BRITISH JOURNAL OF BIOMEDICAL SCIENCE, 2017
VOL. 74, NO. 1, 42–47
http://dx.doi.org/10.1080/09674845.2016.1220708

Assessment of semi-automated nucleic acid testing programme in a Regional Blood Transfusion Centre

Kanjaksha Ghosh, Kanchan Kumar Mishra, Apeksha Trivedi, Sheetal Sosa and Krima Patel

Surat Raktadan Kendra & Research Centre, Surat, Gujarat, India

ABSTRACT

Background: Detection of human immunodeficiency virus type-1 (HIV-1), hepatitis-C (HCV) and hepatitis-B virus (HBV) in the blood donors is crucial. An efficient form of detection is nucleic acid testing (NAT) in blood screening. We assessed the suitability of commercial NAT testing in a developing country, focusing on the Altona RealStar assay and the method of Sacace Biotechnologies.

Methods: We have standardised and validated commercially available NAT kits with a semi-automated system for detection of HBV, HCV and HIV-1 in blood donations. The MP-NAT (mini-pool) assay consists of pooling of sample, virus extraction, amplification and detection with commercially available NAT kits. An internal control (IC) is incorporated in the assay to monitor the extraction, target amplification and detection process.

Results: The sensitivity of the Altona RealStar assay at 10-MP for each viral target was evaluated, HBV showed amplification in all diluted positive samples of 100, 50, 25, 10 and 5 IU/ml. HIV and HCV infected samples showed amplification in all diluted positive samples of 500, 100, 50 and 30 IU/ml. For HIV, out of six diluted samples of 30 IU/ml, five were amplified. A total of 14,170 seronegative blood samples were tested by RealStar PCR kit in 10-MP and 6 (0.042%) samples/pools were positive. A total of 65,362 seronegative blood donations were also tested by kits of Sacace Biotechnologies, in 10-MP and 45 (0.075%) pools were positive. The prevalence of combined NAT yield cases among routine donors was 1 in 1559 donations tested for all the 3 viruses.

Conclusion: The semi-automated combined system for NAT screening assays is robust, sensitive, reproducible, and this gives an additional layer of safety with affordable cost.

ARTICLE HISTORY
Received 24 May 2016
Accepted 26 July 2016

KEYWORDS
Nucleic acid testing (NAT); HIV; HBV; HCV; Semi-automated; seronegative

Introduction

Viral safety from transfusion-transmitted viruses which cause chronic infections, organ failure and morbidity is a preeminent preoccupation of all transfusion medicine centres throughout in the world. The safety is never presumed but is actively pursued by a strategy that includes 100% voluntary donation, donor interview to exclude donors at risk, careful selection of the sites of the blood bank camps, and serological test for Hepatitis B (HBV), Hepatitis C (HCV) and HIV infections.[1,2] Viral infection may be determined by serological methods such as enzyme-linked immunosorbent assay (ELISA) antiviral antibodies, but more recently, one of the most efficient ways of determining and so excluding very early infection is with Nucleic Acid Amplification (NAT) testing.[3–5]

To date, approximately 33 countries in the world [6–9] have adopted NAT testing as one of the most important methods for viral safety in transfused blood or blood products. Using NAT testing, seronegative NAT positive samples in U.K. has reached 1:60,000 for HBV and 1:250,000 donations for HIV and NAT testing has reduced the risk of HCV by 95% and that of HIV by 10%. [10] Similar results are reported from Western European countries and the U.S.A.[7] The transfusion services in India has 2545 private- and government-licensed blood banks in various levels of sophistication,[11] but has not yet been able to include NAT testing as a mandatory test for safe transfusion practice mainly because of financial constraints.[12–14] A small number of private, corporate hospitals in urban areas and government hospitals [3,4] have already implemented NAT testing facility. However, there is growing pressure on transfusion services to adopt NAT testing nationally to improve blood safety, as it is believed that across the country, 10,000 new HIV infections are transfusion related.

