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NAT positivity in seronegative voluntary blood donors from western India

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ABSTRACT

Background and objectives: Prevalence and composition of Hepatitis B, Hepatitis C and HIV-1, NAT positive but seronegative voluntary blood donors from western part of India is yet to be documented.
Material and methods: Over last 2 1/2 years all the seronegative voluntary blood donors were tested using 10 minipools on a semiautomated NAT testing platform. The positively tested donors were followed up for at least five months for development of seropositivity.
Results: 79532 seronegative donations were tested by 10 minipool (MP) NAT leading to 51 positive sample (44 Hep B, 5 HIV 1 and Hep C positive). All the HIV and Hep C NAT positive donors eventually developed seropositivity and out of 44 Hep B NAT positive donors, 31 developed seropositivity within six months of follow up, following counseling of the donors. This data translate into NAT yield of 1:1559 donors for all virus taken together. NAT yield for Hep B 1:1807 donors were much higher than HIV 1 in 1:15906 and HCV yield of 1:39761. Semiautomated minipool NAT testing system was found to be cost effective way for improving blood safety.
Interpretation and conclusion: Seronegative NAT yield in voluntary blood donors are quiet high in western part of India and in line with rest of the country is mainly due to Hepatitis B infection. Implementation of strict donor screening, Hep B vaccination of the population and sample mutation of NAT testing should be under taken on war footing.

1. Introduction

India is the second most population country in the world and presently uses @ 10 million units of blood every year. Seroprevalence of Human immunodeficiency virus (HIV 1), Hepatitis C (Hep C) and Hepatitis B (Hep B) in Indian blood donors are 0.5, 0.4 and 1.4 percent and is not very different from general population prevalence and this is 25–100 times higher than US blood donor data [1–3]. The reason for this difference is several but he most important is that is a crowded blood donation camp with restricted number of staffs. It is extremely difficult to select the donors and Indian blood donors often do not self defer donation rather some of them donates to test their Transfusion Transmitted Diseases (TTD) status and many who should have self deferred but donates blood due to peer pressure.

Under such circumstances experts for long felt an universal Nucleic Acid Tests (NAT) testing is nowhere more urgently needed to be implemented than in India [4–7]. It is believed that at least 10000 individual are newly infected by transfusion every year in India. NAT testing across the country could not be employed for last 2 decades mainly because of cost consideration [8,9].

India has a very heterogeneous population and in an individual city like Surat in western India where their study was conducted has a donor population which is very different [10,11].

The present study was under taken because;

1 Robust NAT yield data from western India that too from a regional transfusion centre is not available.
2 The study also assesses the economic feasibility of using a semi automated NAT testing system in India.

The number of samples used in the NAT testing per each minipool may vary, which usually accommodates the requirements of the blood donation centers for overall sensitivity of the testing as well as for the financial expenditures [5,7,12,13].

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We tested seronegative donations from voluntary blood donors at the Surat Raktadan Kendra & Research Centre and those donating in the outdoor voluntary blood donation camps organized by us in and around Surat, using the commercial available a multiplex NAT kits of Sacace Biotechnologies SRL, Como, Italy, and RealStar HBV, HCV and HIV RT-PCR Kit 1.0, Altona Diagnostics GmbH.

2. Materials and methods

All voluntary blood donors, between April 2013 to Oct 2015 at the Surat Raktadan Kendra & Research Centre, and those donating in the outdoor voluntary blood donation camps organized by us in and around Surat were included in the study.

2.1. Plasma samples

Whole blood from donors was collected in EDTA tubes; plasma was separated by centrifugation at room temperature. Within 8 h of collection, plasma were frozen and then stored at −30 °C and thawed once before use.

2.2. Serological assay

Samples from the donated blood units was go through routine HIV, HBV and HCV screening using a third-generation assay, for HCV Abs with SD HCV ELISA 3.0 test system (Boi SD standard diagnosis Pvt. Ltd); for HIV-1/2 Abs with Microlisa (J. Mitra & Co. Pvt. Ltd) and for HBV Ags with SD HIV ELISA 3.0 test system (Boi SD standard diagnosis Pvt. Ltd).

2.3. Isolation of viral nucleic acid

HBV DNA, HCV RNA, and HIV-1 RNA were isolated from plasma samples either with the use of a High Pure viral nucleic acid kit (MagDEA Viral DNARNA (GC), Precision System Science Co. Ltd., Japan) or with Chemagic Prepito-D automated extractor (Perkin Elmer), in combination with reagents/buffers of the Prepito Viral DNA/RNA Kit.

