Use of nucleic acid testing for blood donor screening of Human Immunodeficiency virus and Hepatitis C virus in the Saudi population

Javed Akhter, *PhD, FIBMS,* ***George T. Roberts,*** *MD, FRCPC,* ***Angela Perry,*** *BSc, RT,*
Julie S. Gaucher, *MBA, MT(ASCP),* ***Peter A. Howman,*** *BSc (Med Lab Sci), Associate Dip (Med Lab Sci).*

ABSTRACT

Objectives: To determine the risk of transfusion associated infection for human immunodeficiency virus and Hepatitis C virus using nucleic acid testing.

Methods: During March 1998, 400 donor blood samples from the Saudi population that were negative by serology were further tested for human immunodeficiency virus 1 and 2 and Hepatitis C virus using nucleic acid testing.

Results: A total of 400 were tested by nucleic acid testing, 381 of these were negative, 4 were indeterminate but were found to be negative on repeat testing and one seronegative sample was found to be positive for Hepatitis C virus.

Conclusion: Due to the low prevalence of human immuno-deficiency virus in the Kingdom of Saudi Arabia, nucleic acid testing of blood donors by serology is adequate for screening. But the higher prevalence of Hepatitis C virus and increased risk of transmission would indicate that nucleic acid testing may be warranted for Hepatitis C virus in the near future.

Keywords: Nucleic acid testing, human immuno-deficiency virus, Hepatitis C virus.

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Blood transfusions are given to replace blood that has been lost or to correct serious conditions resulting from low blood counts. In the United States of America (USA), every 3 seconds someone needs blood, and approximately 40,000 units of blood are used each day. Twenty three million components are made from 14 million blood donations each year. Screening donors for diseases that may be transmitted via blood transfusion has improved the quality of the blood supply in the past 30 years. Even though the number of post-transfusion human immunodeficiency virus (HIV) is very low, public expectation is for zero risk. Post-transfusion Hepatitis C (HCV) has been a problem due to the imprecision of test methodologies and the prevalence of the infection.[1] Currently, prevention of transfusion-associated viral disease depends upon pre-donation evaluation followed by serologic testing for infectious pathogens, including HIV-1 and 2, Hepatitis B virus, and HCV. Cytomegalovirus (CMV) screening is generally performed after blood collection when CMV seronegative products are required. Testing is not routinely carried out for parvovirus B19, Hepatitis A virus, Hepatitis G virus, or Hepatitis E virus. Currently it is not possible to detect very recently infected donors during the pre-conversion "window period" of infection.[2] Estimates of the frequency of transmission from blood

From the Department of Pathology and Laboratory Medicine, King Faisal Specialist Hospital & Research Centre, Riyadh, Kingdom of Saudi Arabia.

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Address correspondence and reprint request to: Dr. Javed Akhter, Department of Pathology and Laboratory Medicine (MBC 10), King Faisal Specialist Hospital & Research Centre, PO Box 3354, Riyadh 11211, Kingdom of Saudi Arabia. Tel. +966 (1) 464 7272 Ext. 35890. Fax. +966 (1) 442 4331.

components on a per donor basis are 1 in 100,000 for HCV, 1 in 63,000 for HBV and 1 in 660,000 for HIV. The aggregate risk of receiving a blood component contaminated with a virus is 1 in 34,000 donations.[3] It must be realized that new emerging transfusion transmitted diseases may be discovered in the future. Hence, complete safety from blood transfusions may never reach zero risk. At King Faisal Specialist Hospital and Research Centre (KFSH), all blood donations are tested for the following infections: Hepatitis B surface antigen (HbsAg), HCV, HIV 1 and 2, HIV p24 antigen, HTLV 1 and 2, and syphilis. A prospective study was carried out in March 1998, performing nucleic acid testing (NAT) for HIV and HCV on seronegative blood donor samples.

Methods. Four hundred blood donor samples were tested at KFSH. This is a 550-bed referral center for the Kingdom of Saudi Arabia. Donors were first evaluated by donor history forms, on which they were asked questions regarding their lifestyle, travel history, country/area of birth and whether they had any tissue implants. Aliquots from donations of qualified donors were then tested by conventional enzyme-linked immunosorbent assay (ELISA) methods followed by NAT testing for HIV and HCV. In house methods using nested polymerase chain reaction (PCR) for HIV and HCV testing were used.

Hepatitis C virus polymerase chain reaction method. Eight primers were selected from the conserved region of the capsid region gene, used as 2 overlapping nested sets. The primer sequences were as follows: A primer 5-CACTCCCCTGTGAGGAAC, B primer 5-CTGTGAGGAACTACTGTC, C primer 5-TGTCTTCACGCAGAAAGC, D primer 5-TTCACG CAG AAA GCG TCT AG, E primer 5- GTT GAT CCA AGA AAG GAC CC, F primer 5-AAC ACT ACT CGG CTA GCA GT, G primer ACT CGC AAG CAC CCT ATC, H primer TCT TTG AGG TTT AGG ATT. Five microliters of serum sample was heat inactivated and then added to a reverse transcriptase mix (type 1 water, 10X vent, nucleotides 10mM, dithiothreitol 100mM, primer 20μm, moloney murineleukermia virus reverse transcriptase 200/μl, Rnade 40 U/μl.) This was incubated for one hour at 37˚C followed by addition of PCR mix (primer 20uM, bovine serum albumen 10mg/ml, vent 2U/μl). This was overlaid with oil and run for 50 cycles with a 7 minute extension at 72˚C for the last cycle. This was followed by nested PCR for 30 cycles with a 7 minute extension at 72˚C for the last cycle. Polymerase chain reaction product was separated by agarose gel electrophoresis using a 1.5% gel. Ten microliters of nested PCR product with 2 μl tracking dye was run including a 100 base pair marker. Bands of 163 base pair (bp) and 250 bp were seen on positive samples.

