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Evaluation of serological transfusion-transmitted viral diseases and mutliplex nucleic acid testing in malaysian blood donors

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ABSTRACT

Background: Transmission of infectious diseases is a recognized complication of blood transfusion and blood products. Nucleic acid testing (NAT) may contribute to improved efficiency of blood screening and thereby increase the safety margin for transfused blood.
Methods: Unscreened blood samples from 1388 randomized donors were selected for this study at the Transfusion Medicine Unit of Hospital Universiti Sains Malaysia (HUSM). Informed consent was obtained from all donors and blood samples were tested for HIV, HBV and HCV serologically and by NAT assay.
Results: Of the 1,388 tested samples, 1,360 were non-reactive for both assays. Four samples (0.29%) were both serologically and NAT reactive. The remaining twenty-four samples were divided into two groups. Of these, five samples (0.366%) were NAT reactive and nineteen samples (1.37%) were serologically reactive. However, serology confirmation tests run on the latter nineteen samples were non-reactive.
Conclusions: Hence, NAT adds benefit of detecting "false positive" reactions via standard serology, the cost of administering NAT also need further consideration and study.

1. Introduction

The supply of safe and efficacious blood and blood components for transfusion or manufacturing involves multiple steps beginning with (1) the selection of blood donors; (2) the collection, processing and testing of blood donors and testing of patient samples; (3) and the issue of compatible blood recipients and its administration. Each step of the "transfusion chain" holds risks of error and failure that have serious implications for recipients of blood and blood products [1]. All donors in Malaysia are unpaid volunteers who are carefully selected by using a donor health questionnaire to ensure safety and reduce the risk of transmitting infectious vectors. In Malaysia as well as in many other countries, blood samples are routinely serologically screened for Hepatitis B virus (HBV), human Immunodeficiency Virus Type 1 (HIV-1), and Hepatitis C Virus (HCV). Transmission of HIV, HCV and HBV by blood and blood components has remarkably declined over the past two decades as a result of implementing sensitive and specific tests for the detection of viral antibodies and antigens [2,3]. Hence, the residual risk of transfusion-transmitted viral infection is considered minimal or negligible. However, attaining 'zero risk' remains a substantial challenge [1,4]. Genomic screening for HBV, HCV and HIV represents a major advance that can eliminate infectious blood donations collected during the pre-seroconversion window period, meaning donations can occur when the donor is infectious but nonreactive to assays for viral antibodies or antigens [3]. The seronegative window phase for

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Hepatitis C is estimated at an average of eighty-two days for second-generation enzyme immunoassays (EIAs) [5], but was reduced to approximately sixty-six days for third generation EIAs [6–8]. Hepatitis B and HIV have window phases of approximately fifty-nine and twenty-two days, respectively [5]. Cases of viral infection have been documented in patients who received blood or blood components from donors during the window phase of infection [9,10]. Other sources of residual risk are rare cases of 'immunosilent' infections, which possibly include a large spectrum of viral variants that are not detectable when using tests designed to identify their common forms. These include: atypical seroconversion as indicated by an unusually prolonged window period or lack of seroconversion, as well as emerging viral mutants. Laboratory error is still another source of residual risk [3,11,12]. The recent introduction of Nucleic Acid Testing (NAT) as a screening tool for blood donors may enhance the safety of the blood supply. Thus, donors that otherwise escape detection by routine serology during an "infectious" window period may be identified via NAT assay [1]. Presently, NAT testing is recommended for government and private organizations in pursuit of providing "zero risk – safe blood" to recipients. Nevertheless, the necessity and feasibility of achieving "zero-risk" for blood transmissible viral agents is currently questioned, especially in view of high cost vs. benefit of the additional measure [1,4,13].

2. Materials and methods

This cross sectional prospective study was conducted over a period of fourteen months from November 2008 through January 2010 at the Transfusion Medicine Unit of Hospital Universiti Sains Malaysia (HUSM). A cohort of 1,388 donors who fulfilled the eligibility criteria for blood donation was selected by a systematic random sampling method applied to registered donors. We estimated a need for five donors daily given an estimated attendance at the blood bank of approximately fifty donors per day. The sampling interval was fixed at every tenth patient and ceased after five samples were taken daily.

This study was approved by the School of Medical Science Research and Ethics Committee. Written informed consent was obtained from all donors after the nature of the study was fully explained.

The NAT assays were conducted at the Transfusion Medicine Unit, HUSM, utilizing the individual test (ID) NAT Assay (Chiron Corporation, Emeryville, CA) according to the manufacturer's instructions. Each sample was tested individually by NAT and concordantly with ELISA. The ID NAT assay is a multiplex test that provides simultaneous detection of HIV-1 RNA, HCV RNA and HBV DNA in human plasma by using transcription mediated amplification technology (TMA).

When a tested sample was reactive by NAT and ELISA, all blood bags were discarded. Any sample with discordant results was followed up prospectively during the next visit with further analysis as follows: Blood was taken directly from the donor and sent for a second run for ELISA confirmation. In cases where the NAT was initially reactive, donor samples were tested in duplicate using the same NAT kit prior to the discriminatory assay. If the ELISA was initially positive, the donor's blood bag was discarded and a second sample was taken directly from the donor (by bleeding) for a second ELISA assay and confirmatory serological testing.

The confirmatory serology test was performed in the microbiology laboratory unit of HUSM in order to identify false positive reactivity during serological screening as it is more sensitive and specific. We performed neutralization tests for HBsAg, INNO-LIA HCV Score for HCV, and particle agglutination for HIV.

The INNO-LIA HCV Score is a Line Immuno Assay (LIA) for the detection of antibodies to Human Hepatitis C Virus in human serum or plasma. It is intended for use as a supplementary test of human serum or plasma specimens found reactive using the anti-HCV screening method.

The discriminatory assay/test was performed when an individual test was initially reactive. The HIV-1, HCV, and HBV discriminatory assays utilize the same three steps taken for the ID NAT Assay (target capture, TMA and HPA). The same assay procedure was followed with one major difference: HIV-1-specific, HCV-specific, and/or HBV-specific probe reagents were used in place of the ID NAT assay probe reagent.

3. Results

Characteristic profiles of the study's cohort are shown in Table 1. The age of donors ranged from eighteen to sixty years of age with mean of 28.9 years (Standard Deviation: 10.95). The majority were Malays (85.37%), followed by Chinese (12.96%), Indian (1.15%), and other races (0.5%). Altogether there were 376 women (27.08%), and 1,012 men (72.91%).

Of the 1388 donor samples studied, the prevalence for 'window case' HIV-1, HCV and HBV in HUSM using NAT was 0.65% (nine cases) which required another procedure to identify the type of virus via discriminatory testing. However, this prevalence was reduced to less than half after the discriminatory test was performed as there were only four positive cases identified (HBV DNA) yielding a prevalence of 0.29% (see: Table 2).

In terms of standard serology technique, twenty-three cases were identified as positive via serological reaction giving a prevalence of 1.66%. Subsequently, nineteen of the twenty-three cases were found negative via serological confirmatory testing, reducing the prevalence to 0.29%. The

Table 1
Characteristic of 1388 donors in HUSM.

Characteristic	Frequency (%)	Mean (SD)
Age (years)		28.9 (10.95)
Ethnic group		
Malay	85.37	
Chinese	12.96	
Indian	1.15	
Others	0.50	
Sex		
Male	72.91	
Female	27.08	