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TITLE: Evaluation of nucleic acid testing for blood donors: One year study

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Short Running Title: Evaluation of NAT for blood donors

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ABSTRACT

Aims:
Blood transfusion is an essential management to save patients' life in healthcare system. Blood is a potential source of transmitted diseases which make the safety of blood products important in laboratory medicine. Investigations of transfusion-transmitted infections (TTI), especially hepatitis viruses and acquired immunodeficiency virus, are essential in all blood bank policies. Several methods were applied to screen blood products for hepatitis B & C viruses and acquired immunodeficiency virus.

Methods:
Blood donor's samples for one year were collected and examined for hepatitis viruses and acquired immunodeficiency virus by using serological and nucleic acid testing (NAT).

Results:
Comparative study showed that NAT is more specific than serologic screening testing for both hepatitis C virus and acquired immunodeficiency virus. However, NAT and serological tests are required to increase the safety of blood components from hepatitis B virus transmission.

Conclusion:
This study is an attempt to evaluate the effectiveness of introducing NAT for examination of blood components. National-wide study is required to evaluate the policy of blood screening program.

Keywords: NOT GIVEN
INTRODUCTION

The safety of blood products is one of the major issues in the area of transfusion medicine. Screening of blood donors for transmissible agents play a major role to decrease the risk of transfusion of infected units. Firstly, testing of antibody/or antigen markers of blood borne pathogens was established. However, limitation of these serological techniques including window period between infection time and detection time, and antigenic variability enhance implementation of nucleic acid testing (NAT).

Nucleic Acid Testing (NAT) for detection of human immunodeficiency virus (HIV), hepatitis C virus (HCV) and hepatitis B virus (HBV) has become a routine part of blood donor infectious screening.

Hepatitis B Virus (HBV) is one of several viruses known to cause viral hepatitis. Over two billion people throughout the world have been infected with HBV and over 350 million of them are chronically infected carriers [1]. Chronic carriers are at high risk of long term complications of infection, including chronic hepatitis, cirrhosis and hepatocellular carcinoma [2-4]. Serologic markers are commonly used as diagnostic and/or prognostic indicators of acute or chronic HBV infection. The most common marker of HBV infection is the presence of HBV surface antigen (HBsAg). Although carriers may clear HBsAg and develop antibody to HBsAg, there appears to still be a risk of serious liver complications later in life [5,6]. HBV entire antigen (HBeAg) is generally used as a secondary marker to indicate active HBV replication associated with progressive liver disease. Failure to clear HBeAg appears to increase the risk of end stage liver disease [7, 8]. Variant strains of HBV can either produce HBeAg that is not detectable in serum or the strain can lose the ability to make HBeAg even when an active infection is present [9]. Therefore, using this marker to monitor disease progression may be of limited utility [10]. Instead, nucleic acid quantification of hepatitis B virus showed low level viremia in seronegative strains and reliable tools to follow up treated cases [11-14]. The methodology can allow the detection of HBV DNA after HBsAg clearance [15] or detection of HBV lacking serologic markers.
[16-17]. However, a relationship between serologic markers and HBV DNA levels has not yet been established.

Hepatitis C virus (HCV) is a small single strand RNA virus belong to Hepacivirus genus in the family Flaviviridae. HCV discovered at 1970 as non-A non-B hepatitis and proved at 1989. There are several subtype of HCV which have different geographical distribution. HCV is a blood borne virus that transmitted through blood transfusion, drug syringe misuse, sexual intercourse and other routes [18]. HCV affected around 3% of world population. The incidence of HCV infection varies from region to other [19]. The presence of HCV antibodies (anti-HCV) in patients infected with HCV has led to the development of immunoserological assays that are specific for these antibodies. The presence of anti-HCV antibodies is a measure of prior exposure to HCV infection, but cannot be considered a marker for current infection. In contrast, detection and quantitation of HCV RNA by Polymerase Chain Reaction (PCR) offers a measure of active viremia [20-22]. It is possible by using PCR to detect HCV viremia prior to immunological seroconversion [23-24] and to detect changes in the viral load in antibody-positive, chronic HCV infected, patients undergoing therapy with interferon [25-27].

