World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
bpgoffice@wjgnet.com
doi:10.3748/wjg.v20.i11.2948

World J Gastroenterol 2014 March 21; 20(11): 2948-2954
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

TOPIC HIGHLIGHT

WJG 20th Anniversary Special Issues (2): Hepatitis C virus

Current testing strategies for hepatitis C virus infection in blood donors and the way forward

Neelam Marwaha, Suchet Sachdev

Neelam Marwaha, Suchet Sachdev, Department of Transfusion Medicine, Post Graduate Institute of Medical Education and Research, Chandigarh 160012, India
Author contributions: Marwaha N planned the review article, wrote the article; Sachdev S crosschecked and took out all the references and proofed the article; Marwaha N and Sachdev S both approved the article before final submission.
Correspondence to: Neelam Marwaha, Professor, Department of Transfusion Medicine, Post Graduate Institute of Medical Education and Research, Sector 12, Chandigarh 160012, India. neelam2918@yahoo.com
Telephone: +91-172-2756481 Fax: +91-172-2744401
Received: September 27, 2013 Revised: December 23, 2013
Accepted: February 20, 2014
Published online: March 21, 2014

Abstract

Screening tests for blood donations are based upon sensitivity, cost-effectiveness and their suitability for high-throughput testing. Enzyme immunoassay (EIAs) for hepatitis C virus (HCV) antibodies were the initial screening tests introduced. The "first generation" antibody EIAs detected seroconversion after unduly long infectious window period. Improved HCV antibody assays still had an infectious window period around 66 d. HCV core antigen EIAs shortened the window period considerably, but high costs did not lead to widespread acceptance. A fourth-generation HCV antigen and antibody assay (combination EIA) is more convenient as two infectious markers of HCV are detected in the same assay. Molecular testing for HCV-RNA utilizing nucleic acid amplification technology (NAT) is the most sensitive assay and shortens the window period to only 4 d. Implementation of NAT in many developed countries around the world has resulted in dramatic reductions in transfusion transmissible HCV and relative risk is now < 1 per million donations. However, HCV serology still continues to be retained as some donations are serology positive but NAT negative. In resource constrained countries HCV screening is highly variable, depending upon infrastructure, trained manpower and financial resource. Rapid tests which do not require instrumentation and are simple to perform are used in many small and remotely located blood centres. The sensitivity as compared to EIAs is less and wherever feasible HCV antibody EIAs are most frequently used screening assays. Efforts have been made to implement combined antigen-antibody assays and even NAT in some of these countries.

Key words: Hepatitis C virus; Screening tests; Blood donors; Immunoassays; Nucleic acid testing

Core tip: This is a review article on development and current status of screening tests for hepatitis C virus (HCV) infection in blood donors. The sensitivity of HCV antibody assays, HCV core antigen assays and combination assays are discussed. Effect of nucleic acid amplification technology implementation of blood safety is highlighted. Future prospects for developed countries and resource constrained countries are presented.

Marwaha N, Sachdev S. Current testing strategies for hepatitis C virus infection in blood donors and the way forward. *World J Gastroenterol* 2014; 20(11): 2948-2954 Available from: URL: http://www.wjgnet.com/1007-9327/full/v20/i11/2948.htm DOI: http://dx.doi.org/10.3748/wjg.v20.i11.2948

INTRODUCTION

Transfusion therapy is an important component of modern health care and is dependant primarily on safe and adequate blood supply. The real challenge of blood

transfusion lies in minimising risks and optimising clinical benefits. The first cases of transfusion associated jaundice were reported in 1943[1,2]. It was only in the 1960's that the causative agent - hepatitis B virus was identified and screening of donor blood for hepatitis B surface antigen (HBsAg) was initiated in the United States (US) in 1971 and became a US federal regulation in July 1972[3]. During the same period a retrospective study was conducted by Grady and Chalmers in 1964 where it was shown that the incidence of transfusion associated hepatitis (TAH) in recipients of blood transfusion from volunteer donors was 0.6 cases/1000 units compared with 2.8 cases/1000 units where blood was collected from a mixture of volunteer and commercial blood donors[4]. This report was followed by a prospective study on recipients of blood transfusion to determine the incidence of TAH when blood was transfused from volunteer or commercial donors and reconfirmed the increased association between commercial blood donors and TAH[5]. The blood banks began switching over to blood collection from voluntary non-paid donors. By the end of 1975 the US Food and Drug Administration made it mandatory to collect blood from only voluntary non-remunerated blood donors[6]. Adoption of these two measures namely voluntary blood donation and screening donor blood for HBsAg led to 70% reduction of TAH and 85% reduction in transfusion associated hepatitis B[7]. Despite HBsAg screening, cases of TAH continued to occur, though majority of these (75%) were not due to hepatitis B virus (HBV). Hepatitis A virus (HAV) was identified in 1973[8] and serological assays for antibody detection became available. It was presumed then, that HAV might be the agent of non-B TAH, but serological screening of recipient and donor samples for anti-HAV showed non-reactivity[9]. Thus began the search for non A non B (NANB) hepatitis agent. During the period of non-discovery of the NANB hepatitis agents, surrogate tests *i.e.*, alanine aminotransferase and anti-HBc were performed for routine blood donation screening[3]. Extensive experimental studies were conducted in chimpanzees[10,11] by inoculating the animals with serum from patients with acute or chronic NANB hepatitis and asymptomatic carriers, and finally in 1989 the virus was cloned and characterized. It was designated as hepatitis C virus (HCV)[12]. World over about 170 million people are chronically infected with HCV (3% of world population)[13]. However the prevalence varies in general population from high (> 10%) in Egypt, Cameroon, Rwanda, Bolivia, Gabon and Burundi, intermediate (2.5%-10%) in Mediterranean countries, South America, Africa and Middle East to low (< 2.5%) in North America, Europe, Australia[14]. Seroprevalence amongst blood donors as reported from different countries varies from 0.4% to 13.3%[15-20].

