Hepatitis C Virus Diagnosis and Testing

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Development of serological and nucleic acid testing (NAT) has revolutionized hepatitis C virus (HCV) diagnosis. Although third generation anti-HCV enzyme immunoassays (EIAs) are very effective for testing high prevalence populations, confirmatory testing is still necessary when these tests are applied to populations with a low HCV prevalence to exclude false positive results. Limitations of third generation anti-HCV EIAs include: the relatively prolonged time between acute infection and detection of seroconversion (which typically requires at least 5-6 weeks); delayed seroconversions in immunocompromised hosts (requiring months to years); and the inability of serological tests to confirm active HCV infection. In contrast, nucleic acid testing (NAT) can directly detect HCV RNA in serum, plasma or tissue and thereby confirm active infection as well as narrow the window between infection and HCV detection to as little as 1-2 weeks. Commercial NAT assays are now highly sensitive, specific, and reproducible and have largely replaced unreliable home brew nucleic acid amplification assays. Qualitative commercial NAT are typically more sensitive than quantitative assays and therefore the method of choice to confirm active infection. Given the efficacy of combination therapy with interferon/ribavirin and newer antiviral agents under development, HCV infection may become curable, which will likely impact future disease transmission. As the therapeutic costs are currently very high, there is clearly a need to assess the utility of quantitative NAT and to further evaluate the role of HCV genotyping to optimize antiviral therapy. Thus for the foreseeable future, a combination of both serological tests and NAT will be required for cost-effective HCV diagnosis and monitoring.

Public health and clinical care management of hepatitis C virus (HCV) infection depends on accurate laboratory diagnosis. Unfortunately clinicians and public health officials are faced with a confusing array of serological and nucleic acid detection tests. This manuscript will explain the strengths and weaknesses of the available diagnostic tests and illustrate how a combination of serological and nucleic acid testing (NAT) is now required for accurate HCV diagnosis and antiviral monitoring.

HCV antibody detection by enzyme immunoassay (EIA)

HCV is typically diagnosed by measuring an immune response to infection by detecting anti-HCV antibody in serum or plasma by enzyme immunoassay (EIA). Modern EIAs use recombinant or synthetic viral antigens to capture circulating anti-HCV antibodies to microtitre plate wells or microparticle beads. These antibodies are then detected by anti-IgG labelled with enzymes which catalyze the transformation of substrates to generate colour or light. The generated signals are compared with controls and the signal intensity is usually proportional to the amount of anti-HCV in the specimen.[1]

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HCV EIA sensitivity and specificity

Current third generation EIAs are substantially more sensitive and specific than the older first and second generation assays.[2,3] However, their overall sensitivity is still highly dependent on the clinical status of the population tested. In chronically infected non-immunocompromised persons, EIA sensitivity approaches 97-99%.[3,4] In contrast, in acutely infected non-immunocompromised individuals, EIA sensitivity is much lower. For example, only 50-70% of acutely infected individuals will be antibody positive at the onset of symptoms,[5] since after acute HCV infection it takes approximately 5-6 weeks to generate a detectable amount of anti-HCV. Figure 1 illustrates the approximate time interval between acute infection and detection by various generation EIAs and NAT in non-immunosuppressed individuals. As can be seen in Figure 1, both NAT and a new HCV antigen test which is under development, can narrow the time between infection and detection to 1-2 weeks. In immunosuppressed individuals, however, the time between infection and detection of an antibody response may be many months to years, or may never occur.[6-8] For these individuals, NAT (or HCV antigen testing if it becomes commercially available) may be required to diagnose infection prior to seroconversion (Table I).[6-8]

The specificity of third generation EIAs is also affected by the population prevalence. For individuals with clinical evidence of HCV infection, i.e., abnormal liver function tests in the absence of other causes of liver disease, third generation assays are 95-98% specific.[3,4] However, in low prevalence populations such as blood donors, the

specificity is 50-60%.[2-4,9] Thus for low prevalence populations, supplemental EIA, immunoblot or NAT is required to correctly identify infected individuals.