At present in India, about 10 million units of blood are collected annually, but as only 0.3 million (3%) are NAT tested, there is an urgent need for a formal assessment of cost-effective NAT testing facilities as an additional layer of viral safety. Accordingly, we evaluated a commercial

CONTACT Kanchan Kumar Mishra kanchan008@gmail.com

semi-automated NAT testing platform with a mini-pool format (Altona RealStar) in comparison with multiplex kits of Sacace Biotechnologies.

Materials and methods

All voluntary blood donors at the Surat Raktadan Kendra & Research Centre, and those donating in the outdoor voluntary blood donation camps in and around Surat between April 2013 and October 2015 were included in the study. Informed consent was obtained prior to the collection. Whole blood from donors was collected in EDTA tubes and plasma was separated by centrifugation at room temperature. Within 8 h of collection, plasma was frozen and then stored at −40 °C and thawed once before use.

Serological assay

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

Isolation of viral nucleic acid

Viral nucleic acid of HBV DNA, HCV RNA and HIV-1 RNA was isolated from individual or pooled plasma samples by Magtration® System 12GC automated nucleic acid extraction instrument, (PSS Co. Ltd., Japan), and was stored at −80 °C until further analysis. Starting volume per pooled plasma sample was 1.0 ml and final elution was in 50 µl (Concentration 20-fold).

Sacace real-time-PCR amplification of viral nucleic acid

HBV viral DNA and HIV and HCV RNA were amplified with multiplex kits of Sacace™ HCV/HBV/HIV Real-TM (V50–100FRT), Sacace Biotechnologies SRL, Como, Italy. As described in the manufacturers protocol, total volume of RT-PCR reaction was 25 µl (10 µl of Reaction Mix and 15 µl of extracted RNA/DNA sample). The PCR was performed on a Smart Cycler II (Cepheid™, Maurens-Scopont, France) using the selected primers and probes.

Quality control

Internal quality controls were performed daily using both positive and negative controls from the manufacturer. In case of any deviation, the root cause analysis was carried out and the corrective action was taken before analysing the samples. Random-selected sample (coded) was exchanged with a centre doing ID-NAT testing using Novartis system as one of the quality assessment system.

Validation of Altona RealStar® HIV, HCV and HBV RT-PCR kit 1.0

Viral nucleic acids (DNA and RNA) were extracted and purified on chemagic Prepito®-D automated extractor (PerkinElmer, Germany), in combination with reagents/buffers of the Prepito Viral DNA/RNA 1 k Kit (catalogue numberCMG-2018). Starting volume per pooled plasma sample was 1.0 ml and final elution in 50 µl (concentration 20-fold).

Amplification of viral DNA/RNA targets and internal control sequence

Viral target sequence was amplified using RealStar® HIV, HCV and HBV RT-PCR Kit 1.0 (Product No. 491003), altona Diagnostics GmbH, Mörkenstraße 12, Hamburg. These assay kits provide all necessary amplification reagents, Quantitation Standards (QS1 – QS4) for calculation of viral concentration to establish the limit of detection (LoD) in IU/ml, water to set up no template control (NTC), as well as synthetic internal control (IC) sequence for spiking of pooled plasma samples during extraction. The IC was added during the sample preparation procedure, the Master Mix were set up according to the following pipetting scheme; Master A 5 µl, Master B 25 µl and 25 µl of the sample (eluate from the nucleic acid extraction) or 25 µl of the controls (positive control or negative control).

All assays were run on the ABI Prism 7500 Real Time PCR System (Life Technologies, USA). The RealStar HCV RT-PCR Kit 1.0 and HIV RT-PCR Kit 1.0 could be run in parallel as both share a common cycling temperature profile, while the RealStar HBV kit 1.0 has to be run independently. All assays were run in standard 25 µl of reaction volume (12.5 µl of extracted nucleic acids +12.5 µl of Master Mix).

Detection of the amplified products

Amplified products were detected by fluorescence and data analysed 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. An internal control is added to each reaction to verify the specimen processing, amplification and detection steps.

