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Performance characteristics of a combined hepatitis C virus core antigen and anti—hepatitis C virus antibody test in different patient groups

Jeng-Fu Yang [a,b], Ya-Yun Lin [a], Meng-Hsuan Hsieh [a], Chiu-Hung Tsai [c], Shu-Fen Liu [c], Ming-Lung Yu [b,d,e], Chia-Yen Dai [a,b,c,d], Jee-Fu Huang [b,c,d,f,*], Wen-Yi Lin [a], Zu-Yau Lin [b,d], Shinn-Chern Chen [b,d], Wan-Long Chuang [b,c,d]

[a] Department of Preventive Medicine, Kaohsiung Medical University Hospital, Kaohsiung, Taiwan
[b] Hepatobiliary Division, Department of Internal Medicine, Kaohsiung Medical University Hospital, Kaohsiung, Taiwan
[c] Graduate Institute of Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan
[d] Faculty of Internal Medicine, College of Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan
[e] Department of Internal Medicine, Kaohsiung Municipal Hsiao-Kang Hospital, Kaohsiung, Taiwan
[f] Department of Internal Medicine, Kaohsiung Municipal Hsiao-Kang Hospital, Kaohsiung Medical University, Kaohsiung, Taiwan

Abstract   We evaluated the performance of a hepatitis C virus (HCV) antigen/antibody combination test [Murex HCV Antigen/Antibody Combination Test (Murex Ag/Ab test)] by comparing it with the current third-generation HCV antibody enzyme immunoassay (anti-HCV). A total of 403 serum samples were consecutively collected from four patient groups: healthy controls ($n = 100$); HCV-infected patients (HCV group, $n = 102$); Human immunodeficiency virus (HIV)/HCV-infected patients (HIV/HCV group, $n = 100$); and patients with uremia (uremia group, $n = 101$). Performances were evaluated for the Murex Ag/Ab, anti-HCV, and HCV RNA in the HIV/HCV and uremia patient groups. In the HCV group, all 102 samples showed concordant positive and negative results for anti-HCV, Murex Ag/Ab, and HCV RNA tests. In the HIV/HCV group, all 100 samples were positive for both anti-HCV and Murex Ag/Ab tests, whereas 88 patients (88%) were HCV RNA positive. In the uremia group, 14 (69.0%) of the 23 anti-HCV-positive patients were HCV RNA positive, whereas 14 (77.8%) of the 18 Murex

Ag/Ab—positive patients were HCV RNA positive. None of anti-HCV-negative or Murex Ag/Ab—negative patients were HCV RNA positive. Based on the HCV RNA assay, the sensitivities for both anti-HCV and Murex Ag/Ab assays were 100%, whereas the specificities of these two assays were 89.7% and 95.4%, respectively. With good sensitivity and specificity, the Murex Ag/Ab assay could be a useful alternative diagnostic tool, especially in immunocompromised populations, such as patients with uremia or those infected with HIV.

Introduction

With an estimated 170 million infected individuals, hepatitis C virus (HCV) infection has an enormous impact on public health worldwide [1—3]. Therefore, an accurate and early diagnosis of active HCV infection is critical not only because of its associated morbidity and mortality but also because early diagnosis is the most important factor for a chance of cure, either spontaneously or by antiviral therapy [4—6].

Commercial assays for anti-HCV antibodies have made early detection of HCV infection possible since 1990 and have prevailed since then [7]. With the widespread application of the anti-HCV test in the past two decades, more endemic areas have been discovered and have benefited from implementation of public health strategies [4,8—11]. Although the anti-HCV test has significantly reduced the risk of HCV transmission, the window period for detection of recent or new infection remains a concern. The anti-HCV antibodies can be detected 7—8 weeks after infection and usually persist for life. However, false-negative results may arise in immunocompromised patients, such as those with human immunodeficiency virus (HIV) infection or uremia. In the context of HIV infection, the HCV seroconversion delay is often prolonged [12] and leads to the failure of reactivity of current anti-HCV antibody detection tests [13]. The window period can be longer in patients on hemodialysis (HD) because these individuals are severely immunocompromised [14]. In such situations, a highly sensitive and reliable test is needed for early detection of HCV infection.