2.4. Real-Time-PCR amplification of viral nucleic acid

HBV viral DNA and HIV and HCV RNA were amplified either by multiplex kits (HIV/HBV/HCV) of Sacace Biotechnologies, Italy, or by RealStar HBV PCR Kit 1.0, RealStar HCV RT-PCR Kit 1.0 and RealStar HIV RT-PCR Kit 1.0 (Altona Diagnostics GmbH) as described in the manufacturer's protocol. The PCR was performed on a Smart Cycler II (Cepheid, France) and ABI Prism 7500 Real Time PCR System (Life Technologies).

2.5. Proficiency testing

The HIV, HCV and HBV RT-PCR NAT Kit was used in this study, contains a heterologous Internal Control (IC), which can be used as a RT-PCR inhibition control as well as a control of the sample preparation procedure (nucleic acid extraction). The IC was added during the nucleic acid extraction in each pool.

Internal quality controls were performed daily by using both positive and negative controls from the manufacturers. In case of any deviation, the root cause analysis was carried out and the corrective action was taken before analyzing the samples. Proficiency testing was maintained with a leading hospital using ID-NAT system with which our Institute has collaboration and both Institutes regularly exchange their NAT coded positive and negative samples.

2.6. Detection of the amplified products

The results of multiplex kits of Sacace Biotechnologies, Italy, were interpreted by the device software of Smart Cycler II, Cepheid, France, through the presence of crossing of fluorescence curve with the threshold line. HCV cDNA was detected on the Fam (Green) channel, HIV cDNA on the Joe (Yellow)/HEX/TET/Cy3 channel, HBV DNA on the Rox (Orange)/TexasRed channel and IC on the Cy5 (Red) channel.

Amplified products of RealStar HBV, HIV, HCV RT-PCR Kit 1.0 (Altona Diagnostics GmbH) were detected by fluorescence and data analyzed using the 7500 SDS software, version 2.3. Fluorescence from the specific amplification of viral target DNA/RNA was captured in the FAM channel, while signal from IC amplification read in JOE channel. During data analysis, ROX was switched on only for HCV/HIV test. Analysis was performed using Auto-Threshold and Auto-Baseline by default.

2.7. Economic feasibility evaluation

Costs of NAT testing by available automated machines were compared with that of the present semiautomated system. However the automated systems are mostly installed in private corporate hospitals and in few government hospital and from there ancillry costs ie technician's salary, machine cost, cost regarding space and other occupancy could not be obtained. Only cost of kits and other ancillary reagents in running the assay was obtained.

3. Results

A total of 79,532 seronegative blood donations from the period of April 2013 to Oct 2015, were tested for the HIV, HCV and HBV by real time-PCR, using commercially available two different Real time-PCR kits of HIV, HBV and HCV. The average of the units tested per months was approximately 2565, and the mean time needed for the screening by real-time PCR was 4.6 h that included preparations of the plasma sample, mini-pooling (manual), and viral DNA/RNA extraction and performing RT-PCR. The entire procedure was easily managed by two skilled technologist, valid runs of NAT testing were explained by testing of the internal controls of HBV, HCV and HIV-1 real time −PCR kits. We started initially our programme with 12-GC Precision System for viral nucleic acid isolation and Sacace kits for RT-PCR, but due to some reason the supply of the kits in our country stopped hence we had to switch to Altona RealStar kits and Chemagic Prepito-D automated extractor system. The differences in the performance between the two kits were not deferent as evidence by Chi-square testing between the two kits.

3.1. HIV, HBV and HCV real time PCR assay by kit of sacace biotechnologies SRL, Italy

Of the 79,532 seronegative blood samples, 65,362 samples were tested by kits of Sacace Biotechnologies SRL, Como, Italy in ten mini-pools (10-MP) and 45 (0.075%) were positive by 10-MP-NAT assay. And among these forty five samples, 40 were positive for HBV DNA (0.067%), 01 for HCV RNA (0.001%) and 04 for HIV-1 RNA (0.007%). Whenever the MP was positive, the reactive sample was identified by testing individual samples. Due to small size of the pool (n = 10), this was not a problem.

3.2. HIV, HBV and HCV real-time PCR assay by kit of alotona RealStar

A total 14,170 seronegative blood samples were tested by Alotona RealStar HBV, HIV and HCV PCR Kit 1.0, Altona Diagnostics