Validation of the assay was done by examining the sensitivity of the NAT kits, using dilution series 100 to 5 IU/ml for HBV, 500 to 30 IU/ml for HCV and HIV-1. Validation was carried out in three stages;

Stage 1 – verification of negative plasma

Plasma pools pretested as negative with existing workflow (12-GC extraction +Sacace Biotechnologies, HBV/HCV/HIV kit, detection on SmartCycler II) were prepared and re-extracted with Prepito-D and reassayed with RealStar kits 1.0 on ABI-7500 as described in material methods. A total of approx. 90 ml of negative plasma was verified (HBV/HCV/HIV not detected in FAM, but all with valid amplified IC) and subsequently used in Stage 3.

Stage 2 – quantification of known positive samples

Aliquots of known positive samples pretested with the existing qualitative workflow (12-GC extraction +Sacace Biotechnologies, HBV/HCV/HIV kit) were re-extracted with Prepito-D and reassayed with RealStar kits 1.0 of HIV, HCV and HBV on ABI7500 to obtain quantitative values. The resulting calculated viral concentration in Table 1 was used as a reference to prepare further dilution series.

Stage 3 – dilution of known positive samples

Quantified known positive samples were diluted with negative plasma to prepare the following dilution series (in sextuplicate per dilution), Table 2.

All dilutions were extracted with Prepito-D and assayed with RealStar kits 1.0 of HIV, HCV and HBV on ABI7500 as described by the manufacturer. The sensitivity of Altona Diagnostics RealStar kits 1.0 is described in Table 3. A run was considered valid when all NTCs were negative, all NTC-ICs were positive.

Table 1. Real-time quantitative PCR assays of Hep-B, Hep-C and HIV-1 positive samples.

S.No.	Sample ID of positive sample	Quantitative measurement of Viremia (IU/ml)
1	Hep-B 30233	2.34×10^7 IU/ml
2	Hep-C 30267	5.66×10^5 IU/ml
3	HIV-1 20199	3.54×10^4 IU/ml

Table 2. Dilution sequence of the positive samples of Hep-B, Hep-C and HIV-1.

S.No.	Sample ID of positive sample	Dilution series prepared (IU/ml)
1	Hep-B 30233	100, 50, 25, 10, 5
2	Hep-C 30267	500, 100, 50, 30
3	HIV-1 20199	500, 100, 50, 30

Table 3. Sensitivity of the Altona RealStar (Hep-B, Hep-C and HIV-1) NAT kits, using dilution series 100 IU/ml to 5 IU/ml for HBV, 500 IU/ml to 30 IU/ml for HCV and HIV-1.

S.No.	Sample ID of positive samples	Dilution series (IU/ml)	Hit rate	Average CT
1	HBV 30233	100	6/6	33.6
		50	6/6	34.8
		25	6/6	35.8
		10	6/6	38.3
		5	6/6	37.8
2	HCV 30267	500	6/6	24.0
		100	6/6	26.3
		50	6/6	28.0
		30	6/6	28.5
3	HIV 20199	500	6/6	24.3
		100	6/6	26.5
		50	6/6	27.4
		30	5/6	28.0

Results

We have applied a RT-PCR-based testing assay for the detection of HIV-1, HCV RNA and HBV DNA in the plasma collected from the donors in a mini-pool of 10. This allowed the screening by RT-PCR of a total of 79,532 donations using the above-mentioned kits. The mean time needed for the screening by PCR was 4.5 h, that included preparations of the plasma sample, mini-pooling (manual), viral DNA/RNA extraction, amplification and detection. The entire procedure was easily managed by one or two skilled technologists, valid runs of NAT testing were determined by testing of the internal controls provided in the kits.

HIV, HBV and HCV NAT assay by kits of Sacace Biotechnologies SRL

A total of 65,362 seronegative blood donations from the period of April 2013 to June 2015 were tested by kits in 10 mini-pools (10-MP). Forty-five (0.075%) pools were found to be positive by 10-MP-NAT assay. These 45 pools were finally resolved into individually samples. Out of these 45 NAT positive samples, 10 samples were randomly chosen and sent to the centre routinely doing ID-NAT using Automated NOVARTIS platform and were confirmed positive. Similarly, from the same centre, 10 samples were sent for testing the NAT status in the system. The results showed 100% concordance.

