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Routine Use of Mini-Pool Nucleic Acid Testing (MP-NAT) Multiplex Assay for Sero-Negative Blood Donors

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ABSTRACT

Background: Until December 2010, HBV-DNA NAT assay was not required by the FDA or recommended by the AABB. The FDA approved automated multiple assays that detect HIV-RNA, HCV-RNA, and HBV-DNA in one reaction chamber. These systems are approved for testing of individual donations and pool of 6-16 donor samples.

Material and Methods: With retrospective study, for blood donors, in Almana General Hospitals, Saudi Arabia, from January 2008 to April 2011 donor's blood was examined by serological assays for HBV, anti-HCV, and HIV. Sero-negative donor blood was then examined by mini-pool nucleic acid test (MP-NAT) multiplex assay. Individual donor NAT reactive samples were discriminated by PCR.

Results: The total number of blood donors was 13,435. Serologically non reactive cases but MP-NAT reactive was eight cases. Seven cases were discriminated by PCR as four HBV cases, two HCV cases and one HIV case. These seven cases became sero-positive when reevaluated after three to six months. So they were considered to be diagnosed in the window period. One case was sero-negative but NAT reactive, non reactive by PCR and persisted sero-negative when followed-up after three, six and twelve months. This case was considered as false positive.

Conclusion: The routine use of MP-NAT multiplex assay for detection of HBV-DNA, HCV-RNA, and HIV-RNA should be mandatory for all sero-negative donor blood to reduce the serologic window phase and increase the safety for the patients. In spite of NAT false positive and cost effective drawbacks should be considered.

Key Words: *Nucleic acid test – Multiplex NAT – HIV – HBV – HCV – Blood donor's safety – Transfusion Transmitted Diseases.*

INTRODUCTION

In October 2004, the implementation of HIV-RNA and HCV-RNA NAT examination of donor blood was added to the FDA guidance **[1]**. It was found to detect infection earlier than antibody or antigen assays. Also the FDA permitted discontinuation of HIV-1 p24 antigen testing with implementation of a licensed HIV-1 NAT assay **[2]**. Until December 2010, HBV-DNA NAT assay was not required by the FDA or recommended by the AABB. Some blood banks have implemented automated multiplex assays that screen for HIV-RNA, HCV-RNA, and HBV-DNA simultaneously **[3]**. In recent years, fully automated NAT systems have been developed. The FDA approved two manufacturers' automated triplex (HIV-1/HCV/HBV) nucleic acid testing (NAT) assays, used either in mini-pools (MP-NAT) of 6 or 16 donations, or for testing individual donations (ID-NAT) that detect HIV-RNA, HCV-RNA, and HBV-DNA in one reaction chamber.

Objective:

This study aimed for the detection of the advantages and limitations of the routine use of mini-pool Nucleic Acid Testing (MP-NAT) for the detection of HBV-DNA, HCV-RNA, and HIV-RNA in one chamber for sero-negative donor blood.

MATERIAL AND METHODS

With retrospective study, for all blood donors, in Almana General Hospitals, Saudi Arabia (SA), between January 2008 to April 2011. Blood donors were examined by serological assays for HBV (HBsAg, and HBc-Antibody), anti-HCV, and HIV Ag/Ab Combo (anti HIV-1, anti HIV-2 and HIV p24) by ARCHITECT system (i-1000, Abbott). Sero-negative donor blood for HBV, HCV, and HIV were then examined by mini-pool Nucleic acid test (MP-NAT) multiplex assay (Cobas, S-201 system, TaqScreen Multiplex (MPX) Test, Roche).

