Comparison of Procleix Ultrio Elite and Procleix Ultrio NAT Assays for Screening of Transfusion Transmitted Infections among Blood Donors in India

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Background. Introduction of nucleic acid testing (NAT) has helped in decreasing window period donations, resulting in increased safety of blood supplies. NAT combines the advantages of direct and highly sequence-specific detection of viral genomes with an analytic sensitivity that is several orders of magnitude greater than that of antigen/antibody/antigen-antibody detection techniques or viral isolation methods. The average window period duration during which immunological assays are unable to detect the anti-HIV-1/anti-HIV-2, anti-HCV, and HBsAg, which is estimated to be between 16, 70, and 45 days for HIV-1, HCV, and HBV, respectively, has been markedly reduced with NAT [2]. Introduction of NAT for screening of blood donors had shortened this “window period,” thus significantly increasing the safety of blood supplies [2].

1. Introduction

Blood transfusion is safer than ever before through continuous improvements in donor recruitment, meticulous screening, testing of donated blood with increasingly sensitive assays, and appropriate clinical use of blood [1]. The technological advancements at the molecular level TTI screening in the field of transfusion medicine have significantly curtailed the potential risks of transmissible infections through blood transfusions.

Nucleic acid testing (NAT) is a newer technology, introduced in the field of transfusion medicine for molecular diagnosis and characterisation of viral infections. NAT combines the advantages of direct and highly sequence-specific detection of viral genomes (DNA or RNA) with an analytic sensitivity that is several orders of magnitude greater than that of antigen/antibody/antigen-antibody detection techniques or viral isolation methods. The average window period duration during which immunological assays are unable to detect the anti-HIV-1/anti-HIV-2, anti-HCV, and HBsAg, which is estimated to be between 16, 70, and 45 days for HIV-1, HCV, and HBV, respectively, has been markedly reduced with NAT [2]. Introduction of NAT for screening of blood donors had shortened this “window period,” thus significantly increasing the safety of blood supplies [2].

The prevalence of HBV, HCV, and HIV in developed nations like Australia is reported to be very low to the tune of 7.55, 5.34, and 0.31 per 100,000-donor population, respectively.
In contrast, developing countries like India still have high prevalence of HBV, HCV, and HIV in the order of 1.8–4%, 0.4–1.09%, and 0.2–1%, respectively [4–9]. High prevalence coupled with inadequate blood screening continues to thwart the blood supply of the nation. Nucleic acid testing along with routine serology is expected to increase the blood safety in countries like India where prevalence of TTI is high [10].

A new-generation individual donor-nucleic acid testing (ID-NAT) based on the principle of transcription-mediated amplification (TMA) assay, namely, the Procleix Ultrio assay (PUA) (previously, Chiron/Gen-Probe, Emeryville/San Diego, CA, USA; now marketed by Grifols Diagnostic Solutions, Inc.), was first approved in 2006 by FDA for TTI screening of blood donors for the simultaneous detection of HIV-1 RNA and HCV RNA and later on extended for detection of HBV in blood donors. Manufacturers improved upon this version and introduced Ultrio Plus assay claiming enhanced sensitivity and automation (on Tigris platform).

Recently, the Procleix Ultrio Elite (PUE) system (previously, Chiron/Gen-Probe, Emeryville/San Diego, CA, USA; now marketed by Grifols Diagnostic Solutions, Inc.) was launched which has the additional ability for the detection of HIV-2 to meet the regional needs in various developing countries like India. The PUE is a multiplex NAT assay designed to detect HIV-1/HIV-2, HBV, and HCV qualitatively in vitro on the fully automated PANTHER instrument.

We evaluated the PUE on the Fully Automated Procleix Panther system and compared the results with routine serology and the earlier PUA.

### 2. Material and Methods

#### 2.1. Study Site

The study was conducted at the Main Blood Bank, All India Institute of Medical Sciences, New Delhi, from January 2014 to October 2014 for a total period of 10 months.

#### 2.2. Donor Population

A total of 10,015 routine blood donations at our blood bank were screened for viral markers which were mostly from replacement donors or family donors. Administration of the donor history questionnaire (DHQ) and meticulous screening of all the donors by a trained physician prior to blood donation were used to exclude those with high risks of exposure, thus increasing the chances of the TTI.

#### 2.3. Serological Testing

All the blood donation units were screened by ELISA using antigen-antibody combination assay (4th-generation kits), namely, Genscreen Ultra HIV Ag-Ab (BIO-RAD), for detection of both HIV-1 and HIV-2, antigen assays (3rd-generation kits), namely, Hepanostika HBsAg Ultra (Bioméricex), for detection of HBV, and antibody assays (3rd-generation kits), namely, Hepanostika HCV Ultra, for detection of HCV infections.