HIV, HBV and HCV NAT assay by Altona RealStar kits1.0

Sensitivity of the Altona RealStar assay at 10-MP for each viral target was evaluated using dilution series (100 to 5 IU/ml for HBV, 500 to 30 IU/ml for HCV and HIV-1 as described in Materials and Methods). As shown in Table 3, HBV showed amplification in all the diluted positive samples, and HIV and HCV were showing amplification in all the diluted positive samples. However for HIV, out of six diluted samples of 30 IU/ml five were amplified, possibly due to inadequate /non-homogenous mixing of the stock sample (HIV 20199) prior to making dilution series.

From July 2015 to Oct 2015, 14,170 seronegative blood samples were tested by RealStar HBV, HIV and HCV PCR Kit 1.0, in 10 mini-pools (MP). Six (0.042%) pools were found to be positive by 10-MP-NAT assay (our unpublished data). These six pools were finally resolved into individual samples and these six samples found to be

positive by MP-NAT assay, were sent to a centre which does ID-NAT using Automated NOVARTIS platform and were confirmed positive. Similarly, from the same centre, 6 NAT positive samples and 50 NAT negative samples were sent to us for testing the NAT status in the system we are using and the results showed 100% concordance.

Discussion

We describe a semi-automated, high- and low-throughput, viral detection system, which detects HBV DNA, HCV RNA and HIV-1 RNA using commercially available RT-PCR kits. The time required for the pooling of plasma samples is ½ h, sample extraction is 2 h and amplification and detection requires additional 2 h. The total time required to process 100 samples is about 4 1/2 h with one positive and one negative control together with internal control. Semi-automated NAT assays are in use in Germany, Austria and Scotland [15–17] improving transfusion safety and limiting the cost of blood.

According to the recommendation by Paul Ehrlich Institute and U.S. Food and Drug Administration, sensitivity of NAT for HCV RNA should be 5000 IU/ml, and 650 copies/ml for HIV and 500 copies/ml for HBV. This viral load is present in pre-seroconversion stage; therefore, sensitivity of mini-pool should be at this level. Kits of Sacace Biotechnologies are able to detect HIV, HCV and HBV up to mini-pools of 16 and 24 at above-given copies and IU/ml. [18] Throughout in this study, we have decided to use 10 mini-pools only and showed 45 cases of 65,362 blood donors diagnosed during the window period (negative serological tests with positive by Sacace Biotechnologies kits in 10-MP-NAT assay). Discrimination of these 45 cases showed HBV-, HCV- and HIV-infected donor in 40, 1 and 4 cases, respectively. This suggests that samples from 10 individuals could be pooled for detection of HIV-1, HCV RNA and HBV DNA with kits of Sacace Biotechnologies, without compromising on sensitivity.

The detection limit of the Altona RealStar HIV/HCV/HBV RT-PCR kits was in accordance with the WHO standards in our evaluations of the combination protocol (Table 3), and present observations of these standards did not show any unsatisfactory result as compared with other studies.[19–23] Present data are reasonably similar to the commercially available two fully automated NAT Solutions i.e. Procleix (now GRIFOLS) and ROCHE.[24–26] After standardisation and validation of Altona RealStar kits, 14,170 seronegative blood samples were tested in 10-MP-NAT assay and 6 were positive. Among these six NAT positive, 4 were positive for HBV DNA, 1 for HCV RNA and 1 for HIV-1 RNA.