Despite the relatively low specificity of anti-HCV EIAs in blood donors who are at low risk of infection, serological screening is very effective and has virtually eliminated post-transfusion HCV infection.[10] This is because the presence of anti-HCV is strongly correlated with active viral replication/infectivity and the fact that most infections (50-85%) will become chronic in the absence of therapeutic intervention.[11-16]

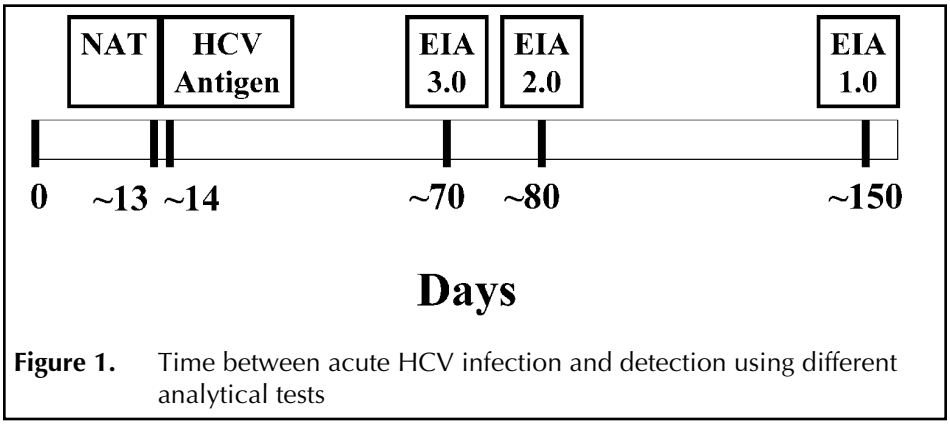

Figure 1. Time between acute HCV infection and detection using different analytical tests

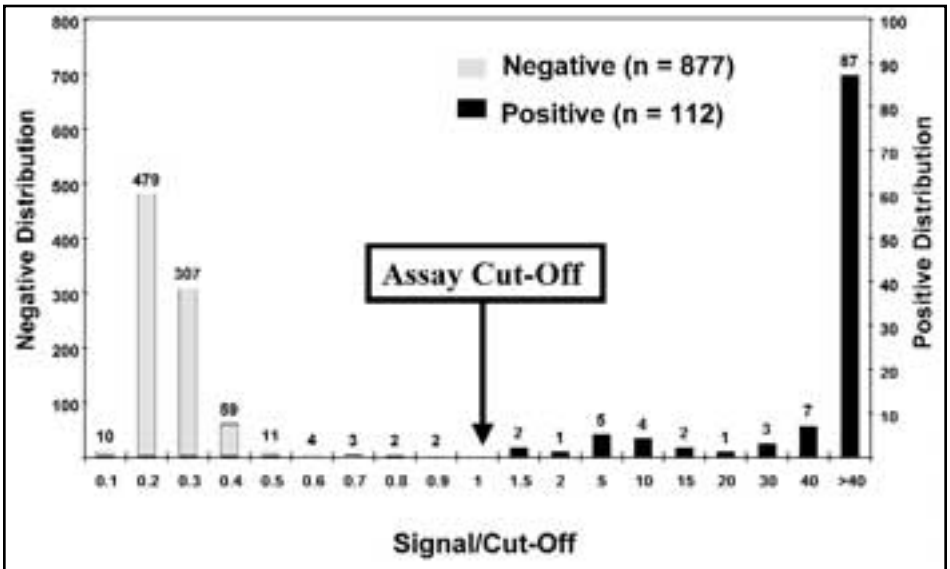

Figure 2. Abbot AxSYM™ third generation anti-HCV EIA signal to cut-off results

HCV immunoblot assays

Immunoblots were developed as supplemental or confirmatory tests to enhance HCV EIA specificity. They consist of recombinant or synthetic HCV proteins coated onto plastic strips that are then exposed to the patient's serum. True anti-HCV positives specifically bind to HCV antigens on the strip and generate a colour reaction. These strips may also contain non-viral antigens that may be co-expressed during the synthetic process used to make the antigens to capture the anti-HCV in the EIA. Including these non-viral antigens on the immunoblot can control for false positive EIA results due to antibody reactivity to these non-viral antigens. Based on the number of specific HCV antigens to which antibody in the specimen reacts on the immunoblot, EIA specimens are confirmed as HCV antibody positive, negative or indeterminate.[3,17-20]

Although immunoblot assays are still available – e.g., Third-Generation Recombinant Immunoblot Assay or Strip Immunoblot Assay (SIA), (RIBA™-3, Chiron), INNO™-LIA Ab III (Innogenetics, NV), DECISCAN™ HCV (Sanofi Pasteur), and LiaTek® HCV (Organon) – given the advances in modern EIAs and NAT discussed below, their value for clinical diagnosis is limited.

