Laboratory diagnosis of Hepatitis C virus infection

A change to common practice

Ali H. Hajeer, *PhD, MRCPath,* ***Ziad A. Memish,*** *FRCPC, FACP,* ***Bandar A. Al-Knawy,*** *FRCPC.*

World Health Organization estimates that 170 million people, 3% of the world's population, are infected with hepatitis C virus (HCV) and are at risk of developing liver cirrhosis and liver cancer, or both.[1] The prevalence of HCV infection in blood bank donors worldwide varies; it is estimated to be between 1-5%. The prevalence is high in some parts of Africa, the Eastern Mediterranean, South-East Asia and the Western Pacific regions compared to countries in North America and Europe.[1] In the Middle East the prevalence of HCV seropositivity in blood donors ranges from 1-12%.[1] While, in the Kingdom of Saudi Arabia (KSA), approximately 500,000 people are infected with HCV, this is based on the estimated 2.7% prevalence rate in the population at large.[2] The most common screening assays used for HCV diagnosis are second and third generation enzyme immunoassays.[3] Results for these tests are given as a quantitative absorbance or as signal/cutoff ratio (S/Co) but they are usually reported simply as negative or positive. High false-positive results are often encountered in areas with low prevalence rates, especially in blood donor settings.[4] A positive HCV antibody is usually confirmed by additional testing. Laboratory diagnosis of HCV infection in asymptomatic patients can be difficult and misleading. This is due to many factors, including, (a) HCV acute infection usually passes unnoticed, early signs and symptoms may mimic flu (b) HCV screening assays have high false positive results, can reach up to 60%[5] (c) There is no protective immunity to HCV; antibodies usually disappear after clearing the infection[6] and (d) Chronic HCV infection is intermittent and goes into phases.[7] Recently the Centers for Disease Control and Prevention (CDC) published new guidelines for testing HCV status.[5] The need for these guidelines stems from the fact that screening assays for HCV antibodies have high false positive rates, especially in regions where the infection rate is low. Specificity of most second and third generation assays available in the market is high, however, false positive rates can be as high as 60% in selected populations.[5] This finding raised the issue of suitability of antibody screening methods in diagnosing HCV infection. It is rather advised that supplementary test should be adopted. The new CDC regulations divide the HCV screening antibody results into negative, low positive and high positive.[5] For chemiluminescent immunoassay the CDC defines low positive as a result of signal to cut off ratio (S/Co) between 1 and 8, and for enzyme immunoassays low positive ranges from 1-3.[8] Dufour et al[8] found that low S/Co ratio is a predictor of low likelihood of HCV infection. Sookoian and Castano[9] tested for HCV antibody by microparticle enzyme immunoassay (MEIA) test and found that an S/Co of 26 showed sensitivity of 99% and specificity of 96% in predicting viremic status of HCV infected individuals. Recently, at King Abdul-Aziz Medical City, we have analyzed prospectively anti-HCV of blood bank donors using Abbott's microparticle enzyme immunoassay screening system (MEIA V3.0).[10] A very high false positive anti-HCV screening result was found. Out of 111 donors with positive anti-HCV screening results, only 16 (14%) were truly positive for anti-HCV by a third generation recombinant immunoblot assay (RIBA). Fifty-six were truly negative and 39 gave indeterminate results by RIBA. Even those who gave indeterminate results when retested by HCV ribonnucleic acid (RNA) were negative (Unpublished data). This result has a major effect on many aspects of HCV infection in KSA. First, the epidemiology of anti-HCV antibody, reported by us[11] and others (reviewed by Al Faleh),[12] showed an average HCV prevalence among blood donors and the community at large of 1%. Our new findings suggest that the true prevalence is only a fraction of what was described before and would be in the range of 0.1%. The second major implication is that in our blood donors, a high false positive rate