#### 2.4. NAT

ID-NAT was performed in parallel for all the corresponding donor samples using PUE (Automated Panther platform) and PUA (semiautomated platform) for the qualitative detection of the genomic sequences of HCV, HBV, HIV-1, and HIV-2 (only in case of PUE and not in PUA). The principle of the TMA was reviewed by Assal et al. [15]. The NAT algorithm (Figure 1) developed for Indian ID-NAT users was employed which was similar to that described in Grabarczyk et al. [16].

#### 2.5. Supplementary Assays

The NAT yield samples were retested for anti-HIV-1/anti-HIV-2, anti-HCV, and HBsAg by an alternative, more sensitive Chemiluminescent Immunoassay (ChLIA) (Architect Plus i1000 SR, Abbott Laboratories.,
Abbott Park, IL, USA) at an accredited laboratory in order to verify the screening results by ELISA. In order to detect the presence of any occult HBV infections (OBI), supplemental testing for anti-HBc, anti-HBs, and anti-HBe was performed by ChLIA (Architect Plus ii000 SR, Abbott Laboratories, Abbott Park, IL, USA). OBI is the presence of HBV DNA in blood or tissues without detectable HBsAg, with or without the presence of anti-HBc antibodies or anti-HBs antibodies, out with the preseroconversion window period [17]. Viral load of all NAT yield samples was also determined using real time quantitative PCR.

3. Results

A total of 213 (2.13%) donations (N = 10,015) were found out to be reactive for either of the markers detected by either serology or NAT (both PUA and PUE). Overall prevalence of viral markers was 1.44% for HBV, 0.4% for HCV, 0.25% for HIV, and 5 (0.05%) coinfections (Table 1). Concordant serological and NAT reactive results were found in 153/213 (71.8%) reactive donations.

Thirty-nine cases (18.3%) were found to be serology reactive which when tested in duplicate were found to be consistent with the initial serological results and thus were labelled as “potential seroyields” (repeatedly seroreactive and NAT negative). These included 5 reactive samples for HBsAg, 22 for anti-HCV, and 12 for anti-HIV-1/anti-HIV-2.

Of note, PUE assay detected 2 HBV cases which escaped detection by PUA but were HBsAg reactive (PUA miss). PUE also labelled 5 samples as coinfections (2 HIV-HBV, 3 HCV-HBV) which were identified as monoinfections in older PUA as well as serology. Three HBV and 2 HCV were flagged as coyields in PUE.

4. Discussion

The introduction of improved ID-NAT and serology tests measuring pathogen-specific humoral immune responses in the donor led to safer blood supply [18]. In Indian context, though NAT is not mandated, many blood centres have started NAT as an additional safety layer for better transfusion services [19]. Makroo et al., in their first Indian multicentric study, evaluated ID-NAT and found a NAT yield of 1 in 2622 donations and implicated that the routine NAT along with serological testing would significantly improve the blood safety in India [10]. Studies done previously at our institution with earlier versions of the assay reported yield of 1: 610 and 1: 628 which were higher than the previous studies [20, 21].

The virus-specific NAT yields for PUA were found to be 1 in 668 for HBV, 1 in 10,015 for HCV, and 1 in 2,003 for coinfections, respectively. However, virus-specific NAT yield for PUA was found to be 1 in 3,338 for HBV only. Hence, PUE system was evidently more efficient in detection of HBV and HCV. Tsoi et al. screened 517,072 and 399,326 consecutive donations for HBV by ID-NAT using Ultrio and Ultrio Plus assays, respectively, and reported enhanced detection of HBV after introduction of a more sensitive Ultrio Plus assay [22]. Vermeulen et al. reported similar results in their study on South African donor population [23]. Higher NAT yield in our study indicated the efficient detection of viral genome with the newer PUE system owing to its efficient target capture chemistry and better sensitivity to detect certain HBV genotypes (especially genotype D which is most prevalent in India) [16, 24].