TaqScreen Test is a qualitative multiplex test that enables the screening and simultaneous detection of HIV-1 Groups M and O RNA, HCV RNA and HBV DNA in infected pooled and individual plasma specimen donations. The Cobas TaqScreen MPX Test uses a generic nucleic acid pre paration technique on the Cobas AmpliPrep Instrument. HIV-1 Groups M and O RNA, HCV RNA and HBV DNA are amplified and detected using automated, real time PCR on the Cobas Taqman Analyser. The test incorporates an Internal Control for monitoring test performance in each individual test as well as the AmpErase (Uracil-N-glycosyl) enzyme to reduce potential contamination by previously amplified material (amplicon). The Cobas TaqScreen Test does not discriminate which virus is detected in a specimen. COBAS Ampliscreen HIV Test v1.5, COBAS Ampliscreen HCV Test v 2.0 and Cobas AmpliScreen HBV Test were used for discrimination of HIV, HCV and HBV respectively. Minipool of 5 samples were prepared and examined for HBV-DNA, HCV-RNA, and HIV-RNA in one chamber according to the manufacture instructions. NAT reactive pools were then resolved to the single donation. Individual donor NAT reactive samples were discriminated by PCR- based diagnostic assay (COBAS Ampliscreen, Roch). Also the NAT reactive donors were followed-up after 3-6 months by serological testing for HBV, HCV and HIV.

RESULTS

From January 2008 to April 2011, blood donors were 13,435. Their age range was between 20 to 59 years old with median age of 35 years. Male: Female ratio was 10:1. Serological assays revealed reactive cases of HBV in 71 (0.53%), HCV in 112 (0.83%), and HIV in 39 (0.29%) donors. Serologically non reactive cases (for HBV, HCV and HIV) but MP-NAT reactive were 8 cases. Seven cases were discriminated by PCR as four HBV cases, two HCV cases and one HIV case. These 7 cases became seropositive when re-evaluated after 3 to 6 months. So these 7 cases were considered to be diagnosed in the window period. One case was sero-negative (for HBV, HCV, and HIV) but NAT reactive and non reactive by PCR for HBV, HCV or HIV and persisted sero-negative when followed-up after 3,6 and 12 months. This case was considered as false positive.

DISCUSSION

Since 1990, the national strategy to eliminate hepatitis B virus (HBV) infection in Saudi Arabia has included obligatory administration of HBV vaccine to all infants. The prevalence of hepatitis B surface antigen (HBsAg) among children before this program was reported to be 6.7% **[4]**. Nowadays, the prevalence of HBV is 0.22%. The prevalence varied by region, ranging from 0.03% to 0.72% with a mean prevalence of 0.15% **[5]**. In a recent study, in the Eastern Province-Saudi Arabia (SA), the incidence of HBV infection was found to be 0.5%. Similarly, HCV prevalence in Saudi Arabia varies in different provinces being highest in the Western and Southern provinces **[7]**. It was reported to be 0.6% in Eastern Province **[6]**. In this current study the prevalence of HCV infection was 0.83%. The latest statistics of the Ministry of Health (MOH) on the numbers of AIDS patients in Saudi Arabia revealed cumulative number of all AIDS cases detected since 1984 and until the end of 2009,15,213 cases including 4,019 Saudis, and 11,194 non-Saudi (i.e. The proportion of non-Saudis represent almost three times the Saudis). A woman to men ratio is 1:4 **[7]**. Human immunodeficiency virus infection prevalence in Saudi Arabia was shown to be 0.02% in a previous study **[8]**. In a current study it showed to be 0.29% which is much higher than general Saudi population study. This may be attributed to regional variation or due to that Eastern Province being too close to Bahrain which is a free country.

On October 2004, the implementation of HIV-RNA and HCV-RNA NAT examination of donor blood was added to the FDA guidance **[1]**. These were found to detect infection earlier than antibody or antigen assays. Also the FDA permitted discontinuation of HIV-1 p24 antigen testing with implementation of a licensed HIV-1 NAT assay **[2]**. Until December 2010, HBV-DNA NAT assay was not required by the FDA or recommended by the AABB. Some blood banks have implemented automated multiplex assays that screen for HIV-RNA, HCV-RNA, and HBV-DNA simultaneously **[3]**. In recent years, fully automated NAT systems have been developed. The FDA approved two manufacturers' automated triplex (HIV-1/HCV/HBV) nucleic acid testing (NAT) assays, used either in mini-pools (MP-NAT) of 6 or 16 donations, or