Nucleic acid testing (NAT) for HCV RNA was developed as a more accurate method for disease diagnosis and monitoring as well as a confirmatory diagnostic tool for anti-HCV assays [15—17]. The introduction of NAT has greatly reduced the risk of HCV transmission [18]. However, its high cost, liability to environmental contamination, and laborious work have also hampered the wide application of NAT in clinical settings. Efforts have been made over the past several years to develop a test that could competently supplement or eventually replace NAT in the diagnosis of HCV infection.

The simultaneous detection of both antigen and antibody instead of viral nucleic acids was the initial logical approach. Such detection has been shown to efficiently shorten the seronegative window for the diagnosis of HIV infection [19]. A similar approach based on the HCV core protein and specific anti-HCV antibody detection has recently been developed for the diagnosis of HCV infection [20—22].

In this study, we evaluated the clinical performance of an HCV antigen/antibody combination test (Murex Ag/Ab test; Abbott Laboratories, North Chicago, IL, USA) as compared with the third-generation HCV antibody enzyme immunoassay (EIA; AxSYM HCV 3.0, Abbott Laboratories). Patients with chronic hepatitis C (CHC), patients with dual HIV/HCV infections, and patients with end-stage renal disease (ESRD) requiring HD were recruited for the study. Healthy subjects without HCV or HIV infection served as controls.

Materials and methods

Patient selection

A total of 403 serum samples were consecutively collected from four patient groups: healthy controls ($n = 100$; 59 men; mean age, 53.4 ± 11.8 years); HCV group ($n = 102$; 41 men; mean age, 54.0 ± 10.5 years); HIV/HCV group ($n = 100$; all men; mean age, 36.3 ± 7.5 years); and uremia group ($n = 101$; 46 men; mean age, 59.0 ± 12.9 years). The individuals in the control group were seronegative for the anti-HCV test, HCV RNA, HIV, and hepatitis B surface antigen (HBsAg), and had alanine aminotransferase (ALT) values within normal limits. The patients in the HCV group were positive for both the anti-HCV test and HCV RNA, negative for HIV infection and HBsAg, and had ALT levels higher than 1.5 times upper normal limit. The patients in the HIV/HCV group were positive for anti-HIV and anti-HCV antibodies, whereas the uremic patients undergoing regular HD were negative for anti-HIV and HBsAg.

Our research conformed to the Helsinki Declaration. The present study was approved by the ethics committee of Kaohsiung Medical University Hospital. All patients gave informed consent to participate in our study.

Laboratory analyses

The sera were removed from clots within 4 hours of collection and stored at $-70°C$ until needed. ALT levels were measured on a multichannel autoanalyzer (Architect ci8200; Abbott Laboratories). HBsAg was detected using a chemiluminescent microparticle immunoassay (Architect ci8200; Abbott Laboratories). Anti-HCV antibody was detected using a third-generation, commercially available enzyme-linked immunosorbent assay kit (AxSYM HCV 3.0; Abbott Laboratories) [23]. Serum HCV RNA was detected using a standardized automated qualitative reverse transcription-polymerase chain reaction assay (COBAS AMPLICOR Hepatitis C Virus Test, version 2.0; Roche, Branchburg, NJ, USA). The detection limit was 50 IU/mL.

Murex Ag/Ab test

The Murex Ag/Ab test is a two-step EIA for the detection of HCV infection [24]. Each sample was tested by a monoclonal antibody sandwich used for HCV core antigen plus recombinant NS3 protein and core sandwich used for antibody detection according to the manufacturer's instructions. Briefly, 50 μL of sample diluents followed by 50 μL of specimens or controls were added to each of the microplate wells. The wells were incubated at 37°C for 60 minutes, then washed five times after which 120 μL of conjugate was added to all wells, which were then incubated for 60 minutes at room temperature (15−28°C). The plate was washed five times and then 80 μL of substrate was added to each of the wells. The microplate was incubated for 30 minutes at 37°C. Finally, the stop solution was added and the plates were read at 450/630 nm. A sample was considered positive when its optical density was greater than or equal to the cutoff value (determined by the mean of the negative control optical density divided by 2 plus 0.2).

