis C virus infection.

Immunoasssay systems used in the diagnosis of hepatitis C virus infections may be divided into four groups.

2.1. Anti-HCV assay.
2.2. Strip immunblott assay (SIA).
2.3. Hepatitis C virus core antigen assay.
2.4. HCV RNA assay.

Diagnostic tests used for the diagnosis of HCV infections may be divided into three groups according to aim of use. [22]

1. Screening test (Anti-HCV tests based on EIA or CIA)
2. Supplemental test (Strip immunoblot assay-SIA)
3. Confirmatory test (HCV RNA) (HCV Core Ag as a pre-confirmative test.).

Detection or quantification of HCV RNA an important molecular assay based on the principle of target amplification used for confirming test results of immunoassay reactives. Reactives used in order to diagnose HCV infections also may be divided in to two groups according to identification method of the test. [32]

1. Indirect tests: (Anti-HCV and Strip immunoblot assays).

2. Direct tests: (HCV RNA, HCV Ag, HCV genotyping assays and sequencing of HCV genome).

2.1 Anti-HCV assay

Diagnostic procedures of hepatitis C virus infection in laboratories are principally based on the detection of antibodies (IgG) against recombinant HCV polypeptides by two main methods: Enzyme immunoassay (EIA) and Chemiluminescence immunoassay (CIA). The anti-HCV assay is used as a screening test. Non-structural and recombinant antigens have been used in the production of immunoassay reactives. Serologic and virologic markers of past or present HCV infection include IgG antibodies (anti-HCV). An assay for Ig M anti-HCV is available, but it does not distinguish between acute and chronic HCV infection.[25]

Three different generations of anti-HCV test kits have been developed to date. The first-generation HCV enzyme immunoassay (EIA) detected only antibodies against the non-structural region 4 (NS4) with recombinant antigen c100-3.[25] With the development of second-generation tests, additional antigens from the core region (c22-3), the NS3 region (c33c) and a part of c100-3 (named 5-1-1) from the NS4 region were used.[17] The third-generation EIA anti-HCV test used today includes an additional antigen from the NS5 region and a reconfiguration of the core and NS3 antigens.[46] Anti-HCV assays have several disadvantages, such as a high rate of false positivity, a lack of sensitivity of detection in the early window periods of 45 to 68 days after infection, the inability to distinguish between acute (ongoing active, viremic), past (recovered) and persistent (chronic) infections and a possibility of false negativity with samples from immmunocompromised patients who may not have an adequate antibody response.[19, 30, 33, 36] Inorder to shorten the duration of the diagnosis of heaptitis C virus infection especially in preseroconversion period being capable of the detection of antibodies against NS 5 proteins means that a third generation reactive is very important for anti-HCV assays. Because there remains a window period, estimated at 82 days with the second-generation assays, at 66 days with the third generation assays, between the infection and the detection of HCV antibodies [12]. NS5, enables the detection of HCV antibodies on an average of 26 days earlier in individuals with transfusion-transmitted HCV infection. Furthermore, sensitivity is also improved (approaching 97%), specifically in a high-prevalence population.[14]

Summarized properties of the fully automated CIA based systems and anti-HCV reacitves shown in **Table 1.**

2.2 Strip immunblot assay (SIA)

SIA rectives used as supplementary test, involving recombinant immunblot assay (RIBA) HCV 3.0 strip immunoassay (SIA) (Chiron Corporation, Emeryville, CA, USA), which contained recombinant antigens (c33c, NS5) and synthetic peptides (5-1-1, c100 and c22); and INNO-LIA™ HCV Score (Innogenetics N. V. Ghent, Belgium) is a 3rd generation line immunoassay which incorporates HCV antigens derived from the core region, the E2 hypervariable region (HVR), the NS3 helicase region, the NS4A, NS4B, and NS5A regions. Both SIA's are based on the principle of an enzyme immunoassay. Recombinant immunoblot assays are used as supplementary test of anti-HCV assays. As types of EIA, recombinant immunoblot assays also have several disadvantages, such as being difficult to perform, a high percentage of 'indeterminate' results and a high cost. Therefore, these anti-HCV assays are not often used in developing countries or in routine diagnostic laboratory procedures. [23]