Figure 2 illustrates EIA signals generated from HCV antibody negative and positive individuals (n=989) tested at the British Columbia Centre for Disease Control (BCCDC) by third generation Abbott AxSYM™ HCV EIA (Abbott). While most specimens are strongly antibody positive or clearly negative based on their signal intensity, a substantial number of specimens display weak signals above the assay cut-off. Of the approximately 70,000 clinical specimens tested each year at the BCCDC, 8-10% will have some degree of antibody reactivity by AxSYM™ third generation EIA on initial testing. The majority, 82-85%, yield strong antibody positive signals (typically 2-3 times or more of the assay cut-off) and 15-18% will yield weak signals.

Strongly positive third generation EIA specimens are typically immunoblot positive. When initial strong HCV EIA reactive specimens are confirmed as anti-HCV positive by a second manufacturer's EIA that uses different recombinant or synthetic proteins, approximately 99% are immunoblot positive.[20-23] Thus immunoblot testing of strongly EIA positive specimens is generally not necessary for confirmation of reactivity.

For individuals with very high levels of anti-HCV, the major clinical question is whether they remain actively infected or have resolved their HCV infection but remain antibody positive. Approximately 95-99% of individuals with abnormal serum transaminases and no other cause of the liver disease who are third generation anti-HCV EIA positive are confirmed as being actively infected by NAT.[3,4] Unfortunately, 25-40% of HCV-infected individuals will have consistently elevated serum transaminases. In these individuals, detection of HCV RNA by NAT or HCV antigen (if available) is the only way to confirm active infection other than performing a liver biopsy (see NAT section).

While immunoblots clearly enhance EIA test specificity by confirming the presence of specific anti-HCV reactivity, their most important limitation is that they are typi-

TABLE I

Test	Comments
Third Generation Anti-HCV Enzyme Immunoassay	➤ Very effective screening test. ➤ In high prevalence populations, sensitivity >97% and specificity >95%. ➤ In low prevalence populations (e.g., blood donors), sensitivity >97%, specificity 50-60%; therefore confirmatory testing is required. ➤ During acute infection, sensitivity is about 50-70%, as detection of seroconversion requires at least 5-6 weeks. Early antibody levels may be insufficient for immunoblot confirmation. NAT should be used to confirm acute infection where appropriate. ➤ The time to seroconversion may be prolonged in immunocompromised individuals (months to years). ➤ The presence of antibody does not confirm active infection, however, 85-95% of individuals who have strongly positive EIA reactivity are NAT positive.
Immunoblot Assay	➤ Can confirm specific HCV antibody. ➤ Are typically less sensitive than EIAs and are therefore poor at confirming acute infection or infection in immunocompromised individuals. ➤ Cannot determine if infection is active and therefore provides limited diagnostic information. NAT should be performed to confirm active infection where appropriate.
Qualitative HCV NAT	➤ Most sensitive NAT, provides a yes or no answer. ➤ Sensitivity 95-99%, specificity 98-99% but requires meticulous laboratory procedures. ➤ Confirms active infection and end of treatment response. ➤ NAT is positive within 1-2 weeks after HCV infection. ➤ Can be used to detect mother-infant transmission as passively transferred maternal antibody can be detected in infants for at least 12-18 months.
Quantitative HCV NAT	➤ May be useful to predict interferon/ribavirin therapeutic outcome, but the predictive value needs to be validated using standardized commercial assays. ➤ May be used to monitor therapy by early assessment of non-responders. Further studies are necessary to document clinical utility of viral load testing. ➤ Typically less sensitive than qualitative assays.
Genotyping Assay	➤ Differentiates the major HCV genotypes based on sequence differences. ➤ HCV genotype I is typically more difficult to treat but it remains controversial whether it is correlated with more severe clinical illness. ➤ Most common commercial method of genotyping involves specific hybridization of the AMPLICOR PCR product to genotype-specific immobilized probes (reverse-hybridization line probe assay (LiPA) INNO-LIPA, INNOGENETICS).
HCV Antigen Test	➤ Under development. ➤ Can detect acute infection within approximately 2 weeks. ➤ May be helpful for serological confirmation or to monitor treatment response.

cally less sensitive than EIAs. This lack of sensitivity is important when trying to determine if a weakly reactive or indeterminate EIA result is due to small amounts of anti-HCV or non-specific cross-reacting antibody. For example, small amounts of anti-HCV can occur when testing during acute infection prior to complete seroconversion, testing immunocompromised hosts who may have blunted antibody responses[6-8] or they may reflect a resolved infection with waning anti-HCV.[4] Given that immunoblots are less sensitive than EIAs, weakly EIA reactive or indeterminate specimens are also typically immunoblot negative or indeterminate. Thus for the specimens where it is most important to distinguish true anti-HCV from non-specific reactions, neither EIA nor immunoblot testing provide a definitive diagnosis of active infection. Diagnosis in weakly reactive individuals typically requires NAT or follow-up testing to confirm seroconversion.[3,4,24,25]