SCREENING BLOOD DONATIONS FOR HCV

The natural course of HCV infection is characterised by the appearance of the following markers in chronological sequence; HCV RNA, HCV antigens and subsequently HCV antibodies. The tests however were introduced in the reverse order for donor screening since the technologies had to be optimised to achieve maximum sensitivity and specificity for blood transfusion safety.

SEROLOGICAL ASSAYS

Serology based assays may detect presence of anti-HCV antibodies, HCV antigen or both simultaneously. The testing platforms could be enzyme immunoassays (EIAs), chemiluminescence (CLIA) or rapid tests. The test principle in EIAs and CLIAs is the same, but end point detection in EIAs is measured as colour change and in CLIAs as luminescence. The CLIA end point detection signal has been reported to have better sensitivity than enzyme based assays. The improvements in assay performance, particularly of the EIAs have been termed as "generations" of the assays. The first generation anti-HCV EIA detected antibodies 12 to 26 wk after exposure, creating a long window period of infectivity and some patients never seroconverted with this assay. The false positivity rate in low risk populations was reported to be as high as 60%. The limited sensitivity of the first generation assay was attributed to the use of the antigen c100-3 (NS4) alone which represents only 12% of the viral genome[21,22]. The second generation anti-HCV EIA incorporated two more epitopes, one each from the core antigen and a non-structural antigen (NS3). The range of window period of infectivity reduced to 10 to 24 wk. The third generation EIAs incorporated additional antigen from the non-structural HCV antigen (NS5) and showed a further reduction in the window period by one week. Despite increase in sensitivity with each successive "generation" of the assay, the prolonged serology negative but 'infectious' window period remained a cause for concern, with second generation assays, the average window period remains around 82 d[23] and with third generation assays it reduces to but still remains around 66 d[24,25].

Window period can be shortened by either detection of viral antigen or the genome. ELISA assays for the detection of HCV core antigen were developed and tested on both blood donors and patients at high risk of acquiring infections *i.e.*, patients on chronic haemodialysis[26-28]. In a study on HCV antibody negative blood donors core antigen assays detected HCV antigen in 11 out of 18 (61%) of the donor samples initially positive for HCV RNA by real-time polymerase chain reaction (RT-PCR). In the 7 cases (39%) where the antigen assay was negative, the HCV core antigen became detectable between 2 and 21 d after the initial testing[27]. In another study[28] HCV core antigen was tested on 6 HCV antibody negative and HCV RNA positive blood donors and 135 serial samples from 28 antibody negative patients on haemodialysis. Five of the six donors (83%) were positive for HCV antigen, while in haemodialysis patients 81 samples tested positive out of 92 HCV RNA

positive samples (88%). A correlation was observed between viral load and the detection of HCV antigen. The positive results were obtained in 96% of the study population when the number of HCV RNA copies per ml was greater than 10^5 and only 53% when the number of HCV RNA copies was lower. Average time from RNA to antigen positivity was estimated at 2 d in contrast to a study in which compared with amplified HCV RNA testing the core antigen only testing increased the window period by 8 d[27]. Thus HCV antigen appears at the earliest within 2-8 d of HCV-RNA positivity. The average window period reduction can be achieved by 49 d. In a recently published meta-analysis the pooled sensitivity of the HCV core antigen assay was 0.84% (95%CI: 0.83-0.85) and the pooled specificity was 0.98% (95%CI: 0.97-0.98)[29]. The HCV core antigen only assay did not find widespread applicability in blood donor screening since two serology based assays had to be performed on the same donor unit. Additional high cost of the assay also precluded its use as a screening test. A sandwich ELISA for the detection of HCV NS3 antigen has been developed indigenously in a resource constrained country[30]. The authors used a high sensitivity and specificity anti-HCV-NS3 antigen monoclonal antibody and a highly efficient, purified anti-HCV polyclone antibody from a chronic HCV patient with high titre anti-HCV (titre-1 in 10000). The HCV NS3 antigen positivity rate in the 173 anti-HCV positive samples and the 3708 HCV-negative samples was 24.3% and 1.3% respectively. Innovations like these may help to make technological advancements affordable to large parts of the developing world. ELISA assays are the most widely used serological screening tests for HCV antibody detection in blood centres. The assays can be run in batches and can be fully automated for testing large numbers of donor samples. About 70%-80% of HCV antibody positive samples are also HCV RNA positive, hence there is high probability of HCV transmission from seropositive donors[16,31].