Immunoassay Sytem	Analytic method	Test speed	Anti-HCV reactive
Abbott Arcihtect USA	Chemiluminescent Microparticle Immunoassay (CMIA)	200 Test/h	NS3, NS4.
Abbott Axsyme Plus USA	Microparticle Enzyme Immunoasay (MEIA)	100 Test/h	NS3, NS4, NS5.
SeimensAdvia Centaur XP Germany	Chemiluminescence (CHEM)	240 Test/h	NS3, NS4, NS5.
Seimens Advia Centaur CP Germany	Chemiluminescence (CHEM)	180 Test/h	NS3, NS4, NS5.
Roche Cobas e601 Switzerland	Electrochemiluminesans (ECLIA)	170 Test/h	NS3, NS4.
Roche Cobas e411 Switzerland	Electrochemiluminescence (ECLIA)	86 Test/h	NS3, NS4.
Beckman Coulter UniCel DxI 600/800 USA	Chemiluminescence (CHEM)	200/400 Test/h	NS3, NS4.
Beckman Coulter Access 2 USA	Chemiluminescence (CHEM)	100 Test/h	NS3,NS4.
Ortho-Clinical Diagnostics Vitros 3600 USA	Enhanced Chemiluminescence (ECHEM)	189 Test/h	NS3, NS4, NS5
Ortho Clinical Diagnostics Vitros ECi/ECiQ USA	Enhanced Chemiluminescence (ECHEM)	90 Test/h	NS3, NS4, NS5
Diasorin Laison Italy	Chemiluminescence (CHEM)	180 Test/h	NS3, NS4, NS5

Table 1. Summarized properties of CLIA based basic assay systems and reactives.

2.3 Hepatitis C virus core antigen (HCV Ag) assay

Total serum HCV core antigen, a surrogate marker of HCV replication, can also be detected and quantified. A commercial assay kit for it is available. HCV core antigen can be detected on average, 1 to 2 days after HCV RNA during the pre-seroconversion period. HCV Ag test is presented as a ''Direct marker for diagnosis of HCV infection''. Sensitivity of HCV core antigen test is slightly lower than HCV RNA assay but many studies carried out with HCV core Ag test compared with HCV RNA, proved that the HCV Ag test is specific, reproducible, highly sensitive and clinically applicable assay. HCV antigen test also showing good correlation comparing with HCV RNA. HCV core antigen test may be used as a second line confirmatory test alternative to HCV RNA.[23, 28, 29] It is also needed as a pre-confirmatory test for anti-HCV results and to distinguish false positives from the accurate ones. This is because it is easy to perform, reliable, has a high specificity and sensitivity rate, is cost effective, is able to shorten the duration of the diagnosis of patients during the window period and has a lower risk of laboratory contamination than assays based on nucleic acid amplification technology. [45, 50]

During the past decade, several HCV Ag tests have been developed as potential alternatives to HCV RNA assay. [5] The first was developed by Tanaka et al.[45] 1995, and then Aeyogi et al.[1] developed a new and 100-fold more sensitive test. In previous studies, the HCV Ag was detected one day after the HCV RNA in patients undergoing seroconversion. [12, 13, 34] The Architect HCV Ag assay (Abbott Laboratories, Diagnostics Division, Abbot Park, IL, USA) is highly specific, sensitive, reliable, easy to perform, reproducible, cost-effective and applicable as a screening, and pre-confirming test for anti-HCV assays in the laboratory procedures used for the diagnosis of hepatitis C virus infection. The Architect HCV Ag assay was performed using the automated Architect® i2000SR CIA system (Abbott Laboratories, Diagnostics Division, Abbot Park, IL, USA). The Architect HCV Ag assay is a two-step chemiluminescent microparticle immunoassay technology for the quantification of the HCV Ag in human serum or plasma samples. The sample volume required is 110 µl, and the total assay time is 36-40 min. The cutoff value is 3.00 fmol/liter (0.06 pg/mL); thus, values <3.00 fmol/l are considered nonreactive, values ≥3.00 fmol/l are considered reactive and values ≥3.00 fmol/l and <10.00 fmol/l are retested in duplicate. If both retest values are nonreactive, the specimen is considered nonreactive for HCV Ag. If one or both of the duplicates have a value ≥3.00 fmol/l, the specimen is considered repeatedly reactive. [29, 38]