Table 1 - Results of nucleic acid testing (NAT) on 400 Saudi blood donors.

Virus	n of Specimens	Serology Negative	Polymerase chain reaction (PCR) Negative	Polymerase chain reaction (PCR) Positive
HIV	400	400	400	0
HCV	400	400	399	1

HIV=human immuno-deficiency virus, HCV=hepatitis C virus, n=number

Human immuno-deficiency polymerase chain reaction method. This method used 2 sets of 4 primers from the envelope and polymerase genes. The primer sequences were as follows: Core protein (GAG) 5-GGA GTA GCA CCC ACC AAG GCA AAG, GAG2 5-AGCAGCAGGAAGCACTATGG, GAG3 5-CCAGACTGTGAGTTGCAACAG, GAG4 5-CTTTAGGTATCTTTCCACAGC, Pol 1 5-TATTGGCAAGCCACCTGGAT, Pol 2 5-ACCTTCTATGTAGATGGGGCAGCTA, Pol 3 5-ATGCATATTGTGAGTCTGTTACTAT, Pol 4 5-CCTTTGTGTGCTGGTACCCAT. Two hundred μl samples of buffy coat from unclotted blood samples were processed using Qiagen extraction. 10 microliters of extracted sample were added to a PCR mix (10X vent buffer, nucleotides 10mM, primer GAG1 [or Pol1] 20 μm, primer GAG4 [or Pol 4] 20 mM, BSA 10mg/ml, vent 2U/μl) a total of 40μl was run for 50 cycles with a 7 minute extension at 72˚C for the last cycle. This was followed by nested PCR (10X vent buffer, nucleotides 10mM, primer GAG2 [or Pol 2] 20mM, primer GAG3 [or Pol3] 20 mM, BSA 10mg/ml, vent 2U/μl) run at 50 cycles with a 7 minute extension at 72˚C for the last cycle. Polymerase chain reaction product was run using agarose gel electrophoresis using a 1.5% gel. A band for the GAG2-GAG3 primers appeared at 142 bp and for the Pol2-Pol 3 primers appeared at 193bp.

Results. Of the 400 donor blood samples screened using NAT for HIV and HCV. It was found that 400 samples were negative for HIV and 399 samples for HCV. Four samples gave indeterminate results which tested negative on repeat testing. One antibody negative sample was found to be positive for HCV by PCR (Table 1).

Discussion. In industrialized countries, the use of sensitive screening tests, donor deferral, and more conservative use of blood was resulted in a dramatic decrease in the transmission of HIV infection by blood transfusion.[4] Despite this low risk, continued public concern has forced policy makers to continue

to search for more sensitive methods for excluding contaminated donations.[5] Human immuno-deficiency virus-1 p24 antigen testing was implemented in the USA in March 1996. In the first 18 months of p24 testing, an estimated 18 million blood donations were tested at a cost of $90 million to detect 3 antigen-positive, antibody negative donations.

According to World Health Organization estimates, approximately 3% of the world population may be infected with the Hepatitis C virus. The main routes are parenteral, causing in approximately 80% of transfusion recipients a chronic carriage state after infection. The current risk of acquiring HCV through fresh frozen plasma is 1 in 100,000 screened blood units.[6]

Many countries such as the USA and Australia have commenced NAT to further reduce the incidence of transfusion associated infection from HIV and HCV. The question remains whether this would be a necessary step in the Saudi Arabian setting. Human immuno-deficiency virus 1 and 2 prevalence in the Kingdom of Saudi Arabia was recorded as 0% (10 from 134,599 specimens) among apparently healthy blood donors in Eastern Saudi Arabia.[7] At present the prevalence of HIV and is very low in the Saudi population and further steps such as an all volunteer blood donor service should aid in protecting the blood supply. Also the enormous added cost of extra screening for HIV is also not warranted. Hence, our results would indicate that conventional ELISA methods may be adequate for HIV donor screening in the Kingdom of Saudi Arabia. However, the prevalence of HCV in the Middle East is significantly higher and varies from 1.5% to 22%, but the prevalence in the Kingdom of Saudi Arabia is 1% (86 from 7424 Saudi donors).[8] It may therefore be a judicious investment to routinely screen all ELISA negative donations for HCV since the chronic stage is asymptomatic, but is associated with chronic liver inflammation.[9] The state of liver transplantations in this country is in its infancy and will add an enormous burden on the health system. Further surveillance studies are necessary to monitor how this status quo changes.

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