Combination antigen-antibody assays were introduced, where two markers of the same infection could be detected simultaneously. These assays came to be known as "fourth generation" or "antigen-antibody combo" tests and appeared more suitable in a blood bank setting where large numbers of donor samples need to be screened in the shortest possible time. The combined antigen-antibody assays are usually sandwich ELISAs where the solid phase and second phase comprise both HCV derived antigens and antibodies against HCV. The performance of these combined assays was evaluated on three panels of antibody negative plasma samples from HCV RNA only positive donors[32]. Assay kits from two manufacturers were compared, there was variability in antigen detection which also seemed genotype dependent and influenced to some extent by the viral load. The detection of HCV infection by these two fourth generation assays was 29% and 50% respectively in HCV RNA positive samples. Combined assays resulted in suboptimal detection of HCV antigen when compared to antigen only assays. Despite this, combination assays are a definite improvement over antibody only assays, as they detect the infection earlier and are capable of detecting immunosilent carriers who are viremic without detectable antibodies in their plasma[33]. In another study, 70.5% of HCV RNA positive and anti-HCV negative samples collected in pre-seroconversion phase were detected by antigen-antibody assays[34]. This study was conducted on 107 sequential samples from 10 HCV seroconversion commercial panels. The specificity was further analysed in 2503 consecutive blood donations and was estimated at 99.8%. The average window period reduction with combined assays was estimated at 26.8 d and this would translate to a reduction of the HCV transfusion transmissible risk by 41%. The authors observed that the assay failed to detect HCV antigen in few samples where the HCV-RNA load was greater than 10^6/mL. They postulated that this might be explained by inaccessibility of the core antigen to monoclonal antibodies included in the assay. Nevertheless these assays provide a viable alternate to molecular methods where cost constraint is a limiting factor.

RAPID TESTS

In many blood centres located within resource-constrained countries the laboratory facilities are limited as regards instrumentation, electricity supply and trained manpower. In such situations rapid tests provide an alternate screening methodology to EIAs. These tests can be performed within few minutes and the results can be read visually and are not dependant on any instruments or electrical supply. They are based upon any one of the following principles; agglutination[35], immunofiltration[36] or immuno-chromatography[37]. The antigens used in the test are usually same as those incorporated in third generation EIAs. Each test strip or cassette has an inbuilt control band for validation. The reported sensitivities with these tests ranged from 98%-100%[35-37], however in the presence of associated human immunodeficiency virus (HIV) coinfection, the test sensitivity was detected to be only 77.5%[38]. An added advantage of rapid tests is to perform a quick risk assessment of source person in case of needle stick injury to health care workers. In a multinational assessment of blood-borne virus testing at 51 blood centres in 17 African countries, the sensitivity of rapid anti-HCV tests varied from 47% to 100%. Samples with the lowest HCV antibody levels were more frequently negative, especially with the rapid tests, thus compromising blood safety. Four samples with high antibody levels which were detected by all EIAs, tested negative with rapid tests[39].

MOLECULAR TESTING OF HCV

In the late 1990s and early 2000s, several developed countries introduced direct viral nucleic acid detection for HCV and HIV- I , following later by HBV. The ear-

lier tests were based largely on in-house PCR and testing was voluntary. As technology advanced and the potential of blood donor screening by viral nucleic acid detection was realised, semi-automated and fully automated high throughput commercial platforms become available. Amplification of the small amounts of viral nucleic acid RNA/DNA present in donor plasma was an integral part of the technology, these tests came to be referred to as nucleic acid amplification technology. Viral nucleic acids are detected by one of the two technologies in blood centres[40] (1) PCR; and (2) transfusion mediated amplification (TMA). PCR is ideal for DNA amplification. For RNA amplification as required for HCV a reverse-transcription step is needed to generate cDNA. Both the steps, that is reverse transcription and DNA amplification can be incorporated into a single step by incorporating reverse transcriptase and DNA polymerase in the same reaction. TMA technology uses two enzymes-RNA polymerase and reverse transcriptase (RT). The RT enzyme creates cDNA which serves as a template to generate RNA by the activity of the enzyme RNA polymerase. In this way about a billion RNA amplicons are produced in less than an hour.