NAT principles

NAT allows direct detection of specific HCV RNA in serum, plasma or tissues independent of the host's immune response. Viral nucleic acid detected in the plasma or serum reflects active HCV replication in the liver[26] which can generate as many as 10^{10-13} virions per day in chronically infected individuals.[13] NAT-based detection of HCV RNA is performed in two major ways. The most familiar to the reader is target amplification. This technique involves *in vitro* synthesis of HCV-specific nucleic acid followed by detection of the amplified product. Examples of target amplification assays include the polymerase chain reaction (COBAS AMPLICOR HCV PCR Test (qualitative), COBAS AMPLICOR HCV Monitor PCR (quantitative) (Roche)),[27] Transcription-Mediated Amplification (TMA, Bayer)[2] and Nucleic Acid Sequence Based Amplification (NASBA, Organon).[28] The other major approach involves signal amplification. For signal amplification the HCV RNA is hybridized to a specific probe which undergoes extensive enzymatic amplification with the output signal corresponding to the amount of input HCV RNA target in the specimen. Branched chain DNA or bDNA (Quantiplex HCV RNA 2.0, and a newer version 3.0 under development, Bayer) are examples of this type of analytical technique.

NAT will play an increasingly important role in clinical diagnosis because direct detection of HCV RNA can (a) narrow the interval between acute infection and detection of anti-HCV from the current 5-6 weeks by third generation EIAs to 1-2 weeks (Figure 1);[29-31] (b) detect infection in immunocompromised individuals with blunted antibody responses; (c) differentiate active from resolved infection in seropositive individuals; (d) determine perinatal infection independent of the presence of passively transferred maternal antibody, and (e) be used to monitor treatment response.[14-16,32]

Although NAT tests have certain advantages over serological tests, high quality NAT is still not widely available, in part because of a number of technical issues that will be resolved in the coming years and the fact that the cost remains prohibitive (approximately six times that of EIA tests).

Technical factors affecting NAT accuracy

Widespread application of NAT for HCV diagnosis has been slow due to technical limitations. To get accurate and reproducible NAT results requires meticulous standardization of the entire analytic process. In the past only in-house or home-brew NATs were available. These tended to be poorly reproducible between centres and often yielded false positive test results.[33] These home-brew assays were largely replaced with commercial assays which were reproducible but varied in their sensi-

tivity. In addition, quantitative results from the same specimen tested by different manufacturers' assays varied by about 10 fold.[4,26,34] These early commercial assays also had difficulty accurately detecting and quantifying certain HCV genotypes.[27]

Within the last year commercial assays have been adjusted to detect and quantify all the known genotypes and/or HCV strains,[27] and have been standardized against a new International HCV standard.[35] One IU/ml corresponds to about 2-8 copies/ml of HCV.[36,37] This is expected to dramatically improve the inter- and intra-assay reproducibility and accuracy of detection. Assay standardization, availability of semi-automated high-throughput instrumentation and use of internal controls to monitor for inhibition of nucleic acid amplification, have increased test capacity, accuracy and reliability.[38,39] A list of various qualitative and quantitative HCV RNA detection assays are listed in Figure 3.