The overall objective was to determine if this new test could be an alternative for the diagnosis of HCV infection during the window period and whether the sensitivity for antibody detection is preserved. For sera with discrepant results between the Murex HCV Ag/Ab combination and AxSYM HCV 3.0, we performed confirmatory tests using the HCV RNA test.

Statistical analysis

Data were analyzed with Chi-square test or the Fisher's exact test, and the Student t test or Mann−Whitney test was used for the baseline characteristics of patients. Differences were considered significant if the p value was less than 0.05. In addition, we also evaluated the results of serum samples from the HIV and uremia patient groups with AxSYM HCV 3.0, Murex HCV Ag/Ab combination, and HCV RNA, for sensitivity and specificity. Quality control procedures, database processing, and analyses were performed using the SPSS 12.0 statistical package (SPSS Inc., Chicago, IL, USA).

Results

Patients' characteristics

The mean ALT level in the HCV group was 127.3 ± 175 IU/L, which was significantly ($p < 0.001$) higher than the levels in the HIV/HCV groups (33.7 ± 31.1 IU/L); uremia group (11.6 ± 14.6 IU/L); and healthy controls (12.8 ± 4.8 IU/L). In the HCV group, all 102 samples showed concordant positive results for anti-HCV, Murex Ag/Ab, and HCV RNA tests. Concordant negative results were also found with respect to these three assays. In the HIV/HCV group, all 100 samples were positive for both anti-HCV and Murex Ag/Ab tests, whereas 88 patients (88%) were HCV RNA positive (Table 1). In the uremia group, 22.8% of patients (23 of 101) were anti-HCV positive, 17.8% (18 of 101) were Murex Ag/Ab positive, and 13.9% (14 of 101) were HCV RNA positive.

Table 1 Comparison of anti-HCV, Murex Ag/Ab, and HCV RNA assays in uremia and HIV/HCV groups

	Uremia group		HIV/HCV group	
	HCV RNA		HCV RNA	
	+	−	+	−
Anti-HCV, n (%)				
+	14 (69.0)	9 (39.1)	88 (88.0)	12 (12.0)
−	0	78 (100)	0	0
Murex Ag/Ab, n (%)				
+	14 (77.8)	4 (22.2)	88 (88.0)	12 (12.0)
−	0	83 (100)	0	0

Anti-HCV: AxSYM HCV 3.0. The third-generation HCV antibody enzyme immunoassay, S/CO ≥1 = positive; <1 = negative.
Murex Ag/Ab: Murex HCV Antigen/Antibody Combination Test, OD for the sample/cutoff OD.
HCV RNA: AMPLICOR HCV Monitor 2.0 assay.
HCV = hepatitis C virus; HIV = human immunodeficiency virus; OD = optical density; S/CO = sample/cutoff, indicating the OD of the sample/the OD of the cutoff.

Comparison of performances among anti-HCV, Murex Ag/Ab, and HCV RNA assays in the uremia and HIV/HCV groups

We further analyzed the performance characteristics among these assays in the uremia group to assess the inconsistent results. In the uremia group, 14 (69.0%) of the 23 anti-HCV-positive patients were HCV RNA positive, whereas 14 (77.8%) of the 18 Murex Ag/Ab−positive patients were HCV RNA positive. None of the anti-HCV-negative or Murex Ag/Ab−negative patients was HCV RNA positive. Taken together, based on HCV RNA assay, the sensitivities of both anti-HCV and Murex Ag/Ab assays were 100%, whereas the specificities of anti-HCV and Murex Ag/Ab assays were 89.66% and 95.40%, respectively (Table 1). The negative predictive values for both anti-HCV and Murex Ag/Ab assays were 100%, and the positive predictive values of anti-HCV and Murex Ag/Ab assays were 82.9% and 86.4%, respectively.