The cases of HIV-1, HCV and HBV detected with above NAT kits and performances of the systems and NAT kits indicated that both kits (Sacace Biotechnologies and RealStar Altona Diagnostics) were acceptable for routine NAT. In November 2012, the U.S. Food and Drug Administration (FDA) issued guidelines regarding HBV DNA detection NAT assays, stipulating that such assays should have a minimum sensitivity of 100 IU/ml (approximately 500 copies/ml).[27] In our study kits of Sacace Biotechnologies, for HIV/HBV/HCV and Altona RealStar HIV/HCV/HBV RT-PCR, both kits have shown their minimum sensitivity as per the guidelines of the FDA.[27,28]

Our experience indicates that mini-pool NAT with pool sample size of 10 is optimum as well as a cost-effective approach for implementation of NAT. With this protocol, the cost of an investigation unit is essentially divided in 10, and subsequently as each blood bag is made into at least 3 components, then the cost on each product is 1/30 of the total NAT Cost.[29] Certainly, it can be commented that it will improve the safety of blood transfusion as well as will be cost effective for developing countries such as India. Furthermore, it has been estimated that ID-NAT screening, even if it increases detection of infected donors, the minimal associated testing cost would be significantly increased.[30,31]

One of the arguments which can be levelled against mini-pool NAT is that it is less sensitive as ID-NAT, considering the fact that howsoever sensitive the system is, it will still miss some samples which may have lesser amount of nucleic acid than can be detected by the system; this is clearly shown by comparing two ID-NAT systems by the same company and tested against the same group of samples in the same institute where one instrument failed to detect half of the HBV infections.[20] Hence, we have to consider that any improvement of the present condition takes us one step nearer to the ideal. It is felt that the mini-pool semi-automated system, as is standardised here, is an eminently workable system in the country. Moreover, in this system, the identification of the causative virus is identified in the first run itself.

At present, the data correspond well to the NAT yield data using ID-NAT and MP-NAT system in several centres [32–38] in this country. Moreover, in the quality control studies, ID-NAT positive samples sent to us were tested as mini-pool with 100% correct results. One important caveat of semi-automated assay is to have good technical manpower and a robust troubleshooting protocol in place. This was achieved in this centre by having a spare set of instruments and regular training programme for the technical staff with troubleshooting protocols.

In conclusion, we have standardised and validated a semi-automated MP-NAT assay, with high and low throughput, and high sensitivity with commercially available HIV, HCV and HBV RT-PCR kits in combination with automated extraction of viral DNA/RNA for the screening of blood and it can be easily applied to the routine daily work of blood bank laboratories. This tool could provide the next large step in improving the safety of blood samples in our country. We recognised the potential benefit of MP-NAT particularly for HBV and HIV, and therefore

believe that NAT test should be adopted. This work represents an advance in biomedical science because it shows that improvised semi-automated mini-pool NAT testing could optimise the viral safety of HIV-1, HBV and HCV with cost effectiveness for both high- and low-volume blood bank.

Summary table

What is known about this subject?

- NAT testing is being practiced in all developed Western countries for about two decades.
- Developing countries like India have not yet been able to include NAT testing as a mandatory test for safe transfusion practice mainly because of cost.
- According to the recommendation by Paul Ehrlich Institute and U.S. Food and Drug Administration, sensitivity of NAT for HCV RNA should be 5000 IU/ml, and 650 copies/ml for HIV and 500 copies/ml for HBV.

What this paper adds:

- We describe a semi-automated, high- and low-throughput, viral detection system, which detects HBV DNA, HCV RNA and HIV-1 RNA.
- Detection limit of the Altona RealStar HIV/HCV/HBV RT-PCR kits was in accordance with the WHO standards in our evaluations of the combination protocol.
- The study also assesses the economic feasibility of using a semi-automated NAT testing system

Acknowledgements

We would like to offer our sincere thanks to Dr Anand Deshpande, P. D. Hinduja National Hospital, Mumbai, India for providing the assistance with ID-NAT positive and negative plasma samples for quality-control programme. The authors thank all individuals from the TTI department of the SRK&RC, who were involved in doing the ELISA of the blood donors. We also acknowledge Dr N. Vasavada, SRK&RC, Deputy Director, who has worked tirelessly for making the NAT testing project viable.

Disclosure statement

No potential conflict of interest was reported by the authors.