2.4 Molecular diagnostic systems, and reactives used for the HCV RNA and HCV genotyping assays

Confirmation test is needed. Although third-generation HCV reactives are more sensitive and specific than older generation assays, they still have a high percentage of false positive reactions, so that it is mandatory to confirm every reactivity, especially with low titers, by anti-HCV CIA or EIA with HCV RNA assay (reactives with a lower limit of detection of 50 IU/mL or less) to avoid false positive results. To minimize the likelihood of false-positive anti-HCV results, the CDC has recommended the confirmation of all anti-HCV results by either RIBA or HCV RNA assay.[28, 29]

HCV RNA is the earliest marker of infection, and a direct indicator of ongoing viral replication. It appears 1 to 2 weeks after infection before any alterations in liver enzyme levels and appearance of anti-HCV antibodies can be detected. If the nucleic acid testing (NAT) result is positive, active HCV infection is confirmed. If NAT result is negative, the HCV antibody or infection status can not be determined. NAT assays are used to detect and quantify HCV RNA. [8, 18, 40]

HCV RNA assay systems can be divided into two groups: qualitative and quantitative HCV RNA.

Qualitative HCV RNA assay. Target amplification methods used qualitative detection of HCV RNA and have lower limits of detection of 5-50 IU/mL: Reverse Transcriptase-PCR (single enzyme RT-PCR, dual enzyme RT-PCR, nested RT-PCR), TMA (transcription-mediated amplification), NASBA (isothermal RNA amplification). This group includes the qualitative RT-PCR, of which the Amplicor™ HCV 2.0 (Roche Molecular Systems, USA) is an FDA- and CE-approved RT-PCR system for qualitative HCV RNA testing that allows detection of HCV RNA concentrations down to 50 IU/ml of all HCV genotypes. [31] Transcription-mediated amplification- (TMA)-based qualitative HCV RNA detection has a very high sensitivity (lower limit of detection 5-10 IU/ml). [20, 41] Transcription Mediated Amplification (TMA) Component System, Versant™ HCV RNA Qualitative Assay (Siemens

Healthcare Diagnostics, Germany) is also commercially available which is accredited by FDA and CE and provides an extremely high sensitivity, superior to RT-PCR-based qualitative HCV RNA detection assays. [21, 42, 43]

Quantitative HCV RNA assay. Quantification of HCV RNA can be determined by target amplification techniques Real-Time PCR assays), or by signal amplification methods (branched DNA- bDNA Assay). Several FDA- and CE-approved standardised systems are commercially available. The Cobas Amplicor™ HCV Monitor (Roche Diagnostics) is based on a competitive PCR technique whereas the Versant™ 440 HCV RNA Assay (Siemens Healthcare Diagnostics) is based on a bDNA technique. Both have restricted lower limits of detection (500-615 IU/ml). More recently, the Cobas TaqMan assay and the Abbott RealTime™ HCV test, both based on real-time PCR technology, have been introduced and now replaced the qualitative and quantitative methods. All commercially available HCV RNA assays are calibrated to the WHO standard based on HCV genotype 1. It has been shown that results may vary significantly between assays with different HCV genotypes despite standardisation. [9, 47]

The Abbott RealTime™ HCV Test provides a lower limit of detection of 12 IU/mL, a specificity of more than 99.5 % and a linear amplification range from 12 to 10,000,000 IU/mL independent of the HCV genotype. [27, 39] VERSANT kPCR Molecular System Siemens Healthcare Diagnostics is also avialable as a real time PCR system for quantification of HCV RNA. Rotor Gene Q real time PCR device and Qiagen HCV RNA kits (Qiagen GmbH, Germany) are used for quantification of HCV RNA by real time PCR method with specificity of 99.0 %, a lower limit of detection 34 IU/ml and capable to detect up to 10, 000,000 IU/mL.

In certain situations HCV RNA result can be negative in persons with active HCV infection. As the titre of anti-HCV increases during acute infection, the level of circulating HCV RNA declines; intermittent HCV RNA positivity has been observed among persons with chronic HCV infection. A negative HCV RNA result can also indicate resolved infection. Follow-up HCV RNA testing is indicated only in persons with serologically confirmed anti-HCV positive results. [8, 18]