An analysis of the false-positive results by anti-HCV or Murex Ag/Ab assays in uremia and HIV/HCV patient groups is shown in Table 2. Five (23.8%) patients (1 man) of the 21 anti-HCV-positive patients were negative for Murex Ag/Ab assay, and all five patients were HCV RNA negative with normal ALT levels. Four (25.0%) of the 16 patients positive for both assays were HCV RNA negative and had normal ALT levels.

Analysis of negative results by HCV RNA assay but positive by AxSYM HCV 3.0 assay or Murex HCV Ag/Ab combination test

In the uremia and HIV groups, 21 samples, 9 in the uremia group and 12 in the HIV/HCV group, had negative results on HCV RNA assays with AMPLICOR HCV Monitor 2.0 assay, but positive results with the AxSYM HCV 3.0 assay or Murex HCV Ag/Ab combination test. A confirmatory test was performed in these samples using a recombinant immunoblot assay

Table 2 Analysis of negative results by HCV RNA assay but positive by AxSYM HCV 3.0 assay or Murex HCV Ag/Ab combination test in uremia and HIV/HCV patient groups

Patient group	Sex/age (yr)	ALT (IU/L)	AxSYM HCV 3.0[a]	Murex HCV Ag/Ab[b]	HCV RNA[c]	RIBA[d]
Uremia	M/52	12	+ (55.55)	+ (8.166)	−	−
Uremia	M/46	8	+ (1.42)	− (0.618)	−	+
Uremia	M/52	8	+ (1.31)	+ (2.269)	−	−
Uremia	F/74	7	+ (2.18)	− (0.558)	−	−
Uremia	F/60	11	+ (1.98)	− (0.897)	−	+
Uremia	M/64	8	+ (89.11)	+ (3.744)	−	−
Uremia	F/51	6	+ (1.34)	− (0.797)	−	−
Uremia	F/65	4	+ (1.01)	− (0.591)	−	−
Uremia	F/55	8	+ (6.02)	+ (1.472)	−	−
HIV/HCV	M/44	14	+ (64.47)	+ (8.836)	−	−
HIV/HCV	M/29	15	+ (40.57)	+ (8.964)	−	−
HIV/HCV	M/35	30	+ (45.33)	+ (8.243)	−	−
HIV/HCV	M/35	6	+ (92.66)	+ (9.272)	−	−
HIV/HCV	M/50	14	+ (131.81)	+ (8.757)	−	−
HIV/HCV	M/35	28	+ (33.03)	+ (9.131)	−	−
HIV/HCV	M/49	20	+ (112.49)	+ (7.364)	−	−
HIV/HCV	M/40	29	+ (44.01)	+ (8.918)	−	−
HIV/HCV	M/28	60	+ (38.82)	+ (9.403)	−	−
HIV/HCV	M/37	12	+ (145.9)	+ (9.334)	−	−
HIV/HCV	M/46	8	+ (93.32)	+ (9.128)	−	−
HIV/HCV	M/35	26	+ (78.15)	+ (8.033)	−	−

[a] The third-generation HCV antibody enzyme immunoassay, S/CO ≥ 1 = positive; <1 = negative.
[b] Murex HCV Ag/Ab combination, OD for the sample/cutoff OD.
[c] AMPLICOR HCV Monitor 2.0 assay.
[d] Confirmatory tests using RIBA (immunoblot assay).

ALT = alanine aminotransferase; F = female; HCV = hepatitis C virus; HIV = human immunodeficiency virus; M = male; OD = optical density; RIBA = recombinant immunoblot assay; S/CO = sample/cutoff, indicating the OD of the sample/the OD of the cutoff.