NAT testing for HCV was the first viral nucleic acid detection screening test introduced in blood centres. Testing was performed on minipools (MP-NAT), with pool size varying from 96 to 6 blood donations[41]. The first country to introduce NAT screening of whole blood and apheresis donations in 1997 was Germany. Initially this technology was started on voluntary basis, but in 1999 HCV NAT was made mandatory, prior to release of blood components[42]. Subsequently HCV NAT became mandated in at least 23 countries across the world[43], although NAT has been implemented in blood centres in over 40 countries. Testing in minipools has been reported to reduce sensitivity and this has led to decrease in pool size, varying from 24 to 6 donations. Many blood centres have progressed to individual donation (ID-NAT) testing. In a comparative study between MP-NAT and ID-NAT testing, the latter was observed to be significantly more sensitive in detecting HCV and HIV RNAs but the difference in sensitivity was limited for HBV DNA[44]. To enhance the sensitivity of MP-NAT, fully automated nucleic acid extraction systems are available. In Germany where MP-NAT is performed on pool size of 96, ultracentrifugation prior to viral extraction has yielded analytical sensitivity of NAT, equivalent to smaller pool size[45]. Many blood centres have progressed to individual donation (ID-NAT) testing. Regardless of minipool or individual donation testing, blood centres are using the multiplex commercial NAT platforms where simultaneous detection of HCV, HBV, HIV-1 and recently even HIV-2 is available. This helps in conserving reagents, reducing sample volume required and gives results for 3 viral agents within the same time frame. The initial NAT reactive results are then confirmed using discriminatory assays for each of the specific virus.

NAT has a high analytical sensitivity for HCV RNA and ID-NAT can detect as low as 2.0 to 9.4 IU/mL[46]. NAT testing for HCV reduced the infectious pre-seroconversion period dramatically to 4 to 6 d. It also helped to detect immunosilent carriers. The residual risk of transfusion-transmitted HCV infection has declined significantly in countries where universal NAT screening has been implemented. In a study from the US, during a 10 year period 66 million donations were screened with HCV antibody and NAT, 244 HCV (1:270000) NAT yield donations were identified and the prevalence of HCV in first time donors decreased by 53%[47]. Enhanced blood safety is also evident from studies published from other countries. In Germany between 1990 and 1998, approximately seven transfusion-transmitted cases occurred annually, but after implementation of NAT, no additional case has been reported, except one where NAT was false negative due to very low viral load[42]. The marked improvement in blood safety is due to the high analytical sensitivity of NAT assays and addition of NAT to serological testing has been instrumental in reducing residual risk of transfusion-transmitted HCV to less than 1 per million donations[48]. NAT is an additional though highly sensitive and significant advance in blood safety. There are situations where NAT is negative but HCV serology is positive. In a recent international survey on NAT testing of blood donations 29.4% of HCV antibody positive donations tested HCV NAT negative[43]. Hence serology and NAT testing are complimentary tests. NAT must be viewed as an additional though highly significant advancement in blood safety.

TESTING IN DEVELOPED COUNTRIES

Developed countries have successfully implemented stringent donor screening, voluntary blood donation and advanced and sensitive technologies for blood donation screening to reach acceptable levels of blood safety. Second/third generation HCV antibody screening EIAs or CLIAs coupled with HCV RNA detection by NAT has resulted in marked reduction in transfusion transmitted risk. The NAT yield rate (HCV RNA positive, antibody negation donations) is lowest (< 1 per million donations screened) in UK, France, Germany, Canada and South Africa. The residual risk (risk of HCV infectious donations entering the blood supply) in these countries varies from 1 in 7.7 million in France to 1 in 31.59 million in United Kingdom[31]. The residual risk though minute remains due to viral load below limit of detection of NAT assays or specific genotype prevalence or mutant variants of the virus. Implementation of NAT has been expensive both in terms of technology and human resource training and despite its low cost effectiveness as evidenced by low NAT yields it continues to be progressively implemented in the interest of the public.

TESTING IN RESOURCE-CONSTRAINED COUNTRIES

The testing scenario is highly variable and depends upon

availability of laboratory infrastructure, instrumentation as well as trained staff and financial resources. These countries are mostly located in Africa, Asia and Latin America and as per WHO database on blood safety, blood donations are still not routinely tested for infectious marker screening including HCV in 39 countries. Forty seven per cent donations are tested in laboratories without quality assurance[49]. The testing technologies vary from rapid tests to EIAs, CLIAs or even NAT as in Egypt or few blood centres within countries as in India. The HCV NAT yield as reported from Egypt was 15 in 15655 donation (1 per 1043) which carries a residual risk of 1 per 65000 donations[50]. In the first multicentre evaluation of NAT in Indian blood donors HCV RNA yield was 1 per 12224 donors[51]. Subsequent studies showed higher HCV NAT yields (3 in 18354 donors)[52]. In a study from China where HCV testing with MP-NAT was employed, the residual risk estimate from NAT yield was 1 in 540541 as compared to HCV antigen-antibody EIA (1 in 320000) and with third generation EIA (1 in 59588)[53]. There is an urgent need for the resource-constrained countries to implement testing where none exists and to replace rapid tests wherever feasible with either sensitive antibody assays or combined antigen-antibody assays to shorten the antibody pre-seroconversion window period. Quality control is essential for both precision and accuracy of test results. NAT testing where affordable and feasible should be introduced since the prevalence of HCV, HBV and HIV-1/2 is higher in these countries and NAT might detect more window period donations than low-prevalence countries.