Detection and quantification of HCV RNA is used as the only one confirmative test of all the anti-HCV, HCV Ag assays and SIA tests. The HCV RNA assay is a reliable method but needs technical skill and may also result in false positivity because of contamination. It is time consuming and expensive. [18, 38] HCV RNA is extensively used to confirm antibody-based screening test results. Amplification methods (target amplification by RT-PCR, transmission-mediated amplification (TMA), and signal amplification by b-DNA-branch-DNA are the most expensive methods (45-50 USD per test for real-time PCR, 10-12 USD per test for HCV Ag CIA, and 5-6 USD per test for anti-HCV CIA) when compared with anti-HCV and HCV Ag tests; and require sophisticated technical equipment and highly trained personnel. One specific problem with the HCV RNA assay is that HCV RNA can be temporarily undetectable because of the transient, partial control of viral replication by the immune response. Patients in a period of non-viremia may be detected as anti-HCV-positive and HCV RNA-negative. In such a situation, the HCV RNA test should be repeated a few weeks later with a new sample. This need for re-testing is a disadvantage of the HCV RNA test. In addition, nucleic acid amplifications are time-consuming methods and have the risk of laboratory contamination. for these reasons, amplification methods are not suitable for wide use in most laboratories, especially in developing countries. [2, 10, 24, 38, 44]

HCV Genotyping assay. HCV has six genotypes represented by digits (1-6) and multiple subtypes represented by letters (a, b, c…) and most recently a seventh HCV genotype have been characterized. HCV genotyping should be carried out in every patient before antiviral therapy.[6] Both direct sequence analysis and reverse hybridisation technology allow HCV genotyping. Reverse-hybridization method and kits (The VERSANT HCV Genotype Assay (LiPA Line Prob Assay) 2.0, Bayer HealthCare LLC, Tarrytown, NY, USA) also exist for hepatitis C virus genotype assay. The test is mainly based on biotinylated DNA, generated by RT-PCR amplification of the 5'untranslated region (5'UTR) of HCV RNA, is hybiridized to immobilized oligonucleotide probes. The VersantTM HCV Genotype 2.0 System (Siemens Healthcare Diagnostics) is suitable for indentifying genotypes 1-6 and more than 15 different subtypes and is currently the preferred assay for HCV genotyping. By simultaneous analyses of the 5'UTR and core region, a high specificity is achieved especially to differentiate the genotype 1 subtypes (1a versus1b). The TruGene direct sequence assay determines the HCV genotype and subtype by direct analysis of the nucleotide sequence of the 5'UTR region. Incorrect genotyping rarely occurs with this assay. However, the accuracy of subtyping is poor. The current Abbott RealTime™ HCV Genotype II assay is based on real-time PCR technology, which is less time-consuming than direct sequencing. Preliminary data reveal a 96% concordance at the genotype level and a 93% concordance on the genotype 1 subtype level when compared to direct sequencing of the NS5B and 5'UTR regions.

Interpretation of HCV and acute hepatitis C test results are presented in **Tables 2** and **3**.

Immunoassay for anti-HCV	**Nucleic acid test for HCV RNA**	**Supplemental test for anti-HCV**	**Interpretation of HCV status**
Negative	Not applicable	Not applicable	Never infected with HCV
Positive	Not done	Not done	Unknown, positive screening test needs confirmation
Positive	Positive	Positive/not done	Active HCV infection
Positive	Negative	Not done	Unknown, single negative HCV RNA result cannot determine infection status; perform RIBA to rule out screening test false-positive
Positive	Negative	Positive	Has been infected with HCV;repeat testing for HCV RNA to rule out active infection as HCV RNA levels may fluctuate
Positive	Not done	Positive	Has been infected with HCV; follow-up testing for HCV RNA, liver enzymes is indicated to determine current infection status
Positive	Not done/ Negative	Negative	Never infected with HCV

Table 2. Interpretation of HCV test results.[26]

Anti HCV[1]	HCV-RNA[2]	Interpretation
Negative	Negative	Not acute hepatitis C
Negative	Positive	Acute hepatitis C
Positive	Negative	Probably not an acute hepatitis C* (retesting needed)
Positive	Positive	Difficult to discriminate from chronic hepatitis C**

[1]Third-generation EIA, [2]HCV RNA assay with a lower limit of detection ≤50 IU/mL.
*Generally seen in patients who have recovered from a past HCV infection. RIBA should be used. A positive RIBA with two or more HCV-RNA positive results suggest that HCV infection resolved. A negative RIBA result indicates the false positivity of the EIA result, in the both no further testing is needed.
**Acutely infected patients can also have HCV RNA and anti-HCV at the time of diagnosis. It is difficult in these cases to distinguish acute hepatitis C from an acute exacerbation of chronic hepatitis or acute hepatitis of another cause in a patient with chronic hepatitis C.