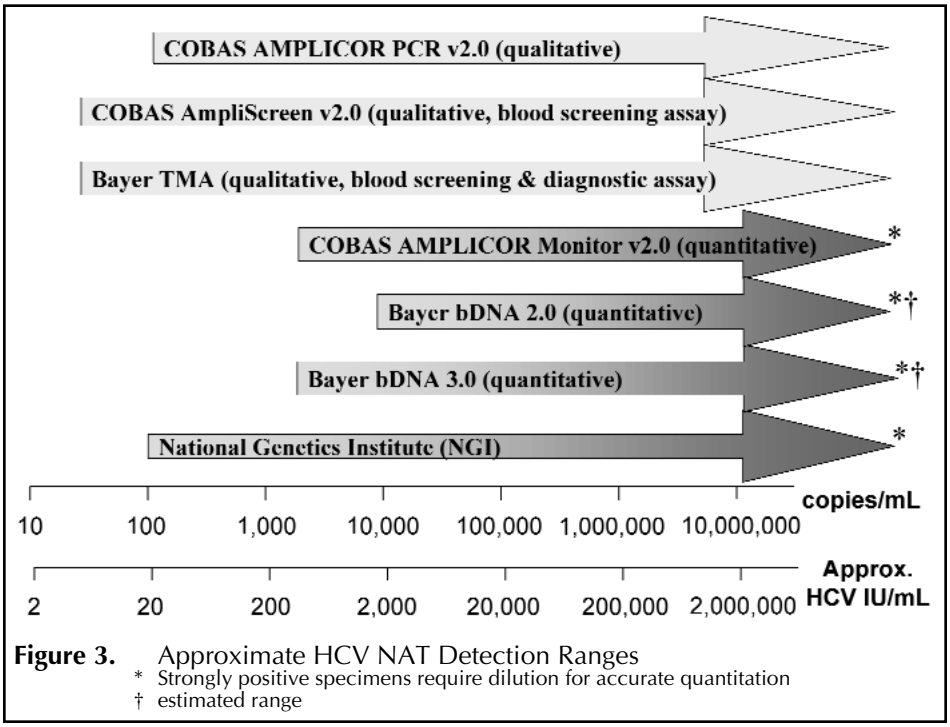

Figure 3. Approximate HCV NAT Detection Ranges
* Strongly positive specimens require dilution for accurate quantitation
† estimated range

Combining serology and NAT to document active HCV infection

As discussed above, at the present time serology alone cannot determine if an individual is actively infected with HCV. Individuals can be grouped into those who demonstrate (Figure 2): 1) strong anti-HCV antibody responses by third generation EIA (e.g., 2-3 times or more than the cut-off of the assay), the vast majority of whom are immunoblot positive and NAT positive; and, 2) weak serological responses, who are generally immunoblot negative or indeterminate and are usually NAT negative.

Of the approximately 70,000 specimens tested by third generation HCV EIA at the BCCDC in 1999, 7,700 (11%) demonstrated some degree of seroreactivity. Of these individuals, 82-85% demonstrated strong EIA signals confirmed by testing on a second manufacturers' third generation EIA. When individuals with strong third generation anti-HCV EIA seroreactivity are tested by NAT, e.g., AMPLICOR (qualitative), 85-95% are NAT positive (Figure 3).[3,18-20]

In contrast, for individuals with weak anti-HCV responses (which represent 15-18% of seroreactive specimens tested at the BCCDC in 1999), immunoblot testing was typically negative or indeterminate and therefore was of limited diagnostic value. When these individuals are tested by AMPLICOR (qualitative), using specimens collected and handled appropriately for NAT, approximately 5-10% are positive. Most of the NAT positive antibody weakly reactive or indeterminate specimens were either undergoing acute seroconversion or had a blunted antibody response from immunosuppression.

Which NAT assay should be used for clinical diagnosis?

As discussed previously, the literature regarding the sensitivity, specificity, reproducibility and standardization of the various commercial NAT is very confusing. Given the adoption of the International HCV Standard (IU), it is expected that manufacturers will soon be able to agree on the relative amount of HCV RNA in a given specimen and the relative sensitivity of their assays.[36,37] Figure 3 demonstrates the relative sensitivities and range of detection of available NATs as well as those under development.

As can be seen in Figure 3, qualitative NAT (AMPLICOR & TMA) are more sensitive than the quantitative assays. The AmpliScreen (Roche) and TMA (Bayer) assays that have been developed for blood donor screening are the most sensitive.[40] Qualitative tests are currently 10-100 fold more sensitive than quantitative assays and can reliably detect 5-50 IU/ml (approximately 10-100 copies/ml). Qualitative assays are able to provide a yes or no answer regarding the presence of HCV RNA in the specimen and, as outlined in Table I, should be used to: 1) detect acute infection prior to seroconversion, 2) resolve weakly reactive or indeterminate serological results and, 3) determine if an individual is actively infected and/or has responded to therapy.

It is difficult to assess the clinical sensitivity and specificity of qualitative NATs given the lack of a clinical "gold standard" of HCV infection. Based on well-characterized HCV-infected and control populations, the sensitivity and specificity of commercial qualitative NAT approach 95-99% and 98-99% respectively.[4] These figures are dependent, however, on meticulous laboratory procedures.