FUTURE DEVELOPMENTS

Serological and NAT testing has contributed dramatically to improving blood safety. However, a minimal residual risk still remains. In addition, emerging and re-emerging pathogens and bacterial contamination continue to pose further risks to blood safety. Recognition of any new infectious threat necessitates development of appropriate assays systems, regulatory approvals and staff training. One approach is to inactivate pathogens in donated blood. Pathogen reduction technologies (PRT) are available for platelets and plasma and lead to 4-6 log reduction of transfusion transmissible HCV, HIV, HBV, CMV as well as syphilis organisms[54,55]. Issues of concern with PRT are high costs, neo-antigen formation in the product and post PRT yields and function of platelets or plasma coagulation factors. PRT for red cells is not yet licensed. Attempts are on-going to develop pathogen inactivation process for whole blood[56,57]. This step may obviate the need for multiple PRT platforms for blood components. Second approach is to consider microarray platforms for all potential transfusion transmissible infections or combination technologies[42] (antibody, antigen and NAT) as one platform.

"Zero risk" blood supply still remains an elusive goal. The blood transfusion services continue to be challenged by mutations in known viruses, immunosilent carriers and emerging infectious agents.

CONCLUSION

In conclusion the best strategy for HCV screening should ideally include a sensitive antibody assay coupled with a direct viral detection method, either HCV antigen or HCV RNA. "Fourth generation" combined antigen-antibody assays can provide a single serological platform in resource-constrained settings.

REFERENCES

1 **Beeson PB**. Jaundice occurring one to four months after transfusion of blood or plasma. Report of seven cases. *JAMA* 1943; **121**: 1332-1334 [DOI: 10.1001/jama.1943.02840170016005]

2 **Morgan HV**, Williamson DA. Jaundice following Administration of Human Blood Products. *Br Med J* 1943; **1**: 750-753 [PMID: 20784888 DOI: 10.1136/bmj.1.4302.750]

3 **Tobler LH**, Busch MP. History of posttransfusion hepatitis. *Clin Chem* 1997; **43**: 1487-1493 [PMID: 9265899]

4 **GRADY GF**, CHALMERS TC. RISK OF POST-TRANSFUSION VIRAL HEPATITIS. *N Engl J Med* 1964; **271**: 337-342 [PMID: 14171801 DOI: 10.1056/NEJM196408132710702]

5 **Walsh JH**, Purcell RH, Morrow AG, Chanock RM, Schmidt PJ. Posttransfusion hepatitis after open-heart operations. Incidence after the administration of blood from commercial and volunteer donor populations. *JAMA* 1970; **211**: 261-265 [PMID: 5466902 DOI: 10.1001/jama.1970.03170020025005]

6 **Domen RE**. Paid-versus-volunteer blood donation in the United States: a historical review. *Transfus Med Rev* 1995; **9**: 53-59 [PMID: 7719038 DOI: 10.1016/S0887-7963(05)80030-6]

7 **Alter HJ**, Holland PV, Purcell RH, Lander JJ, Feinstone SM, Morrow AG, Schmidt PJ. **Posttransfusion hepatitis after** exclusion of commercial and hepatitis-B antigen-positive donors. *Ann Intern Med* 1972; **77**: 691-699 [PMID: 4628213 DOI: 10.7326/0003-4819-77-5-691]

8 **Genêt F**, Poiraudeau S, Revel M. [Effectiveness and compliance to a center-based short rehabilitation program with a home-based program for chronic low back pain]. *Ann Readapt Med Phys* 2002; **45**: 265-272 [PMID: 12076853 DOI: 10.1126/science.182.4116.1026]

9 **Dienstag JL**, Purcell HR, Alter HJ, Feinstone SM, Wong DC, Holland PV. **Non-A, non-B post-transfusion hepa**titis. *Lancet* 1977; **1**: 560-562 [PMID: 65657 DOI: 10.1016/S0140-6736(77)91996-1]

10 **Tabor E**, Gerety RJ, Drucker JA, Seeff LB, Hoofnagle JH, Jackson DR, April M, Barker LF, Pineda-Tamondong G. Transmission of non-A, non-B hepatitis from man to chimpanzee. *Lancet* 1978; **1**: 463-466 [PMID: 76018 DOI: 10.1016/S0140-6736(78)90132-0]

11 **Bradley DW**, McCaustland KA, Cook EH, Schable CA, Ebert JW, Maynard JE. Posttransfusion non-A, non-B hepatitis in chimpanzees. Physicochemical evidence that the tubule-forming agent is a small, enveloped virus. *Gastroenterology* 1985; **88**: 773-779 [PMID: 2981754]

12 **Choo QL**, Kuo G, Weiner AJ, Overby LR, Bradley DW, Houghton M. Isolation of a cDNA clone derived from a blood-borne non-A, non-B viral hepatitis genome. *Science* 1989; **244**: 359-362 [PMID: 2523562 DOI: 10.1126/science.2523562]

13 **Perz JF**, Armstrong GL, Farrington LA, Hutin YJ, Bell BP. The contributions of hepatitis B virus and hepatitis C virus infections to cirrhosis and primary liver cancer worldwide. *J Hepatol* 2006; **45**: 529-538 [PMID: 16879891 DOI: 10.1016/j.jhep.2006.05.013]