ORCID

Kanchan Kumar Mishra http://orcid.org/0000-0001-8499-9971
Apeksha Trivedi http://orcid.org/0000-0002-5213-7899

References

[1] Adeoti FM, Oyourou AO, Sirancy-Bogui L, et al. Evolution of the residual risk of transmitting HIV, HCV and HBV in the blood transfusion from 1998 to 2009 in Cote d'Ivoire. J. Vaccines Vaccin. 2012;3:1–4.
[2] Marwaha N. Voluntary blood donation in India: achievements, expectations and challenges. Asian J. Transfus. Sci. 2015;9:1–2.
[3] Shyamala V, Sandison TG, Holmberg JA. Individual donation nucleic acid technology testing to minimize human immunodeficiency virus-1, hepatitis C virus, and hepatitis B virus transfusion transmitted infections. Asian J. Transfus. Sci. 2014;8:68.
[4] Albertoni G. Mini review: current molecular methods for the detection and quantification of hepatitis B virus, hepatitis C virus, and human immunodeficiency virus type-1. Int. J. Infect. Dis. 2014;25:145–149.
[5] Hans R, Marwaha N. Nucleic acid testing-benefits and constraints. Asian J. Transfus. Sci. 2014;8:2–3.
[6] Stramer SL, Glynn SA, Kleinman SH, et al. Detection of HIV-1 and HCV infections among antibody-negative blood donors by nucleic acid-amplification testing. N. Engl. J. Med. 2004;351:760–768.
[7] Laperche S. Blood safety and nucleic acid testing in Europe. Euro Surveill. 2005;10:3–4.
[8] Stramer SL, Krysztof DE, Brodsky JP, et al. Sensitivity comparison of two Food and Drug Administration-licensed, triplex nucleic acid test automated assays for hepatitis B virus DNA detection and associated projections of United States yield. Transfusion. 2011;51:2012–2022.
[9] Stramer SL, Zou S, Notari EP, et al. Blood donation screening for hepatitis B virus markers in the era of nucleic acid testing: are all tests of value? Transfusion. 2012;52:440–446.
[10] Soldan K, Davison K, Dow B. Estimates of the frequency of HBV, HCV, and HIV infectious donations entering the blood supply in the United Kingdom, 1996 to 2003. Euro Surveill. 2005;10:17–19.
[11] El Ekiaby M, Lelie N, Allain J-P. Nucleic acid testing (NAT) in high prevalence-low resource settings. Biologicals. 2010;38:59–64.
[12] Central Drug Standard Control Organisation. State wise list of the approval blood banks in the country up to no; 2014. Available from: http://www.cdsco.nic.in/forms/list.aspx?lid=1836&ld=8
[13] Department of Health Schedule. The drugs and cosmetics act, 1940 and the drugs and cosmetics rules, 1945, as amended up to 2005 June 30. [Last accessed on 2013 Apr 04]. Government of India. Ministry of Health and Family Welfare; p. 326. Available from: http://www.cdsco.nic.in
[14] Jain R, Aggarwal P, Gupta GN. Need for nucleic acid testing in countries with high prevalence of transfusion-transmitted infections. ISRN Hematol. 2012;2012:718671.
[15] Chandra T. Implementation of Nucleic Acid Testing in a government blood bank setting in India. Vox sang. 2013;105:82–87.
[16] Jain R, Aggarwal P, Gupta GN. Need for nucleic acid testing in countries with high prevalence of transfusion-transmitted infections. ISRN Hematol. 2012;2012:5.
[17] Roth WK, Busch MP, Schuller A, et al. International survey on NAT testing of blood donations: expanding implementation and yield from 1999 to 2009. Vox Sang. 2012;102:82–90.
[18] Roth WK, Seifried E. The German experience with NAT. Transfus. Med. 2002;12:255–258.
[19] Kleinman SH, Strong DM, Tegtmeier GGE. Hepatitis B virus (HBV) DNA screening of blood donations in minipools with the COBAS AmpliScreen HBV test. Transfusion. 2005;45:1247–1257.
[20] Eiras A, Sauleda S, Planelles D, et al. HCV screening in blood donations using RT-PCR in mini-pool: the experience in Spain after routine use for 2 years. Transfusion. 2003;43:713–720.
[21] Wendel S, Levi JE, Takaoka DT, et al. Primary screening of blood donors by NAT testing for HCV-RNA: development of an 'in-house' method and results. Rev. Inst. Med. Trop. Sao Paulo. 2007;49:177–185.
[22] Glaubitz J, Sizmann D, Simon CO, et al. Accuracy to 2nd International HIV-1 RNA WHO Standard: Assessment of three generations of quantitative HIV-1 RNA nucleic acid amplification tests. J. Clin. Virol. 2011;50:119–124.
[23] Yalamanchili N, Syed R, Chandra M, et al. A latest and promising approach for prediction of viral load in hepatitis B virus infected patients. Indian J. Hum. Genet. 2011;17:17–21.