for testing individual donations (ID-NAT) that detect HIV-RNA, HCV-RNA, and HBV- DNA in one reaction chamber. These recent FDA licensures may offer an opportunity to further reduce the risk of transfusion-transmitted infection. Furthermore, it has been estimated that ID-NAT screening would minimally increase detection of infected donors, whereas the associated testing cost would be significantly increased **[9]**. An additional important concern is that donors would be deferred for false-positive results much more frequently with ID-NAT screening than MP-NAT screening **[2]**. Both licensed assay systems appeared to perform adequately in terms of analytical sensitivity and specificity, and when applied to contemporary US donors they generate incremental yields of 1:300,000 to 1:600,000 HBV DNA-positive donations not detected by current serological tests (HBsAg and anti-HBc). This rate is similar to the yield rate of HCV MP-NAT, and substantially higher than that for HIV MP-NAT. The HBV yield donations tend to contain low copy numbers of HBV genome that are not detected by currently available ultrasensitive HBsAg assays **[10]**.

Transfusion- transmission of HIV, HCV, and HBV is now so rare that the rate of transmission cannot be measured by prospective clinical studies. The primary cause of residual transmissions however is thought to be related to donations made by individuals in the window period of early infection, before serological test results are positive **[2]**.

The FDA requires donor screening for HBsAg and for anti-HBC (IgM and IgG antibody). It is difficult to estimate the HBV residual risk of transmission from donor because neither the window period duration nor the incidence for donor HBV infection is precisely known. The duration of the infectious window period before HBsAg has recently been estimated at 30 to 38 days. A recent publication estimated US HBV transfusion-transmission risk to be between 1/280.000 **[11,12]** and 1/357.000 **[13]** units. Also current donor screening for HIV includes serologic testing for antibodies to HIV-1 and HIV-2 (both IgG and IgM) and NAT testing for HIV-RNA. This gives risk of HIV transmission of 1 of 1.5 million for a unit of blood obtained from general donor population, but the risk is too much higher in high risk donor (1 of 4100) which could be missed by current screening methods despite the short window period due to inclusion of donors with high risk of acquiring HIV **[14,15]**. Current FDA donor screening for HCV includes NAT testing for HCV RNA and serologic testing for antibodies to HCV. The average window period between exposure and detection of infection by MP-NAT is estimated to be 7.4 days **[9]**. The serologic test detects only IgG antibody, a relatively late marker of infection, and therefore they may be a significant lag (1.5 to 2 months) between detection of RNA and detection of antibody **[16]**. The current estimated US risk of HCV transmission by transfusion after application of NAT testing is extremely low approximately 1 in 1.1 million. Accordingly, questioning of donors for risk to minimize window-period donations continue to be critical for preserving blood safety **[2]**.

Overtime, the window periods have been shortened by implantation of donor screening tests that detect earlier infection. However, because there are no tests that will give a positive result instantly after an individual acquires an infection, the window period remains. With mini-pool NAT (MP-NAT), the average duration of the window period for HIV and HCV infections is estimated to be 9.4 and 7.4 days, respectively **[9]**. The window period for HBV is longer. The use of NAT assay allows the differentiation between new infections and established infections and it has been found that new HIV and HCV infections are two to four folds more common among first-time donors compared to repeat donors **[13,14,17]**. Current study showed 7 cases out of 13,435 blood donor diagnosed during the window period (negative serological tests with positive NAT assay). Discrimination of these 7 cases showed HBV, HCV, and HIV infected donor in 4,2,1 cases respectively. Those 7 cases became sero-positive when followed-up after 3 to 6 months so this confirms their diagnosis early after few days of exposure to infection and during the window phase.

Blood donations collected at the National Blood Center, the Thai Red Cross Society, Bangkok, in 2007 were tested by nucleic acid amplification technology (NAT) using the Chiron TIGRIS/Procleix Ultrio test and the Roche Cobas s 201/cobas TaqScreen multiplex (MPX) test. The sensitivity, specificity, and robustness were determined by testing 486,676 sero-