(RIBA). In the uremia group, all of the results by AxSYM HCV 3.0 assay were discriminative to the HCV RNA assay, and only two results by the AxSYM HCV 3.0 assay were consistent with the results of RIBA. For the Murex HCV Ag/Ab combination test, five cases matched with the HCV RNA assay and three cases matched with RIBA (Table 2). In HIV/HCV group, all 12 patients with positive results in the AxSYM HCV 3.0 assay and Murex HCV Ag/Ab combination test revealed negative results in the HCV RNA assay and RIBA.

Discussion

HCV infection is one of the most important causes of liver cirrhosis and hepatocellular carcinoma with a high impact on health worldwide [6]. Therefore, early diagnosis of HCV infection remains a persistent need for identification of the characteristically asymptomatic viral infection. The current study covering four different groups of patients in a clinical setting demonstrated that the Murex Ag/Ab assay possessed a comparable performance with the anti-HCV assay in the diagnosis of HCV infection. The concordant results from the healthy control and HCV groups may further confirm that it is a useful complementary tool in screening patients or when NAT is unavailable. Moreover, the Murex Ag/Ab assay had a higher specificity for detection in uremic patients, indicating that the Murex Ag/Ab assay is a competent alternative for detecting HCV infection, particularly in immunocompromised hosts.

Diagnosis of HCV infection largely relies on classical serologic methods of anti-HCV antibody by EIAs, and it is confirmed by a positive result obtained using an immunoblot assay or by the presence of HCV RNA [25]. However, the value of anti-HCV antibody detection assays is somewhat limited during the early stages of infection because of the slow development of specific antibodies. In addition, patients may fail to develop a strong and rapid specific immune response against HCV because of their immunocompromised status. In the window period for diagnosis, the viral load is very low and despite improvements in the assay for anti-HCV, the infection goes undetected, more so in immunocompromised patients, such as those with ESRD on maintenance HD [26] and patients coinfected with HIV [27,28]. Previous studies have demonstrated that the ratio of HCV viremia in HCV-seronegative patients on HD was 1−15% [29,30]. Some studies mentioned that HCV particles may pass through the dialysis membrane [29] and may even be destroyed in the process of dialysis; these circumstances might lead to the low viral load observed in this group of patients on HD. The reported prevalence of chronic seronegative HCV infection among anti-HCV-infected, HIV-infected patients has varied between 0% and 13.2% [27,28,31,32]; this variation is possibly the result of immunosuppression, in which case, patients are unable to mount or maintain HCV antibody titers for detection by standard serodiagnostic tests [33−35]. The development of new assays that combine both antigen and antibody detection, similar to what has been done in the field of HIV detection

[19], may prove useful in reducing the long window of HCV seronegativity or in compensating for the absence of a specific antibody response. This is particularly true in the immunocompromised patients with HIV coinfection and ESRD on HD, where long periods of seroconversion have been described [29,36]. The window period may extend up to 6—12 months in immunocompromised patients [37].

In our analysis, we found no significant difference for detection of HCV infection between the Murex Ag/Ab and anti-HCV assays in patients with CHC without an immuno-compromised status. This implies that these two tests may be equally reliable in non-immunocompromised patients with CHC. In the patients in the uremia group in our study, Murex Ag/Ab and anti-HCV assays had a comparable performance in terms of sensitivity, when compared with the HCV RNA assay, and the Murex Ag/Ab assay had better specificity than the anti-HCV assay. We also found that the Murex Ag/Ab assay had a higher matching rate with the HCV RNA assay in discordant results than the anti-HCV assay in the uremia group. The sensitivity of Murex Ag/Ab assay was identical with that of the anti-HCV assay in the HIV group (100%). The Murex Ag/Ab assay may have good performance in detecting HCV infection, especially in immunocompromised patients, such as those with uremia or HIV infection.