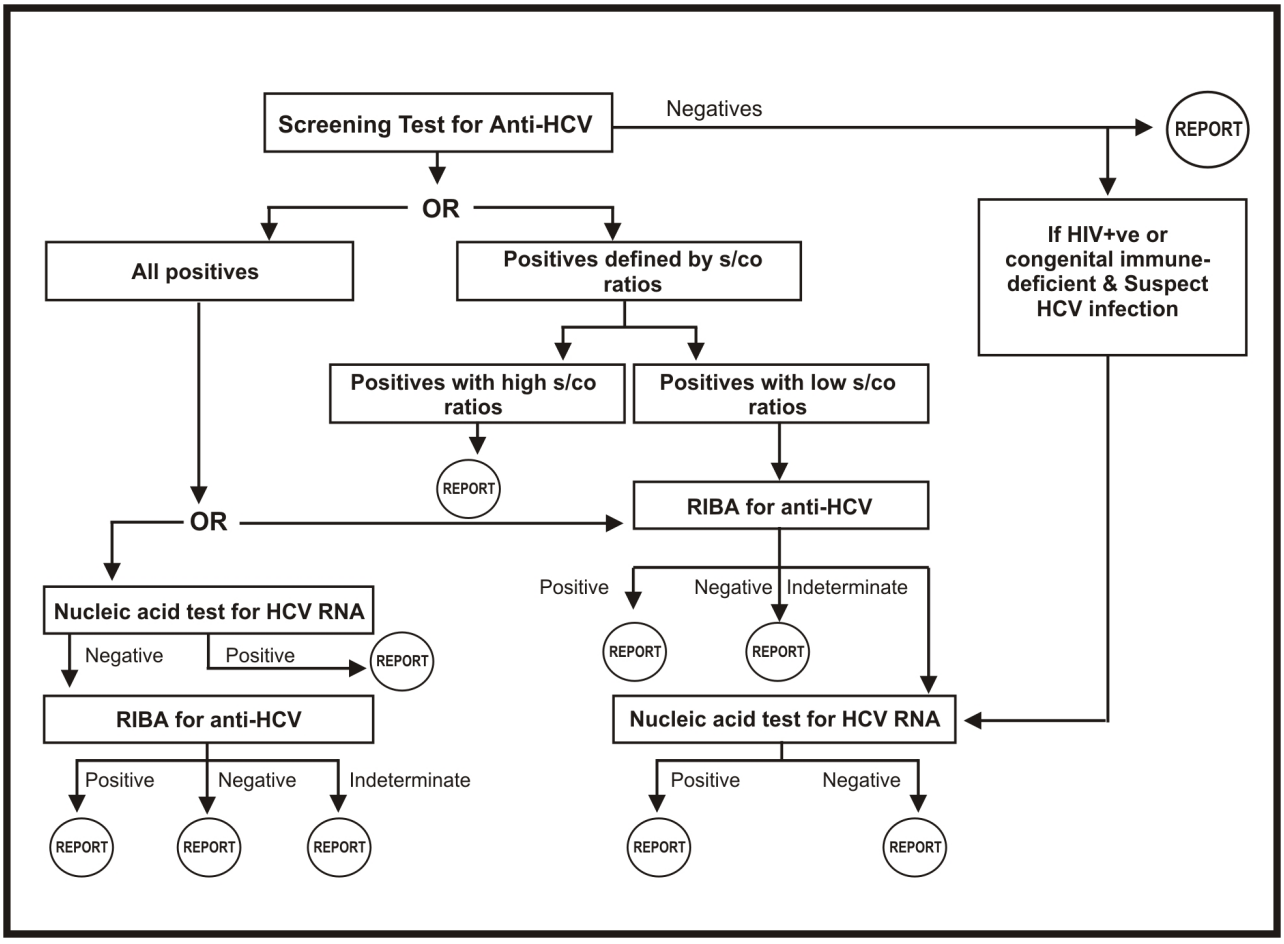

Figure 1 - Laboratory algorithm for anti-hepatitis C virus testing and result reporting, based on Alter et al[5] with modifications

Table 1 - Interpretation of laboratory results for the diagnosis of hepatitis C virus, based on Alter et al[5] with modifications.

Screening test	RIBA	HCV RNA	Anti-HCV status	HCV infection status
Negative	NA	NA	Negative	Not infected unless recent infection is suspected
Positive	Negative	NA	Negative	Not infected unless recent infection is suspected
Positive (high S/Co)	ND	ND	Positive	Past or present infection
Positive	Positive	ND	Positive	Past or present infection
Positive	ND	Negative	Unknown	Unknown*
Positive	Positive	Negative	Positive	Past or present infection**
Positive	Positive/ND	Positive	Positive	Active infection
Negative	NA	Positive	Negative	Active infection, acquired or congenital immunodeficiency should be investigated, flase negative antibody for other reasons
Positive (low S/Co)	Indeterminate	Negative	Indeterminate	Probably false positive screening test, no HCV infection

RIBA - recombinant immunoblot assay, HCV - hepatitis C virus, RNA - ribonnucleic acid
NA - not applicable, ND - not done,
* single negative HCV RNA results cannot determine infection status
** HCV RNA can be intermittent, repeat HCV RNA