HCV genotyping

The term genotype refers to the sequence-based phylogenetic clustering of HCV types and subtypes found in patients. At present, there are at least 11 types and greater than 90 subtypes.[2,41]

HCV genotypes are typically differentiated by the commercially available reverse-hybridization line probe assay (INNO-LIPA HCV II, Innogenetics, Belgium). This involves specific hybridization of the AMPLICOR PCR product to genotype-specific immobilized probes on the line probe assay which generate a colour reaction (a nucleic acid equivalent of an immunoblot). Alternative techniques to determine the genotype include restriction fragment-length polymorphism of PCR products and direct sequencing.[42]

Although infection with certain genotypes (e.g., genotype I) appear to be more difficult to treat,[14,15,32] whether this represents an intrinsic feature of genotype I virulence remains controversial.[2,43]

Quantitative NAT for antiviral monitoring

Combination therapy with interferon and ribavirin has revolutionized the treatment of HCV infection. Treatment for 24-48 weeks can eliminate detectable HCV RNA from blood and improve hepatic histopathology in approximately 40% of HCV-infected individuals who have elevated serum transaminases.[14,15,32] Of particular importance is the fact that most virologic responders remain serum HCV RNA negative for at least two years after therapy is stopped.[16] Correlates of therapeutic response include: sex, age, degree of hepatic fibrosis, the amount of detectable HCV RNA in the serum (viral load) and the genotype of the virus. Approximately 30% of individuals infected with genotype I will respond to combination therapy after 48 weeks of treatment. In contrast, 60% of individuals with non-genotype I will respond with 24 weeks of combination therapy. Although response to combination therapy correlates with low pretreatment viral loads (< 2,000,000 copies/ml as measured by the National Genetics Institute assay) for genotype I-infected individuals, that correlation was based on viral load measurements using a non-commercially available assay which is not directly comparable to currently available commercial assays.[4] In addition, the predictive value of HCV load determinations was less important than the infecting viral genotype.[2,14,15,32,43]

Unlike HIV infection where HIV viral load predicts outcome and serves as a strong surrogate marker of treatment response, this is not the case for HCV viral load. In general, outcome for HCV infection is most strongly correlated with the degree of hepatic fibrosis.[2,43,44] A number of studies are currently underway to determine if monitoring HCV load during combination therapy can be used to rapidly identify non-responders early so that ineffective therapy can be stopped early to reduce the overall therapeutic costs. The results of these studies should be available within the next year. Because commercial quantitative assays are less sensitive than qualitative assays, they should only be used for therapeutic monitoring and not to confirm active infection or to determine if the therapy has been effective.

HCV antigen detection

Another method of HCV detection that is under commercial development, involves detection of HCV antigens in plasma or serum.[45] One version of this test detects free HCV antigen in the specimen. This may be of particular benefit in detecting acute seroconversions. Based on studies in blood donors, HCV antigen can be detected within the first 2 weeks of acute infection, which is quite similar to NAT (Figure 1).[29] However, given the greater sensitivity of NAT and its general availability, the role of HCV antigen testing for routine clinical diagnosis remains unclear. Other versions of the HCV antigen test are designed to detect HCV antigen in the presence of HCV antibody, which may be useful to confirm active infection in chronically infected individuals or to monitor response to antiviral therapy if the test performance proves satisfactory.

SUMMARY

HCV diagnosis and testing is clearly a rapidly evolving field. This paper outlines how anti-HCV serology and NAT can be combined to provide a definitive answer as to whether or not an individual has been or is actively infected. The strengths and weaknesses of the various tests are outlined in Table I. In the future, one can expect more accurate and reproducible NAT which will increase diagnostic accuracy and guide therapeutic intervention on an individual basis.

Although clinical therapeutics is not typically considered the domain of public health officials, it is important to understand that a number of new therapeutic agents are currently under development for the treatment of HCV. These include Pegylated and Consensus Interferon which are more potent than the current interferon alpha used in combination with ribavirin.[46] Given the promising data from the current interferon alpha and ribavirin combination and preliminary data from these new antiviral agents, HCV may well become a curable illness where both prevention and treatment are required to minimize the risk of transmission and reduce the burden of illness in the population.[47,48]

ACKNOWLEDGEMENTS

I thank Darrel Cook and Dr. Robert Brunham for reviewing the manuscript.