Human Immunodeficiency Virus (HIV) is the etiologic agent of Acquired Immunodeficiency Syndrome (AIDS) [28;29]. HIV infection can be transmitted by sexual contact, exposure to infected blood or blood products, or by an infected mother to the fetus [30]. There are 3 main stage of HIV infection. In acute infection stage, many of infected patient develop Flu-like symptoms including fever, swollen glands, sore throat, rash, muscle and joint lethargy, fatigue etc. Patient’s blood also contain high amount of HIV in their blood [31-34]. After acute stage, patient undergo chronic stage, called also clinical latency stage, which is asymptomatic with very low level of HIV replication. [35-36]. This stage is clinically asymptomatic stable phase which can last for years [37-39]. Late stages of HIV infection called symptomatic HIV infection and AIDS. patient will develop opportunistic infections and HIV infection-related cancers. CD4 cells in AIDS stage decrease dramatically below 200 cells/mm3. HIV patient's life expectancy shortened without treatment [40-41].
the main objective of this study is to compare the sensitivity of NAT by using
individual test and serological assays by using enzyme immunoassays.

MATERIALS AND METHODS

Study site & duration of the study:
This cross sectional study was conducted in Molecular pathology unit (NAT lab), king
Fahad Hufof Hospital, Al-ahsa, Kingdom of Saudi Arabia. The study group are the
donors sample which are send for screening in serological and NAT labs. This study
used the archived row data which kept in the lab, the duration of the research was
one year between August 2009 and July 2010.

Sample size:
The total number of donors sample to be screened is (7620) samples. After
extraction of the serum from the participants the following tests are used:

1. Serological tests:
   • Hepatitis B surface antigen (BIO-RAD, France) is a one step enzyme immunoassay
technique of the sandwich type (using monoclonal and poly clonal antibodies) for the
detection of the surface antigen of the hepatitis B virus (HBsAg) in the human serum
   or plasma.
   • Hepatitis B core antibody (BIO-RAD, France) is an immunoassay technique of the
   sandwich type (using monoclonal and poly clonal antibodies) for the detection of the
   core antigen of the hepatitis B virus (HBcAg) in the human serum or plasma.
   • Hepatitis B surface antibody (BIO-RAD, France) is an immunoassay technique of the
   sandwich type (using monoclonal and poly clonal antibodies) for the detection of the
   antibody of the hepatitis B virus surface antigen (HBsAb) in the human serum or
   plasma, in some situations.
   • HIV antibody (BIO-RAD, France) is an enzyme immunoassay kit (ELISA) for the
detection of HIVp24 antigen and antibodies to HIV-1 (group M and O) and HIV-2 in
human serum or plasma, so it can be used for both HIV Ag and Ab screening. This
test is based on the principle of the sandwich technique.
   • INNO - LIAHIVI/II Score (Innogenetics, Belgium) is a immunoassay using western
blot technique (WB) to confirm the presence of antibodies against the HIV-1,
including group O, and HIV2 in human serum or plasma. Also it differentiates
between HIV-1 and HIV-2 infections and it is intended as a supplementary assay on specimens found to be reactive using an anti HIV screening procedure.

- HCV antibody (Diasorin, Italy) is an enzyme immunoassay (IA) for the detection of antibodies to hepatitis C virus (HCV) in human serum or plasma.
- INNO-LIA HCV Score (Innogenetics, Belgium) is used for detection of antibodies to human hepatitis C virus in human serum or plasma, and as supplementary test on human serum or plasma specimen found to be reactive using an anti-HCV screening procedure. It is 3rd generation line immunoassay which incorporates HCV antigens derived from core region, the E2 hypervariable region (HVR), the NS3 helicase region, the NS4A, NS4B and NS5A regions.