14 **Zaltron S**, Spinetti A, Biasi L, Baiguera C, Castelli F. Chronic HCV infection: epidemiological and clinical relevance. *BMC Infect Dis* 2012; **12** Suppl 2: S2 [PMID: 23173556 DOI: 10.1186/1471-2334-12-S2-S2]

15 **Sharma RR**, Cheema R, Vajpayee M, Rao U, Kumar S, Marwaha N, Agnihotri SK. Prevalence of markers of transfusion transmissible diseases in voluntary and replacement blood donors. *Natl Med J India* 2004; **17**: 19-21 [PMID: 15115227]

16 **Thakral B**, Marwaha N, Chawla YK, Saluja K, Sharma A, Sharma RR, Minz RW, Agnihotri SK. Prevalence & significance of hepatitis C virus (HCV) seropositivity in blood donors. *Indian J Med Res* 2006; **124**: 431-438 [PMID: 17159264]

17 **Makroo RN**, Walia RS, Chowdhry M, Bhatia A, Hegde V, Rosamma NL. Seroprevalence of anti-HCV antibodies among blood donors of north India. *Indian J Med Res* 2013; **138**: 125-128 [PMID: 24056566]

18 **Khattak MF**, Salamat N, Bhatti FA, Qureshi TZ. Seroprevalence of hepatitis B, C and HIV in blood donors in northern Pakistan. *J Pak Med Assoc* 2002; **52**: 398-402 [PMID: 12532573]

19 **Nagalo BM**, Bisseye C, Sanou M, Kienou K, Nebié YK, Kiba A, Dahourou H, Ouattara S, Nikiema JB, Moret R, Zongo JD, Simpore J. Seroprevalence and incidence of transfusion-transmitted infectious diseases among blood donors from regional blood transfusion centres in Burkina Faso, West Africa. *Trop Med Int Health* 2012; **17**: 247-253 [PMID: 21988100 DOI: 10.1111/j.1365-3156.2011.02902.x]

20 **Dessie A**, Abera B, Wale F. Seroprevalence of major blood-borne infections among blood donors of Felege Hiwot referral hospital, Northwest Ethiopia. *Ethiop J Health Dev* 2007; **21**: 68-69 [DOI: 10.4314/ejhd.v21i1.10034]

21 **Kobayashi S**, Koike T, Yoshiki K, Shirai S. [Problems in determination of T and B cells in SLE]. *Nihon Rinsho* 1978; **36**: 3689-3693 [PMID: 310478 DOI: 10.1002/hep.1840150228]

22 **Klein HG**, Anstee DJ. Mollison's Blood Transfusion in Clinical Medicine. 11th ed. Oxford: Blackwell Publishing Ltd, 2005: 712-713

23 **Busch MP**, Korelitz JJ, Kleinman SH, Lee SR, AuBuchon JP, Schreiber GB. **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**: 903-910 [PMID: 8604486 DOI: 10.1046/j.1537-2995.1995.351196110893.x]

24 **Barrera JM**, Francis B, Ercilla G, Nelles M, Achord D, Darner J, Lee SR. **Improved detection of anti-HCV in post-transfusion** hepatitis by a third-generation ELISA. *Vox Sang* 1995; **68**: 15-18 [PMID: 7536987 DOI: 10.1111/j.1423-0410.1995.tb02538.x]

25 **Couroucé AM**, Pillonel J. Transfusion-transmitted viral infections. Retrovirus and Viral Hepatitis Working Groups of the French Society of Blood Transfusion. *N Engl J Med* 1996; **335**: 1609-1610 [PMID: 8927113]

26 **Peterson J**, Green G, Iida K, Caldwell B, Kerrison P, Bernich S, Aoyagi K, Lee SR. Detection of hepatitis C core antigen in the antibody negative 'window' phase of hepatitis C infection. *Vox Sang* 2000; **78**: 80-85 [PMID: 10765142 DOI: 10.1046/j.1423-0410.2000.7820080.x]

27 **Grant PR**, Sims CM, Tedder RS. **Quantification of HCV RNA** levels and detection of core antigen in donations before seroconversion. *Transfusion* 2002; **42**: 1032-1036 [PMID: 12385415 DOI: 10.1046/j.1537-2995.2002.00161.x]

28 **Couroucé AM**, Le Marrec N, Bouchardeau F, Razer A, Maniez M, Laperche S, Simon N. Efficacy of HCV core antigen detection during the preseroconversion period. *Transfusion* 2000; **40**: 1198-1202 [PMID: 11061855 DOI: 10.1046/j.1537-2995.2000.40101198.x]

29 **Gu S**, Liu J, Zhang H, Gu B, Lai H, Zhou H, He C, Chen Y. Core antigen tests for hepatitis C virus: a meta-analysis. *Mol Biol Rep* 2012; **39**: 8197-8208 [PMID: 22544611 DOI: 10.1007/s11033-012-1667-z]

30 **Xie L**, Wu XD, Huang DZ, Chen HL, He LX, Wang J, Han DK. Clinical application and analysis of hepatitis C virus NS3 antigen detection by ELISA in human serum. *Chin Med J* (Engl) 2007; **120**: 294-299 [PMID: 17374280]