Table 3. Interpretation of acute hepatitis C test results.[8]

3. False positivity problem and reasons of false positive results of anti-HCV immunoassay reactives

Although the present third generation EIA tests have better sensitivity and specifity rates than their predecessors, there still exists a high prevelance of false-positive results, especially among low risk group, immunocompromised patients or populations without liver diseases, leading to unnecessary cost-effective health expenditures and confusing diagnostic challenges. The most common problem in the laboratory screening assay of anti-HCV is the false positivity of low titers.[3, 7]

Among immunocompromised populations (e.g., hemodialysis patients) the average rate of false-positive results is approximately: 15 %. False positive anti-HCV results obtained from both CIA and EIA-based reactives can be explained by the fact that no structural antigens and proteins have been derived from HCV up to now. HCV has not been cultured and natural viral proteins are not available. Confirmatory test should be used in order to discriminate the false positive results from the accurate ones. [49]

3.1 Reasons of false results of anti-HCV immunoassay reactives.

The amino acid sequence and the purity of the HCV antigen used for assay development are significant factors influencing both the specifity and the sensitivity of anti-HCV immunoassays. Because of the high IgG concentration in human blood (>5 mg/ml)-e.g. in paraproteinemia or auto-antibody production or after Ig G denaturation caused by repeated freezing and thawing or by heat-inactivation of serum samples , there is a strong tendency for some of the IgG molecules to be bound to the micro-well surface by direct adsorbtion or by indirect capture via the surface molecules, and then arouse a signal, giving false-positive results. This problem might be more serious when the samples are from patients with systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), portal cirrhosis, and some infectious diseases due to the very complicated, higher concentration of immunoglobulin components in their blood. [49]

4. References

[1] Aoyagi K, Ohue C, Iida K. et al., Development of a simple and highly sensitive enzyme immunoassay for hepatitis C virus core antigen. J Clin Microbiol 1999; 37:1802–8.

[2] Agha S, Tanaka Y, Saudy N. et al. Reliability of Hepatitis C Virus Core Antigen Assay for Detection of Viremia in HCV Genotypes 1, 2, 3, and 4 Infected Blood Donors: A Collaborative Study Between Japan, Egypt, and Uzbekistan. J Med Vir 2004;73: 216-22.

[3] Ansari MHK, Omrani MD. Evalutaions of diagnostic value of ELISA method (EIA) & PCR in diagnosis of hepatitis C virus in hemodialysis patients. Hepatitis Monthly 2006;6:19-23.

[4] Bartenschlager R. The NS3/4A proteinase of the hepatitis C virus: unravelling structure and function of an anusual enzyme and a prime target for antiviral therapy. J Viral Hepat 1999;6:165-81.

[5] Bouvier-Alias M, Patel K, Dahari H. et al., Clinical utility of total HCV Ag quantification:a new indirect marker of HCV replication. Hepatology 2002;36:211–8.

[6] Bowden DS, Berzsenyi MD. Chronic hepatitis C virus infection: genotyping and its clinical role. Future Microbiol 2006;1:103-12.

[7] Centers for Disease Control and Prevention. Guidelines for laboratory testing and result reporting of antibody to hapatitis C virus. MMWR Morbid Mortal Wkly Rep 2003;52:1-13.

[8] Chevaliez S, Pawlotsky JM. Use of virologic assays in the diagnosis and management of hepatitis C virus infection. Clin Liver Dis 2005;9:371-82.

[9] Chevaliez S, Bouvier-Alias M, Brillet R, Pawlotsky JM. Overestimation and underestimation of hepatitis C virus RNA levels in a widely used real-time polymerase chain reaction-based method. Hepatol 2007;46:22-31.

[10] Chevaliez S, Pawlotsky JM. Hepatitis C virus serologic and virologic tests and clinical diagnosis of HCV-related liver disease. Int J Med Sci 2006; 3:35 – 40.

[11] Choo Q-L, Richman KH, Han JH, et al. Genetic organization and diversity of the hepatitis C virus. Proc Natl Acad Sci USA 1991; 88: 2451 –5.

[12] Courouce AM, Marrec NL, Bouchardeau F. Et al. Efficacy of HCV core antşgen detection during the preseroconversion period. Transfusion 2000;40:1198-202.

[13] Daniel HD, Vivekanandan JP, Raghuraman S. et al., Significance of the hepatitis C virus (HCV) core antigen as an alternative plasma marker of active HCV infection. Indian J Med Microbiol 2007;1:37-42.