2. Nucleic Acid Test (NAT):

NAT performed by using the Cobas Taq Screen MPX (Roche Diagnostic, USA). Test which is a qualitative multiplex test that enables the screening and simultaneous detection of HIV-1 Group M and O RNA, HIV-2 RNA, HCV RNA, and HBV DNA in infected pooled and individual plasma specimen donations, here automated Specimen pooling and control pipetting by using Hamilton MICROLAP STAR/STAR let IVD pipette. The cobas Taq Screen MPX Test uses a generic nucleic acid preparation technique on the COBAS Ampliprep instrument. HIV-1 Group M and O RNA, HIV-2 RNA, HCV RNA, and HBV DNA are amplified and detected using automated, real time PCR on the COBAS Taq Man Analyzer. The Automated Data Management by using the pooling and Data Management (PDM) software.

RESULTS

Among 7620 donors, one sample (0.04%) was reactive by NAT and serological test, enzyme-linked immunoassay (ELISA) and Western blot (INNO - LIAHIVI/II Score) techniques, of HIV. Five samples were positive by ELISA, but they were negative by western blot and non-reactive by NAT test. None of the samples detected positive by NAT test only. Also, there was no confirmed positive sample by western blot (INNO - LIAHIVI/II Score) that was non-reactive by NAT test (table 1).
Seven samples were detected positive for HCV by serological tests and reactive by NAT test. Another 13 (0.3%) samples were positive by immunoassay examination while, they were negative by immunoblot (LIA) technique and non-reactive with NAT testing. There was no single confirmed positive serologically, which is non-reactive in NAT testing (table 2).

Donors sample were screened for HBV by 3 serological markers, in addition to NAT testing. Serologically, all Samples were investigated for HBsAg and HBcAb. Positive samples for HBcAb were further tested for HBsAb. However, all samples were tested by NAT techniques.

Two samples were found positive for HBsAg, negative for HBV core antibody (HBcAb) and non-reactive by NAT testing. Five samples were positive for three serological markers (HBsAg +, HBcAb +, HBsAb+) and also reactive by NAT test. Forty four samples were positive for HBsAg and HBcAb, but they were negative for HBsAb (HBsAg +, HBcAb +, HBsAb-). Among these 6 samples were non-reactive with NAT test, while the rest (38 samples) were reactive.

Number of donors developing HBsAb and HBcAb without HBsAg (HBsAg -, HBcAb +, HBsAb+) was 537. Tow donors had HBV DNA (reactive NAT test) while the rest (535 donors) showed non-reactive NAT test. Other donors (69 donors) develop HBcAb without HBsAb (HBsAg -, HBcAb +, HBsAb-). However, HBV DNA could not detect in their samples.

Rest of the donors, 6963 (91.37%), were free from all the markers (table 3).

DISCUSSION

WHO estimated the HCV infection in Saudi Arabia by 1.8% [42]. Prevalence of HCV infection of blood donors in Saudi Arabia was varied. El-Hazmi [43] reported 0.4% of blood donors have HCV in central region of Saudi Arabia. Other studies demonstrated a decline of HCV infection to 0.08% at 2006 in the same region [44]. Our study showed the prevalence of HCV infection among blood donors in Al-ahsa region is 0.013 %. In contrast to a study reported one positive for HCV from 400 seronegative samples from Saudi population [45]. There is no difference between the number of cases detected by serological tests including confirmatory examination and NAT techniques. However, screening serology test alone may lead
to false positive results. Similarly, Bamaga group could not detect HCV RNA by NAT testing in seronegative blood donors [46]. Thus, Nucleic acid test could replace traditional serologic test.

The prevalence of HIV cases in Saudi population varies between regions from 74 to 2 cases per 100,000 populations [47]. One sample (0.013%) was detected positive in blood donors by both serological and NAT testing. However, there was no NAT positive sample in seronegative period. Similar finding was reported by Bamaga study [46]. Moreover, NAT testing was more specific than serological screening test. All the samples screened positive serologically, while they were negative by NAT testing, confirmed negative by western blotting. Replacing serological tests with NAT testing for HIV screening will saves time and cost.