REFERENCES

1. Chien DY, Arcangel P, Medina-Selby A, et al. Use of a novel hepatitis C virus (HCV) major-epitope chimeric polypeptide for diagnosis of HCV infection. *J Clin Microbiol* 1999;37(5):1393-97.
2. Schiff ER, de Medina M, Kahn RS. New perspectives in the diagnosis of hepatitis C. *Semin Liver Dis* 1999;19(Suppl 1):3-15.
3. Gretch DR. Diagnosis tests for hepatitis C. *Hepatology* 1997;26:43S-47S.
4. Pawlotsky JM. Diagnostic tests for hepatitis C. *J Hepatol* 1999;31(Suppl 1):71-79.
5. National Institutes of Health Consensus Development Conference Panel statement: Management of hepatitis C. *Hepatology* 1997;26(3 Suppl 1):2S-10S.
6. Bukh J, Wantzin P, Krogsgaard K, et al. High prevalence of hepatitis C virus (HCV) RNA in dialysis patients: Failure of commerically available antibody tests to identify a significant number of patients with HCV infection. *J Infect Dis* 1993;168:1343-48.
7. Ridzon R, Gallagher K, Ciesielski C, et al. Simultaneous transmission of human immunodeficiency virus and hepatitis C virus from a needlestick injury. *N Engl J Med* 1997;336(13):919-22.
8. Thio CL, Nolt KR, Astemborski J, et al. Screening for hepatitis C virus in human immunodeficiency virus-infected individuals. *J Clin Microbiol* 2000;38(2):575-77.
9. Cuthbert JA. Hepatitis C: Progress and problems. *Clinical Microbiology Reviews* 1994;7(4):505-32.
10. Goodnough LT, Brecher ME, Kanter MH, AuBuchon JP. Transfusion medicine. First of two parts—blood transfusion. *N Engl J Med* 1999;340(6):438-47.
11. Hoofnagle JH. Hepatitis C: The clinical spectrum of disease. *Hepatology* 1997;26:15S-20S.
12. Vogt M, Lang T, Frosner G, et al. Prevalence and clinical outcome of hepatitis C infection in children who underwent cardiac surgery before the