of the anti-HCV antibodies is evident. Only those with low positive results are required to have RIBA testing, **Table 1** for results interpretation. Based on the recent CDC guidelines,[5] our experience[10] and results from other studies[9,13] with HCV diagnosis, we introduced the following guidelines **(Figure 1)**. 1. Epidemiological studies. Screening is not a useful marker and should be confirmed with supplementary tests to prove true positive cases. 2. Blood bank donors. Screening test results can be divided into 3 categories, (a) negative, no need for confirmatory testing, (b) low positive, a confirmatory test is required **(Table 1)**, and (c) high positive (S/Co> 16 in MEIA), no need for confirmatory testing to confirm antibody status for blood bank use. 3. Laboratory walk-in subjects (no referrals). Should follow on as with the blood bank donors. However, if confirmed positive for HCV antibodies, referral to a specialist is required for follow up and treatment. 4. Subjects with suspected HCV infection. If screening is negative, no further testing is required. Low positive screening results should be confirmed **(Figure 1)** and strong positive screening is indicative of HCV infection. Hepatitis C virus RNA testing is recommended for treatment and follow-up (see below). 5. HCV RNA test. There are qualitative and quantitative HCV RNA polymerase chain reaction tests. The later is mainly used to monitor treatment in HCV infection. A positive HCV RNA qualitative result is suggestive of active HCV infection. HCV RNA test can be negative (undetectable by current assays), in an individual with positive antibody results. This case can be difficult to interpret. As it can be found in an individual with inactive disease, or in someone who is clearing the infection. Interpretation of this test as well as with the other HCV laboratory tests should not be carried out independent of the clinical and other laboratory findings. Repeat testing of HCV RNA at different time intervals is recommended. There are odd cases where HCV RNA test is positive but the screening antibody test is negative **(Table 1)** and in this case acquired or congenital immunodeficiency should be investigated.[14]

Finally, we suggest reporting HCV screening test results as S/Co value with interpretation as, negative, low positive or high positive.

References

1. Global surveillance and control of hepatitis C. Report of a WHO Consultation organized in collaboration with the Viral Hepatitis Prevention Board, Antwerp, Belgium. ***J Viral Hepat*** 1999; 6: 35-47.
2. Shobokshi OA, Serebour FE, Al Drees, Z, Mitwalli AH, Qahtani A, Skakni LI. Hepatitis C virus seroprevalence rate among Saudis. ***Saudi Med J*** 2003; 24 Suppl 2: S81-S86.
3. Gretch DR. Diagnostic tests for hepatitis C. ***Hepatology*** 1997; 26: 43S-47S.
4. Sakugawa H, Nakasone H, Nakayoshi T, Kinjo F, Saito A, Yakabi S et al. High proportion of false positive reactions among donors with anti-HCV antibodies in a low prevalence area. ***J Med Virol*** 1995; 46: 334-338.
5. Alter MJ, Kuhnert WL, Finelli L. Guidelines for laboratory testing and result reporting of antibody to hepatitis C virus. Centers for Disease Control and Prevention. ***MMWR Morb Mortal Wkly Rep*** 2003; 52: 1-13, 15.
6. Proust B, Dubois F, Bacq Y, Le Program S, Rogez S, Levillain R et al. Two successive hepatitis C virus infections in an intravenous drug user. ***J Clin Microbiol*** 2000; 38: 3125-3127.
7. Fabrizi F, Martin P, Dixit V, Brezina M, Cole MJ, Vinson S et al. Biological dynamics of viral load in hemodialysis patients with hepatitis C virus. ***Am J Kidney Dis*** 2000; 35: 122-129.
8. Dufour DR, Talastas M, Fernandez MD, Harris B, Strader DB, Seeff LB. Low-positive anti-hepatitis C virus enzyme immunoassay results: an important predictor of low likelihood of hepatitis C infection. ***Clin Chem*** 2003; 49: 479-486.
9. Sookoian S, Castano G. Evaluation of a third generation anti-hcv assay in predicting viremia in patients with positive HCV antibodies. ***Ann Hepatol*** 2002; 1: 179-182.
10. Abutaleb A, Abed, E, Qasem L, Memish Z, Hajeer A. Improved efficiency of HCV antibody testing algorithm in blood bank donors: results from Saudi Arabia. ***Clin Diag Lab Immunol*** 2004 (in press).
11. Tamimi W, Hajeer A, Qasem L, Alkhashan A, Alsohabani A, Bernvil S et al. Expansion of Saudi blood donor pool by better screening and vaccination practices. ***Clin Diagn Lab Immunol*** 2003; 10: 1159-1160.
12. Al Faleh FZ, Ramia S. Hepatitis C virus (HCV) infection in Saudi Arabia: A review. ***Ann Saudi Med*** 1997; 17: 77-82.
13. Dufour DR, Talastas M, Fernandez MD, Harris B, Strader DB, Seeff LB. Low-positive anti-hepatitis C virus enzyme immunoassay results: an important predictor of low likelihood of hepatitis C infection. ***Clin Chem*** 2003; 49: 479-486.
14. George SL, Gebhardt J, Klinzman D, Foster MB, Patrick KD, Schmidt WN et al. Hepatitis C virus viremia in HIV-infected individuals with negative HCV antibody tests. ***J Acquir Immune Defic Syndr*** 2002; 31: 154-162.