There is a significant decline of HBV infection after implementation of national-wide vaccination program. The prevalence of HBV infection before program started at 1989 was 6.7% which decreased dramatically to 0 % at 2007/8 within vaccinated group [44]. In central region of Saudi Arabia, El-Hazmi reported that 1.5% of blood donors had HBV infection between 2000 and 2002 [43]. In our al-ahsa area, previous study showed 1.9% of blood donors were HBsAg positive, 3.2% HBcAb positive and 10.1% develop both HBsAb and HBcAb [48]. Our result demonstrated a decreased of blood donors that have HBsAg, HBcAb, or both HBsAb and HBcAb to 0.67 %, 0.9%, and 7.04%, respectively.

Introducing of NAT testing for blood donors screening could not detect HBV in early seronegative period. Moreover, NAT test missed two cases (0.026%) which are positive for HBsAg and 6 cases (0.078%) that have both HBsAg and HBcAb. Occult HBV infection, which develops HBcAb without HBsAg in serum, could not detect by NAT testing. However, two donors developed both HBsAb and HBcAb, showed the presence of HBV DNA by NAT.

Thus, combining testing of blood donors for HBV by both serological and NAT techniques are required to improve blood transfusion safety.

This study presented important data about NAT testing of blood donors. Effectiveness of nucleic acid testing for blood donors screening is a debating area in transfusion medicine [49, 50]. Wide-national study is required to assess the safety and cost-effectiveness of using traditional and NAT testing to screen blood donors.
CONCLUSION

Combining testing of blood donors for HBV by both serological and NAT techniques are required to improve blood transfusion safety.

This study presented important data about NAT testing of blood donors. Effectiveness of nucleic acid testing for blood donors screening is a debating area in transfusion medicine [49, 50]. Wide-national study is required to assess the safety and cost-effectiveness of using traditional and NAT testing to screen blood donors.

CONFLICT OF INTEREST

There is no conflict of interest

AUTHOR’S CONTRIBUTIONS

NIL

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ABBREVIATION

NAT = Nucleic Acid Testing
HIV = Human immunodeficiency virus
AIDS = Acquired Immunodeficiency Syndrome
HCV = Hepatitis C virus
HBV = Hepatitis B Virus
HBsAg = HBV surface antigen
HBsAb = HBV surface antibody
HBcAb = HBV core antibody
HBeAg = HBV entire antigen
RNA = Ribose nucleic acid
REFERENCES


### TABLES

#### Table 1: Result of HIV testing for blood donors using NAT an serologic techniques.

<table>
<thead>
<tr>
<th>Serology</th>
<th>ELISA + WB +</th>
<th>ELISA + WB -</th>
<th>ELISA - WB -</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reactive</td>
<td>1 (0.013%)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Non-reactive</td>
<td>-</td>
<td>5 (0.065%)</td>
<td>7614 (99.94%)</td>
</tr>
</tbody>
</table>

ELISA = HIV antibody is an enzyme immunoassay screening test

WB = HIV western blot confirmatory test (INNO-LIAHIVI/II Score)

#### Table 2: Result of HCV testing for blood donors using NAT an serologic techniques.

<table>
<thead>
<tr>
<th>Serology</th>
<th>HCV (IA) + LIA +</th>
<th>HCV (IA) + LIA -</th>
<th>HCV (IA) - LIA -</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reactive</td>
<td>7 (0.091%)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Non-reactive</td>
<td>-</td>
<td>13 (0.17%)</td>
<td>7600 (99.7%)</td>
</tr>
</tbody>
</table>

HCV (IA) = HCV antibody is an enzyme immunoassay screening test

LIA = HCV supplementary test (INNO-LIA HCV Score)
<table>
<thead>
<tr>
<th>Serology</th>
<th>( \text{HBsAg}^+ )</th>
<th>( \text{HBsAg}^+ \text{HBcAb}^+ )</th>
<th>( \text{HBsAg}^+ \text{HBsAb}^+ )</th>
<th>( \text{HBsAg}^- \text{HBcAb}^+ )</th>
<th>( \text{HBsAg}^- \text{HBsAb}^- )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reactive</td>
<td>38</td>
<td>5</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-reactive</td>
<td>2</td>
<td>6</td>
<td>535</td>
<td>69</td>
<td>6963</td>
</tr>
</tbody>
</table>

Table 3: Result of HBV testing for blood donors using NAT an serologic techniques.