[14] Denoyel G, Van Helden J , Bauer R, Preisel-Simmons B. Performance of a new hepatitis C assay on the Bayer Advia Centaur® Immunoassay system. Clin Lab 2004;50:75-82.

[15] Einav S, Elazar M, Danieli T, et al. A nucleotide binding motif in hepatitis C virus (HCV) NS4B mediates HCV RNA replication. J Virol 2004;78:11288-95.

[16] Feinstone SM, Kapikian AZ, Purcell RH, et al: Transfusion-associated hepatitis not due to viral hepatitis type A or B. N Eng J Med 1975; 292: 767 – 70.

[17] Feucht HH, Zöllner B, Polywka S, et al: Study on reliability of commercially available hepatitis C virus antibody tests. J Clin Microbiol 1995, 33: 620 – 4.

[18] Fiebelkorn KR, Nolte SH. RNA virus detection. In Persing DH editor in chief. Molecular Microbiology Diagnostic Principles and Practise. ASM Press, Washington DC, USA. 2004, pp.441-474.

[19] Glynn SA, Wright DJ, Kleinman SH, et al. Dynamics of viremia in early hepatitis C virus infection. Transfusion 2005, 45:994-1002.

[20] Hendricks DA, Friesenhahn M, Tanimoto L, et al. Multicenter evaluation of the VERSANT HCV RNA qualitative assay for detection of hepatitis C virus RNA. J Clin Microbiol 2003;41:651-6.

[21] Hofmann WP, Dries V, Herrmann E, et al. Comparison of transcription mediated amplification (TMA) and reverse transcription polymerase chain reaction (RT-PCR) for detection of hepatitis C virus RNA in liver tissue. J Clin Virol 2005;32:289-93.

[22] Kesli R. Evaluation of assay methods and false positive results in the laboratory diagnosis of hepatitis C virus infection. Arch Clin Microbiol 2011, 2:1-4.

[23] Kesli R, Ozdemir M, Kurtoglu MG, Baykan M, Baysal B. Evaluation and comparison of three different anti-HCV reactives based on chemiluminescence and enzyme immunoassay method used in the diagnosis of Hepatitis C infections in Turkey. J Int Med Res 2009; 37: 1420- 9.

[24] Krajden M, Shivji R, Gunadasa K. et al. Evaluation of the core antigen assay as a second-line supplemental test for diagnosis of active hepatitis C virus infection. J Clin Microbiol 2004; 42:4054–9.

[25] Kuo G, Choo QL, ALter HJ, et al: An assay for circulating antibodies to a major etiologic virus of human non-A, non-B hepatitis. Science 1989; 244:362 –4.

[26] Lemon SM, Walker C, Alter MJ, et al: Hepatitis C virus. In: Fields Virology, 5th ed, Vol 1 (Knipe DM, Howley PM, eds). Philadelphia: Wolters Kluwer -Lippincott Williams & Wilkins, 2007; pp 1253-304.

[27] Michelin BD, Muller Z, Stezl E, Marth E et al. Evaluation of the Abbott Real Time HCV assay for quantificative detection of hepatitis C virus RNA. J Clin Virol 2007;38:96-100.

[28] Miedouge M, Saune K, Kamar N, Rieu M, Rostaing L, Izopet J. Analytical evaluation of HCV core antigen and interest for HCV screening in haemodilysis patients. J Clin Virol, 2010;48:18-21.

[29] Morota K, Fujinamia R, Kinukawaa H, Machidab T, Ohnob K, Saegusab H, Takeda K. A new sensitive and automated chemiluminescent microparticle immunoassay for quantitative determination of hepatitis C virus core antigen. J Virol Methods 2009;157:8-14.

[30] National Institutes of Health.. NIH consensus statement on management of hepatitis C: 2002. NIH Consens. State Sci Statements 19:1–46.

[31] Nolte FS, Fried MW, Shiffman ML, et al. Prospective multicenter clinical evaluation of AMPLICOR and COBAS AMPLICOR hepatitis C virus tests. J Clin Microbiol 2001;39:4005-12.

[32] Pawlotsky JM. Use and interpretation of hepattis C virus diagnostic assays. Clin Liver Dis 2003;7:127-37.

[33] Pawlotsky, JM. Diagnostic tests for hepatitis C. J Hepatol 1999; 31:71–9.