Twenty-one serum samples with negative results by the HCV RNA assay but positive results by the anti-HCV assay or Murex Ag/Ab assay were found in our study. This result is consistent with recent data showing that significant numbers of samples were anti-HCV positive but NAT nega-tive when tested in a minipool [38]. For example, approx-imately 30% of anti-HCV-positive donors in Australia had no detectable HCV RNA [39]. It should be recognized that the level of viremia declines and fluctuates widely in patients after seroconversion or during remission [40,41]. Specimens from these patients may transiently test negative for viral nucleic acid or viral antigen but may still be infectious despite the presence of HCV antibodies. In addition, 2 RIBA-reactive uremic patients were both positive for AxSYM HCV 3.0 but negative for the Murex Ag/Ab assay. These four assays have different related target regions in the HCV viral genome: AxSYM HCV 3.0: NS3,NS4; Murex Ag/Ab assay: core, NS3; HCV RNA assay (COBAS AMPLICOR Hepatitis C Virus Test, version 2.0): 5'-untranslated region; and RIBA: NS5, NS3 (c33c, c100p, 5-1-1p, and c22p), which may result in different reactive patterns in the immunoblot assay. This may be the reason these two RIBA-reactive uremic patients were positive on the AxSYM HCV 3.0 but negative on the Murex Ag/Ab assay. Therefore, NAT will supplement immunoassays rather than replace them. The HCV Ag/Ab combination assay was reported to provide earlier detec-tion of exposure to HCV compared with the anti-HCV anti-body test; HCV NAT provides even earlier detection than is afforded by the HCV Ag/Ab combination assay [21,42]. This newly developed assay was reported to present an improvement for the detection of HCV infection, especially in the early phase of infection when antibodies are unde-tectable. The reductions in the diagnostic window period observed with the new test and HCV RNA assays were equal, on average 24 days and 34.4 days, respectively [42]. Because of a cross-sectional design, we could not assess the data on the reduction in the window period in our analysis.

In conclusion, the Murex Ag/Ab assay has good sensitivity and specificity and could be a useful alternative tool in the diagnostic setting where procedures to reduce the window period, such as HCV RNA detection, are not currently rec-ommended. It could be proposed for use especially in patients with uremia or those with HIV infection, who have a high risk of infection and in whom an early diagnosis allows better management. This assay may improve the diagnosis of HCV infection in addition to the current serologic assays.

Acknowledgments

References

[1] Yang JF, Lin CI, Huang JF, Dai CY, Lin WY, Ho CK, et al. Viral hepatitis infections in southern Taiwan: a multicenter community-based study. Kaohsiung J Med Sci 2010;26:461—9.

[2] Leone N, Rizzetto M. Natural history of hepatitis C virus infection: from chronic hepatitis to cirrhosis, to hepatocel-lular carcinoma. Minerva Gastroenterol Dietol 2005;51:31—46.

[3] Frank C, Mohamed MK, Strickland GT, Lavanchy D, Arthur RR, Magder LS, et al. The role of parenteral antischistosomal therapy in the spread of hepatitis C virus in Egypt. Lancet 2000;355:887—91.

[4] Chuang WL, Yu ML, Dai CY, Chang WY. Treatment of chronic hepatitis C in southern Taiwan. Intervirology 2006;49:99—106.

[5] Yu ML, Dai CY, Huang JF, Hou NJ, Lee LP, Hsieh MY, et al. A randomised study of peginterferon and ribavirin for 16 versus 24 weeks in patients with genotype 2 chronic hepatitis C. Gut 2007;56:553—9.

[6] Yu ML, Chuang WL. Treatment of chronic hepatitis C in Asia: when East meets West. J Gastroenterol Hepatol 2009;24:336—45.

[7] Tobler LH, Stramer SL, Lee SR, Masecar BL, Peterson JE, Davis EA, et al. Impact of HCV 3.0 EIA relative to HCV 2.0 EIA on blood-donor screening. Transfusion 2003;43:1452—9.

[8] Yu ML, Chuang WL, Lu SN, Chen SC, Wang JH, Lin ZY, et al. The genotypes of hepatitis C virus in patients with chronic hepa-titis C virus infection in southern Taiwan. Kaohsiung J Med Sci 1996;12:605—12.