negative blood donations. Samples from each day of collection were divided into two sets; the odd-numbered samples were tested individually on the TIGRIS and the even-numbered samples were tested in pools of 6 on the Cobas s 201. The status of reactive samples was confirmed by duplicate testing of samples from the plasma bag to calculate the test specificity. Reactive samples were tested on the alternate system and followed-up. The analytical sensitivity of both systems met the 95% limits of detection claimed by the respective package inserts. No cross contamination was seen with either system. Test specificity was 99.93 and 99.90% for the Procleix Ultrio and Cobas TaqScreen tests, respectively. The NAT yield rates for human immunodeficiency virus Type 1 (HIV-1), hepatitis C virus (HCV), and hepatitis B virus (HBV) were 1:97,000, 1:490,000, and 1:2800, respectively. Several occult HBV donors, the majority of whom were detected by both tests, were also identified. The HIV-1 and HCV window cases were detected with both tests. The performances of the systems and tests indicated that both were acceptable for routine NAT by the National Blood Center, the Thai Red Cross Society. However, the Procleix Ultrio test appeared to be less sensitive than the cobas TaqScreen test for HBV **[18]**.

In a pilot study in Taiwan among 10,727 sero-negative donations, 12 HBV NAT yield cases (0.11%) and one HCV NAT yield case (0.01%) were detected. Follow-up results for 1 to 8 months showed that the HCV yield case was a window case and all HBV NAT yield cases were occult carriers. The use of NAT detected occult HBV and reduced HCV window period. The yield rate, especially occult HBV, was 10 to 100 fold higher than that in developed, HBV non-endemic countries. Therefore, NAT implementation for routine donor screening in a more cost-effective manner would contribute to safer blood transfusion in Taiwan **[19]**. A one year pilot study conducted by the University of North Carolina in collaboration with the North Carolina Department of Health and Human Services (NC Study) showed that testing HIV serologically negative individuals using NAT can help in the early identification of primary HIV infection. The NC Study performed NAT in pools of 90 samples on more than 100,000 HIV serologically negative samples tested by the state, with a positivity rate of approximately one in 5,000 screened samples **[9]**. South African Blood service screening 732,250 donations by Individual donor-NAT (ID-NAT) showed 16 HIV, 20HBV, and one HCV window phase donations **[20]**.

In current study, one other case was sero-negative for HBV, HCV and HIV but showed MP-NAT reactive. This case was non reactive for HBV, HCV or HIV infection when tested by the discrimination PCR-based assay and also when followed-up after 3,6,12 months where no sero-conversion happened. This case was considered as false positive result which is the main drawback of the use of NAT assay. In a retrospective survey done in China, NAT was used to analyze 28,800 HBsAg-negative samples by ELISA from blood donors in Dongguan city from August, 2006 to August, 2007 with Roche Cobas AmpliScreen systems; and follow-up research including NAT for HBV-DNA, ELISA for HBsAg and multiple factors analysis for HBV infection was carried out on HBV NAT screening-positive crowd. Ten positive pooling were screened from 28,800 samples; after further detection, 2 of these positive pooling were HBV-DNA negative and 8 HBV-DNA positive samples were found. They concluded that NAT is more sensitive than ELISA in screening HBV, but the probability of being false positive of NAT cannot be ignored at the same time. On the other hand, only screening HBsAg for HBV is a relative limitation in high infection region of China **[21]**.

Steven Kleinman **[10]** states that it is reasonable for FDA licensed blood establishments to implement HBV MP NAT on a voluntary basis until the FDA mandates such testing. This mandate should be consistent with FDA-approved labeling of the two manufacturers' tests that allows NAT in MPs of up to 6 or 16 donations. There is no benefit to smaller MP sizes from either modeling studies or clinical trials. We recognized the potential benefit of MP NAT for HBV, and therefore believe that this test should be adopted. As a final comment, the absence of effective reimbursement mechanisms by which hospitals can recover the increased costs of blood safety initiatives, implemented voluntarily or after an FDA recommendation, remains a serious flaw in the regulatory process. HBV NAT is an example of such an initiative that will come as an unfunded mandate if FDA recommends its use.

In conclusion, the routine use of MP-NAT multiplex assay for detection of HBV-DNA, HCV-RNA, and HIV-RNA should be mandatory for all sero-negative donor blood to reduce the serologic window phase and hence reduce the incidence of transfusion transmission of viral infection and increase the safety for the patients. It should be considered in spite of NAT false positive and cost effective drawback.

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