31 **Busch MP**, Kleinman SH. Hepatitis C infection: recent insights relevant to transfusion safety. *ISBT Sci Ser* 2009; 4: 72-79 [DOI: 10.1111/j.1751-2824.2009.01214.x]

32 **Lagardère B**. [Future vaccines in parasitology]. *Bull Soc Pathol Exot* 1991; **84**: 926-934 [PMID: 1819438]

33 **Laperche S**, Le Marrec N, Girault A, Bouchardeau F, Servant-Delmas A, Maniez-Montreuil M, Gallian P, Levayer T, Morel P, Simon N. Simultaneous detection of hepatitis C virus (HCV) core antigen and anti-HCV antibodies improves the early detection of HCV infection. *J Clin Microbiol* 2005; **43**: 3877-3883 [PMID: 16081925 DOI: 10.1128/JCM.43.8.3877-3883.2005]

34 **Laperche S**, Elghouzzi MH, Morel P, Asso-Bonnet M, Le Marrec N, Girault A, Servant-Delmas A, Bouchardeau F, Deschaseaux M, Piquet Y. Is an assay for simultaneous detection of hepatitis C virus core antigen and antibody a valuable alternative to nucleic acid testing? *Transfusion* 2005; **45**: 1965-1972 [PMID: 16371051 DOI: 10.1111/j.1537-2995.2005.00648.x]

35 **Jourbert JJ**, Dewar JB, Weinberg J, De Beer M, Parker JS, Steele AD. **A cost-effective particle agglutination assay to detect** viral antibodies in dried blood spots--a simple solution to HIV and HCV screening. *Cent Afr J Med* 2003; **49**: 127-130 [PMID: 15301465]

36 **Daniel HD**, Abraham P, Raghuraman S, Vivekanandan P, Subramaniam T, Sridharan G. Evaluation of a rapid assay as an alternative to conventional enzyme immunoassays for detection of hepatitis C virus-specific antibodies. *J Clin Microbiol* 2005; **43**: 1977-1978 [PMID: 15815036 DOI: 10.1128/JCM.43.4.1977-1978.2005]

37 **Montebugnoli L**, Borea G, Miniero R, Sprovieri G. A rapid test for the visual detection of anti-hepatitis C virus antibodies in whole blood. *Clin Chim Acta* 1999; **288**: 91-96 [PMID: 10529461 DOI: 10.1016/S0009-8981(99)00146-1]

38 **Desbois D**, Vaghefi P, Savary J, Dussaix E, Roque-Afonso AM. Sensitivity of a rapid immuno-chromatographic test for hepatitis C antibodies detection. *J Clin Virol* 2008; **41**: 129-133 [PMID: 18069058 DOI: 10.1016/j.jcv.2007.11.002]

39 **Laperche S**. Multinational assessment of blood-borne virus testing and transfusion safety on the African continent. *Transfusion* 2013; **53**: 816-826 [PMID: 22804482 DOI: 10.1111/j.1537-2995.2012.03797.x]

40 **Levi JE**. Current concepts in molecular testing. *ISBT Sci Ser* 2011; **6**: 67-71 [DOI: 10.1111/j.1751-2824.2011.01438.x]

41 **Roth WK**, Seifried E. The German experience with NAT. *Transfus Med* 2002; **12**: 255-258 [PMID: 12220254 DOI: 10.1046/j.1365-3148.2002.00383.x]

42 **Schmidt M**, Seifried E. Current concepts in serological testing - TTID. *ISBT Sci Ser* 2011; **6**: 61-66 [DOI: 10.1111/j.1751-2824.2011.01437.x]

43 **Roth WK**, Busch MP, Schuller A, Ismay S, Cheng A, Seed CR, Jungbauer C, Minsk PM, Sondag-Thull D, Wendel S, Levi JE, Fearon M, Delage G, Xie Y, Jukic I, Turek P, Ullum H, Tefanova V, Tilk M, Reimal R, Castren J, Naukkarinen M, Assal A, Jork C, Hourfar MK, Michel P, Offergeld R, Pichl L, Schmidt M, Schottstedt V, Seifried E, Wagner F, Weber-Schehl M, Politis C, Lin CK, Tsoi WC, O'Riordan J, Gottreich A, Shinar E, Yahalom V, Velati C, Satake M, Sanad N, Sisene I, Bon AH, Koppelmann M, Flanagan P, Flesland O, Brojer E, Lętowska M, Nascimento F, Zhiburt E, Chua SS, Teo D, Stezinar SL, Vermeulen M, Reddy R, Park Q, Castro E, Eiras A, Gonzales Fraile I, Torres P, Ekermo B, Niederhauser C, Chen H, Oota S, Brant LJ, Eglin R, Jarvis L, Mohabir L, Brodsky J, Foster G, Jennings C, Notari E, Stramer S, Kessler D, Hillyer C, Kamel H, Katz L, Taylor C, Panzer S, Reesink HW. International survey on NAT testing of blood donations: expanding implementation and yield from 1999 to 2009. *Vox Sang* 2012; **102**: 82-90 [PMID: 21933190 DOI: 10.1111/j.1423-0410.2011.01506.x]