[9] Huang JF, Lu SN, Chue PY, Lee CM, Yu ML, Chuang WL, et al. Hepatitis C virus infection among teenagers in an endemic township in Taiwan: epidemiological and clinical follow-up studies. Epidemiol Infect 2001;127:485—92.

[10] Wu TC, Chuang WL, Dai CY, Huang JF, Hsieh MY, Hou NJ, et al. Hepatitis C virus infection among children in aboriginal areas in Taiwan. Trans R Soc Trop Med Hyg 2008;102:935—8.

[11] Huang CF, Huang JF, Dai CY, Yu ML, Lu SN, Hsieh MY, et al. Changing prevalence of hepatitis C virus infection among teenagers in an endemic area in Taiwan. Trans R Soc Trop Med Hyg 2008;102:929—34.

[12] Ridzon R, Gallagher K, Ciesielski C, Ginsberg MB, Robertson BJ, Luo CC, et al. Simultaneous transmission of human immunodeficiency virus and hepatitis C virus from a needle-stick injury. N Engl J Med 1997;336:919—22.

[13] Pawlotsky JM, Bastie A, Pellet C, Remire J, Darthuy F, Wolfe L, et al. Significance of indeterminate third-generation hepatitis

C virus recombinant immunoblot assay. J Clin Microbiol 1996; 34:80—3.

[14] Lok AS, Chien D, Choo QL, Chan TM, Chiu EK, Cheng IK, et al. Antibody response to core, envelope and nonstructural hepatitis C virus antigens: comparison of immunocompetent and immunosuppressed patients. Hepatology 1993;18:497—502.

[15] Mine H, Emura H, Miyamoto M, Tomono T, Minegishi K, Murokawa H, et al. High throughput screening of 16 million serologically negative blood donors for hepatitis B virus, hepatitis C virus and human immunodeficiency virus type-1 by nucleic acid amplification testing with specific and sensitive multiplex reagent in Japan. J Virol Methods 2003;112: 145—51.

[16] Stramer SL, Glynn SA, Kleinman SH, Strong DM, Caglioti S, Wright DJ, et al. Detection of HIV-1 and HCV infections among antibody-negative blood donors by nucleic acid-amplification testing. N Engl J Med 2004;351:760—8.

[17] Huang JF, Dai CY, Lin YY, Yu ML, Liu SF, Lin IL, et al. Performance characteristics of a real-time RT-PCR assay for quantification of hepatitis C virus RNA in patients with genotype 1 and 2 infections. Clin Chem Lab Med 2008;46:475—80.

[18] Soldan K, Davison K, Dow B. Estimates of the frequency of HBV, HCV, and HIV infectious donations entering the blood supply in the United Kingdom, 1996 to 2003. Euro Surveill 2005;10:17—9.

[19] Ly TD, Laperche S, Brennan C, Vallari A, Ebel A, Hunt J, et al. Evaluation of the sensitivity and specificity of six HIV combined p24 antigen and antibody assays. J Virol Methods 2004;122:185—94.

[20] Tanaka E, Ohue C, Aoyagi K, Yamaguchi K, Yagi S, Kiyosawa K, et al. Evaluation 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.

[21] Laperche S, Elghouzzi MH, Morel P, Asso-Bonnet M, Le Marrec N, Girault A, et al. Is an assay for simultaneous detection of hepatitis C virus core antigen and antibody a valuable alternative to nucleic acid testing? Transfusion 2005;45:1965—72.

[22] Nubling CM, Unger G, Chudy M, Raia S, Lower J. Sensitivity of HCV core antigen and HCV RNA detection in the early infection phase. Transfusion 2002;42:1037—45.

[23] 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—82.

[24] Laura D, Keith P, editors. Evaluation of Murex HCV Ag/Ab Combination. London: Microbiological Diagnostics Assessment Service Evaluations and Standards Laboratory; 2007.

[25] Pawlotsky JM. Use and interpretation of virological tests for hepatitis C. Hepatology 2002;36:S65—73.