[34] Peterson J, Gren G, Iida K, et al. Detection of hepatitis C core antigen in the antibody negative 'window' phase of hepatitis C infection. Vox Sang 2000;78:80-5.

[35] Reed KE, Rice CM. Overview of hepatitis C virus genome structure, polyprotein processing, and protein properties. Curr Top Microbiol Immunol 2000;242:55-84.

[36] Richter SS. Laboratory assays for diagnosis and management of hepatitis C virus infection. J Clin Microbiol 2002;40:4407–12.

[37] Rosen HR, Pawlotsky JM. Scientific advences in hepatitis C virus. Clin Liver Dis 2003; 7:xiii-xv.

[38] Ross RS, Viazov S, Salloum S. et al., Analytical performance characteristics and clinical utility of a novel assay for total hepatitis C virus core antigen quantification. J Clin Microbiol 2010; 48:1161–8.

[39] Sabato MF, Shiffman ML, Langley MR, et al. Comparison of Performance Characteristics of three Real Time Reverse Transcription-PCR test systems for detection and quantification of hepatitis C virus. J Clin Microbiol 2007;45:2529-36.

[40] Saldanha J, Lelie N, Heath A, and WHO Collaborative Study Grouop. Estabilishment of the first international standart for nucleic acid amplification technology (NAT) assays for HCV RNA. Vox Sang 1999;76:149-58.

[41] Sarrazin C. Highly sensitive hepatitis C virus RNA detection methods: molecular backgrounds and clinical significance. J Clin Virol 2002;25:S23-9.

[42] Sarrazin C, Teuber G, Kokka R, et al. Detection of residual hepatitis C virus RNA by transcription-mediated amplification in patients with complete virologic response according to polymerase chain reaction-based assays. Hepatology 2000; 32:818-23.

[43] Sarrazin C, Hendricks DA, Sedarati F, Zeuzem S. Assessment, by transcription-mediated amplification, of virologic response in patients with chronic hepatitis C virus treated with peginterferon alpha-2a. J Clin Microbiol 2001;39:2850-5.

[44] Tanaka E, Ohue C, Aoyagi K, Evalution of a new enzyme immunoassay for hepatitis C virus (HCV) core antigen with clinical sensitivity approximating that of genomic amplification of HCV RNA. Hepatology 2000; 32: 388– 93

[45] Tanaka TJ, Lau Y, Mizokami M, et al. Simple fluorescent EIA for detection and quantification of hepatitis C viremia. J Hepatol 1995; 23: 742–745.

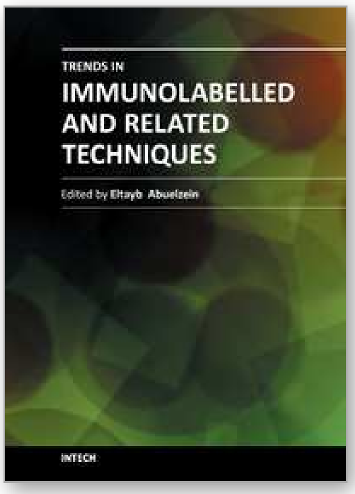
[46] Thomas DL, Ray SC, Lemon SM: Hepatitis C. In: Mandell, Douglas and Bennett's Principles and Practice of Infectious Diseases, 6th ed. (Mandell GL, Bennett JE, Dolin R, eds). Philadelphia: Churchill Livingstone, 2005; pp 1950 – 81.

[47] Vehrmeren J, Kau A, Gärtner B, et al. Differences between two real-time PCR based assays (Abbott RealTime HCV, COBAS AmpliPrep/COBAS TaqMan) and one signal amplification assay (VERSANT HCV RNA 3.0) for HCV RNA detection and quantification. J Clin Microbiol 2008;46:880-91.

[48] Wasley A, Alter MJ. Epidemiology of hepatitis C: geographic differences and temporal trends. Semin Liver Dis 2000; 20:1-16.

[49] Wu FB, Ouyan HQ, Tang XY, Zhou ZX. Double-antigen sandwich time-resolved immunoflourometric assay for the detection of anti-hepatitis C virus total antibodies with improved specifity and sensitivity. J Medical Microbiol 2008; 57:1-7.

[50] Yokosuka O, Kawai S, Suzuki Y, Evaluation of clinical usefulness of secondgeneration HCV Ag assay: comparison with COBAS AMPLICOR HCV MONITOR assay version 2.0. Liver International 2005;25:1136–41.



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