44 **Assal A**, Barlet V, Deschaseaux M, Dupont I, Gallian P, Guit-

ton C, Morel P, David B, De Micco P. Comparison of the analytical and operational performance of two viral nucleic acid test blood screening systems: Procleix Tigris and cobas s 201. *Transfusion* 2009; **49**: 289-300 [PMID: 19000230 DOI: 10.1111/j.1537-2995.2008.01965.x]

45 **Roth WK**, Weber M, Buhr S, Drosten C, Weichert W, Sireis W, Hedges D, Seifried E. Yield of HCV and HIV-1 NAT after screening of 3.6 million blood donations in central Europe. *Transfusion* 2002; **42**: 862-868 [PMID: 12375658 DOI: 10.1046/j.1537-2995.2002.00129.x]

46 **Busch MP**, Glynn SA, Stramer SL, Strong DM, Caglioti S, Wright DJ, Pappalardo B, Kleinman SH. A new strategy for estimating risks of transfusion-transmitted viral infections based on rates of detection of recently infected donors. *Transfusion* 2005; **45**: 254-264 [PMID: 15660836 DOI: 10.1111/j.1537-2995.2004.04215.x]

47 **Zou S**, Dorsey KA, Notari EP, Foster GA, Krysztof DE, Musavi F, Dodd RY, Stramer SL. Prevalence, incidence, and residual risk of human immunodeficiency virus and hepatitis C virus infections among United States blood donors since the introduction of nucleic acid testing. *Transfusion* 2010; **50**: 1495-1504 [PMID: 20345570 DOI: 10.1111/j.1537-2995.2010.02622.x]

48 **Candotti D**, Allain JP. Molecular virology in transfusion medicine laboratory. *Blood Transfus* 2013; **11**: 203-216 [PMID: 23356973]

49 World Health Organisation - Blood safety. Key global fact and figures in 2011. Last accessed 26 September 2013. Available from: URL: http://www.who.int/bloodsafety/global_database/en/index.html

50 **El Ekiaby M**, Laperhe S, Moftah M, Burnouf T, Lelie N. The impact of different HCV blood screening technologies on the reduction of transfusion transmitted HCV infection in Egypt. *Vox Sang* 2009; **96** Suppl 1: 23-24

51 **Makroo RN**, Choudhury N, Jagannathan L, Parihar-Malhotra M, Raina V, Chaudhary RK, Marwaha N, Bhatia NK, Ganguly AK. Multicenter evaluation of individual donor nucleic acid testing (NAT) for simultaneous detection of human immunodeficiency virus -1 & amp; hepatitis B & amp; C viruses in Indian blood donors. *Indian J Med Res* 2008; **127**: 140-147 [PMID: 18403791]

52 **Chatterjee K**, Coshic P, Borgohain M, Premchand RM, Chakroborty S, Sunder S. Individual donor nucleic acid testing for blood safety against HIV-1 and hepatitis B and C viruses in a tertiary care hospital. *Natl Med J India* 2012; **25**: 207-209 [PMID: 23278777]

53 **Shang G**, Seed CR, Wang F, Nie D, Farrugia A. Residual risk of transfusion-transmitted viral infections in Shenzhen, China, 2001 through 2004. *Transfusion* 2007; **47**: 529-539 [PMID: 17319836 DOI: 10.1111/j.1537-2995.2006.01146.x]

54 **Stramer SL**, Hollinger FB, Katz LM, Kleinman S, Metzel PS, Gregory KR, Dodd RY. Emerging infectious disease agents and their potential threat to transfusion safety. *Transfusion* 2009; **49** Suppl 2: 1S-29S [PMID: 19686562 DOI: 10.1111/j.1537-2995.2009.02279.x]

55 **Teo D**, Lam S. Pathogen inactivation. *ISBT Sci Ser* 2011; **6**: 499-453 [DOI: 10.1111/j.1751-2824.2011.01531.x]

56 **Mufti NA**, Erickson AC, North AK, Hanson D, Sawyer L, Corash LM, Lin L. Treatment of whole blood (WB) and red blood cells (RBC) with S-303 inactivates pathogens and retains in vitro quality of stored RBC. *Biologicals* 2010; **38**: 14-19 [PMID: 19995680 DOI: 10.1016/j.biologicals.2009.10.019]

57 **Goodrich RP**, Doane S, Reddy HL. Design and development of a method for the reduction of infectious pathogen load and inactivation of white blood cells in whole blood products. *Biologicals* 2010; **38**: 20-30 [PMID: 20093041 DOI: 10.1016/j.biologicals.2009.10.016]

P- Reviewers: Kelesidis T, Rajeshwari K, Snyder N
S- Editor: Gou SX **L- Editor**: A **E- Editor**: Liu XM

百世登
Baishideng®

Published by **Baishideng Publishing Group Co., Limited**
Flat C, 23/F., Lucky Plaza,
315-321 Lockhart Road, Wan Chai, Hong Kong, China
Fax: +852-65557188
Telephone: +852-31779906
E-mail: bpgoffice@wjgnet.com
http://www.wjgnet.com

ISSN 1007-9327
9 771007 932045 1 1>