[26] Hanuka N, Sikuler E, Tovbin D, Mostoslavsky M, Hausman M, Orgel M, et al. Hepatitis C virus infection in renal failure patients in the absence of anti-hepatitis C virus antibodies. J Viral Hepat 2002;9:141—5.

[27] 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—62.

[28] Hall CS, Charlebois ED, Hahn JA, Moss AR, Bangsberg DR. Hepatitis C virus infection in San Francisco's HIV-infected urban poor. J Gen Intern Med 2004;19:357—65.

[29] Dalekos GN, Boumba DS, Katopodis K, Zervou E, Sferopoulos G, Elisaf M, et al. Absence of HCV viraemia in anti-HCV-negative haemodialysis patients. Nephrol Dial Transplant 1998;13: 1804—6.

[30] Barril G, Bartolome J, Traver JA, Cabrerizo M, Selgas R, Carreno V. Autoclaving eliminates hepatitis C virus from a hemodialysis monitor contaminated artificially. J Med Virol 2000;60:139—43.

[31] Bonacini M, Lin HJ, Hollinger FB. Effect of coexisting HIV-1 infection on the diagnosis and evaluation of hepatitis C virus. J Acquir Immune Defic Syndr 2001;26:340—4.

[32] Thio CL, Nolt KR, Astemborski J, Vlahov D, Nelson KE, Thomas DL. Screening for hepatitis C virus in human immunodeficiency virus-infected individuals. J Clin Microbiol 2000; 38:575—7.

[33] Cribier B, Rey D, Schmitt C, Lang JM, Kirn A, Stoll-Keller F. High hepatitis C viraemia and impaired antibody response in patients coinfected with HIV. AIDS 1995;9:1131—6.

[34] Marcellin P, Martinot-Peignoux M, Elias A, Branger M, Courtois F, Level R, et al. Hepatitis C virus (HCV) viremia in human immunodeficiency virus-seronegative and -seropositive patients with indeterminate HCV recombinant immunoblot assay. J Infect Dis 1994;170:433—5.

[35] Sorbi D, Shen D, Lake-Bakaar G. Influence of HIV disease on serum anti-HCV antibody titers: a study of intravenous drug users. J Acquir Immune Defic Syndr Hum Retrovirol 1996;13: 295—6.

[36] Morand P, Dutertre N, Minazzi H, Burnichon J, Pernollet M, Baud M, et al. Lack of seroconversion in a health care worker after polymerase chain reaction-documented acute hepatitis C resulting from a needlestick injury. Clin Infect Dis 2001;33: 727—9.

[37] Icardi G, Ansaldi F, Bruzzone BM, Durando P, Lee S, de Luigi C, et al. Novel approach to reduce the hepatitis C virus (HCV) window period: clinical evaluation of a new enzyme-linked immunosorbent assay for HCV core antigen. J Clin Microbiol 2001;39:3110—4.

[38] Busch MP, Tobler LH, Gerlich WH, Schaefer S, Giachetti C, Smith R. Very low level viremia in HCV infectious unit missed by NAT. Transfusion 2003;43:1173—4.

[39] Seed CR, Margaritis AR, Bolton WV, Kiely P, Parker S, Piscitelli L. Improved efficiency of national HIV, HCV, and HTLV antibody testing algorithms based on sequential screening immunoassays. Transfusion 2003;43:226—34.

[40] Fang CT, Tobler LH, Haesche C, Busch MP, Phelps B, Leparc G. Fluctuation of HCV viral load before seroconversion in a healthy volunteer blood donor. Transfusion 2003;43:541—4.

[41] Kuramoto IK, Moriya T, Schoening V, Holland PV. Fluctuation of serum HCV-RNA levels in untreated blood donors with chronic hepatitis C virus infection. J Viral Hepat 2002;9:36—42.

[42] Ansaldi F, Bruzzone B, Testino G, Bassetti M, Gasparini R, Crovari P, et al. Combination hepatitis C virus antigen and antibody immunoassay as a new tool for early diagnosis of infection. J Viral Hepat 2006;13:5—10.