implementation of blood-donor screening. *N Engl J Med* 1999;341(12):866-70.
13. Neumann AU, Lam NP, Dahari H, et al. Hepatitis C viral dynamics in vivo and the antiviral efficacy of interferon-alpha therapy. *Science* 1998;282(5386):103-7.
14. McHutchison JG, Gordon SC, Schiff ER, et al. Interferon alfa-2b alone or in combination with ribavirin as initial treatment for chronic hepatitis C. Hepatitis Interventional Therapy Group. *N Engl J Med* 1998;339(21):1485-92.
15. Poynard T, Marcellin P, Lee SS, et al. Randomised trial of interferon alpha2b plus ribavirin for 48 weeks or for 24 weeks versus interferon alpha2b plus placebo for 48 weeks for treatment of chronic infection with hepatitis C virus. International Hepatitis Interventional Therapy Group (IHIT). *Lancet* 1998;352(9138):1426-32.
16. Schvarcz R, Glaumann H, Reichard O, Weiland O. Histological and virological long-term outcome in patients treated with interferon-alpha2b and ribavirin for chronic hepatitis C. *J Viral Hepat* 1999;6(3):237-42.
17. Damen M, Zaaijer HL, Cuypers HTM, et al. Reliability of the third-generation recombinant immunoblot assay for hepatitis C virus. *Transfusion* 1995;35(9):745-49.
18. Wilber JC, Polito A. Serological and virological diagnostic tests for hepatitis C virus infection. *Seminars in Gastrointestinal Disease* 1995;6(1):13-19.
19. Zein NN, Germer JJ, Wendt NK, et al. Indeterminate results of the second-generation hepatitis C virus (HCV) recombinant immunoblot assay: Significance of high-level c22-3 reactivity and influence of HCV genotypes. *J Clin Microbiol* 1997;35(1):311-12.
20. Krajden M, Zhao J, Bourke C, et al. Detection of hepatitis C virus by PCR in second-generation enzyme immunoassay-seropositive blood donors by using matched pairs of fresh frozen plasma and pilot tube sera. *J Clin Microbiol* 1996;34:2191-95.
21. Goubau P, Reynders M, Beuselinck K, et al. Confirmatory strategy of hepatitis C serology based on two screening assays in a diagnostic setting. *Acta Clinica Belgica* 1997;52(1):31-35.
22. Allain JP, Kitchen A, Aloysius S, et al. Safety and efficacy of hepatitis C virus antibody screening of blood donors with two sequential screening assays. *Transfusion* 1996;36:401-5.
23. Anderson SC, Hathaway T, Kuramoto IK, et al. Comparison of two second-generation antihepatitis C virus ELISA on 21431 US blood donor samples. *J Viral Hepatitis* 1995;2:55-61.
24. Lok ASF, Gunaratnam NT. Diagnosis of hepatitis C. *Hepatology* 1997;26:48S-56S.
25. Pawlotsky JM, Lonjon I, Hezode C, et al. What strategy should be used for diagnosis of hepatitis C virus infection in clinical laboratories? *Hepatology* 1998;27(6):1700-2.
26. Pessoa MG, Terrault NA, Detmer J, et al. Quantitation of hepatitis G and C viruses in the liver: Evidence that hepatitis G virus is not hepatotropic. *Hepatology* 1998;27(3):877-80.
27. Mellor J, Hawkins A, Simmonds P. Genotype dependence of hepatitis C virus load measurement in commercially available quantitative assays. *J Clin Microbiol* 1999;37(8):2525-32.
28. Lunel F, Cresta P, Vitour D, et al. Comparative evaluation of hepatitis C virus RNA quantitation by branched DNA, NASBA, and monitor assays. *Hepatology* 1999;29(2):528-35.
29. Peterson J, Green 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(2):80-85.
30. Alter HJ, Purcell RH, Shih JW, et al. Detection of antibody to hepatitis C virus in prospectively followed transfusion recipients with acute and chronic non-A, non-B hepatitis. *N Engl J Med* 1989;321(22):1494-1500.
31. Busch MP, Korelitz JJ, Kleinman SH, et al. Declining value of alanine aminotransferase in screening of blood donors to prevent posttransfusion hepatitis B and C virus infection. The Retrovirus Epidemiology Donor Study. *Transfusion* 1995;35(11):903-10.
32. Davis GL, Esteban-Mur R, Rustgi V, et al. Interferon alfa-2b alone or in combination with ribavirin for the treatment of relapse of chronic hepatitis C. International Hepatitis Interventional Therapy Group. *N Engl J Med* 1998;339(21):1493-99.
33. Zaaijer HL, Cuypers HTM, Reesink HW, et al. Reliability of polymerase chain reaction for detection of hepatitis C virus. *Lancet* 1993;341:722-24.
34. Pawlotsky J-M, Bastie A, Lonjon I, et al. What technique should be used for routine detection and quantification of HBV DNA in clinical samples? *J Virological Methods* 1997;65:245-53.
35. Martinot-Peignoux M, Boyer N, Le Breton V, et al. A new step toward standardization of serum hepatitis C virus-RNA quantification in patients with chronic hepatitis C. *Hepatology* 2000;31(3):726-29.
36. Saldanha J, Lelie N, Heath A. Establishment of the first international standard for nucleic acid amplification technology (NAT) assays for HCV RNA. WHO Collaborative Study Group. *Vox Sang* 1999;76(3):149-58.
37. Saldanha J. Standardization: A progress report. *Biologicals* 1999;27(4):285-89.
38. Martell M, Gomez J, Esteban JI, et al. High-throughput real-time reverse transcription-PCR quantitation of hepatitis C virus RNA. *J Clin Microbiol* 1999;37(2):327-32.
39. Albadalejo J, Alonso R, Antinozzi R, et al. Multicenter evaluation of the Cobas Amplicor HCV assay, an integrated PCR system for rapid detection of hepatitis C virus RNA in the diagnostic laboratory. *J Clin Microbiol* 1998;36(4):862-65.
40. Sun R, Schilling W, Jayakar H, et al. Simultaneous extraction of hepatitis C virus (HCV), hepatitis B virus, and HIV-1 from plasma and detection of HCV RNA by a reverse transcriptase-polymerase chain reaction assay designed for screening pooled units of donated blood. *Transfusion* 1999;39(10):1111-19.
41. Stuyver L, Wyseur A, van Arnhem W, et al. Second-generation line probe assay for hepatitis C virus genotyping. *J Clin Microbiol* 1996;34(9):2259-66.
42. Germer JJ, Rys PN, Thorvilson JN, Persing DH. Determination of hepatitis C virus genotype by direct sequence analysis of products generated with the Amplicor HCV test. *J Clin Microbiol* 1999;37(8):2625-30.
43. Zeuzem S, Franke A, Lee JH, et al. Phylogenetic analysis of hepatitis C virus isolates and their correlation to viremia, liver function tests, and histology. *Hepatology* 1996;24(5):1003-9.
44. Pontisso P, Bellati G, Brunetto M, et al. Hepatitis C virus RNA profiles in chronically infected individuals: Do they relate to disease activity? *Hepatology* 1999;29(2):585-89.
45. 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(6):1802-8.
46. Weiland O. Interferon and ribavirin combination therapy: Indications and schedules. *Forum* (Genova) 2000;10(1):22-28.
47. Garnett GP, Bartley LM, Cameron DW, Anderson RM. Both a 'magic bullet' and good aim are required to link public health interests and health care needs in HIV infection [news]. *Nat Med* 2000;6(3):261-62.
48. Gordon FD. Cost-effectiveness of screening patients for hepatitis C. *Am J Med* 1999;107(6B):36S-40S.