[24] Albertoni G, Castelo Girao MJ, Schor N. Mini review: current molecular methods for the detection and quantification of hepatitis B virus, hepatitis C virus, and human immunodeficiency virus type 1. Int. J. Infect. Dis. 2014;25:145–149.
[25] Makroo RN, Chowdhry M, Bhatia A, et al. Evaluation of the Procleix Ultrio Plus ID NAT assay for detection of HIV 1, HBV and HCV in blood donors. Asian J. Transfus. Sci. 2015;9:29–30.
[26] Jackson JB, Smith K, Knott C, et al. Sensitivity of the Procleix HIV-1/HCV assay for detection of human immunodeficiency virus type 1 and hepatitis C virus RNA in a high-risk population. J. Clin. Microbiol. 2002;40:2387–2391.
[27] FDA approves Roche's blood screening assay for simultaneous detection and identification of three major viral targets. Media Release, Basel, 2015 Jan 9. Available from: http://www.roche.com/media/store/releases/med-cor-2015-01-09.htm
[28] US Food and Drug Administration. Guidance for industry: use of nucleic acid tests on pooled and individual samples from donors of whole blood and blood components, including source plasma, to reduce the risk of transmission of hepatitis B virus. US FDA. 2012. Available from: http://www.fda.gov/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/Blood/ucm327850.htm
[29] US Food and Drug Administration. Guidance for industry: nucleic acid testing (NAT) for human immunodeficiency virus type 1 (HIV-1) and hepatitis C virus (HCV): testing, product disposition, and donor deferral and reentry. Rockville, MD: US FDA. 2010.
[30] Chandrashekar S. Half a decade of mini-pool nucleic acid testing: cost-effective way for improving blood safety in India. Asian J. Transfus. Sci. 2014;8:35–38.
[31] Busch MP, Glynn SA, Stramer SL, et al. A new strategy for estimating risks of transfusion-transmitted viral infections based on rates of detection of recently infected donors. Transfusion. 2005;45:254–264.
[32] Galel SA. Infectious disease screening. Tech. Manual. AABB. 2011;17:239–270.
[33] Chatterjee K, Coshic P, Borgohain M, et al. Individual donor nucleic acid testing for blood safety against HIV-1 and hepatitis B and C viruses in a tertiary care hospital. Natl. Med. J. India. 2012;25:207–209.
[34] Pathak S, Chandrashekhar M. Nucleic acid testing: redefining safety in blood screening. e-Max Med. J. 2010; 1: 1.
[35] Makroo RN, Chowdhry M, Bhatia A, et al. Evaluation of the Procleix Ultrio Plus ID NAT assay for detection of HIV 1, HBV and HCV in blood donors. Asian J. Transfus. Sci. 2015;9:29–30.
[36] Kumar R, Gupta S, Kaur A, et al. Individual donor-nucleic acid testing for human immunodeficiency virus-1, hepatitis C virus and hepatitis B virus and its role in blood safety. Asian J. Transfus. Sci. 2015;9:199–202.
[37] Shyamala V. Factors in enhancing blood safety by nucleic acid technology testing for human immunodeficiency virus, hepatitis C virus and hepatitis B virus. Asian J. Transfus. Sci. 2014;8:13–18.
[38] Chigurupati P, Murthy KS. Automated nucleic acid amplification testing in blood banks: An additional layer of blood safety. Asian J. Transfus. Sci. 2015;9:9–11.