In 9 out of 21 NAT yield cases, viral load could not be quantified. This could be attributed to the small amount of target present and sampling variability consistent with Poisson’s distribution and also to the differences in sensitivities of the assay as there is large variation in limit of detection (LOD) for different strains and genotypes. We assessed the ability of ID-NAT to detect occult HBV infections by qualitative assessment of anti-HBc antibody on NAT yield cases. Out of combined NAT yield of 18 HBV cases, we detected 17 occult

| Infectious marker | Serology and NAT concordant results | Seroyield | Total NAT yield | NAT yield | PUA | PUE | Total |
|-------------------|------------------------------------|-----------|-----------------|-----------|-----|-----|-------|
| HBV               | 124                                | 5         | 15              | 3         | 15  | 144 (1.44%) |
| HCV               | 16                                 | 22        | 1               | 0         | 1   | 39 (0.4%)  |
| HIV               | 13                                 | 12        | 0               | 0         | 0   | 25 (0.25%) |
| Coinfection       | —                                  | —         | 5^a             | 5^a       | 5^a | 5 (0.05%)  |
| Total             | 153                                | 39        | 21              | 3         | 21  | 213 (2.14%)|

^2 HIV/HBV coinfections (2 HBV cases detected only by NAT) and 3 HCV/HBV coinfections (1 HBV and 2 HCV cases detected only by NAT).
Table 2: Detailed serology results and viral load of NAT yields detected by both PUA and PUE assay.

| S. number | Sample ID | UE | Ultrie | Test summary | total samples tested | [0015] |
|-----------|-----------|----|--------|--------------|----------------------|-------|
|           |           |    |        |              |                      |       |
| S. number | Sample ID | UE | Ultrie | Test summary | total samples tested | [0015] |
|-----------|-----------|----|--------|--------------|----------------------|-------|
|           |           |    |        |              |                      |       |
| 1         | 3301      | HBV| NR     | NR          | NR                   | 12 R  |
| 2         | 3404      | HBV| NR     | NR          | NR                   | ND R  |
|            |           |    |        |              |                      |       |
| 3         | 4004      | HIV/HBV| NV     | Anti-HIV    | Anti-HIV             | HBV-ND| Equivocal*|
| 4         | 4278      | HBV| NR     | NR          | NR                   | 68 R  |
| 5         | 4737      | HBV| NR     | NR          | NR                   | 13 R  |
| 6         | 6326      | HBV| NR     | NR          | NR                   | 105 R |
| 7         | 7750      | HIV/HBV| HIV    | Anti-HIV    | Anti-HIV             | HBV-35|            |
| 8         | 4863      | HBV| NR     | NR          | NR                   | ND R  |
| 9         | 9020      | HBV| NR     | NR          | NR                   | <10 R |
| 10        | 10267     | HBV| NR     | NR          | NR                   | 20 R  |
| 11        | 4502      | HBV/HCV| HBV    | HBsAg       | HBsAg                | ND R  |
| 12        | 10187     | HCV| NR     | NR          | NR                   | ND R  |
| 13        | 10489     | HBV/HCV| HBV    | HBsAg       | HBsAg                | ND R  |
| 14        | 30765     | HBV| NR     | NR          | NR                   | ND R  |
| 15        | 31956     | HBV| NR     | NR          | NR                   | ND R  |
| 16        | 31257     | HBV| NR     | NR          | NR                   | 110 R |
| 17        | 31317     | HBV| RR & DNR| NR          | NR                   | 35 R  |
| 18        | 32029     | HBV| NR     | NR          | NR                   | 30 R  |
| 19        | 32112     | HBV| NR     | NR          | NR                   | 26 R  |
| 20        | 30992     | HBV| NR     | NR          | NR                   | 12 R  |
| 21        | 30589     | HCV/HBV| HCV    | HCV         | HCV                  | HBV-ND| R R R |

Equivocal (even after repeat testing in duplicate); R: reactive; NR: nonreactive; RR: repeat reactive; DNR: discriminatory nonreactive; ND: not detected; PUE: Procleix Ultrio Elite; PUA: Procleix Ultrio assay.
HBV infections and 1 window period donation which escaped detection by serology. Doda et al. also reported HBV NAT yield of 18 in which 12 cases were OBI and 6 WP (WP yield) donations [25].

We detected one WP HBV infection and 3 WP HCV infections (Table 2). HBV was detected with both assays whereas HCV infections were detected only by PUE. The results were in agreement with the claims of the manufacturer stating that newer PUE has enhanced capability of target capture along with increase in LOD in comparison to the PUA.

OBI is a disparate group of HBV-related conditions with a low level of circulating HBV DNA, and though the infectivity of such donors is a long-known fact, it is impractical to implement routine anti-HBc screening to curtail OBI transmission of such donors is a long-known fact, it is impractical to implement routine anti-HBc screening to curtail OBI transmission. Routine donor follow-up is not possible as most of the donors do not turn up at transfusion facility upon receiving notifications of their TTI status [21]. Thus, it is difficult to ascertain the seroconversion in most of the donors and the transfused recipients as well. The strengthening of screening tests with incorporation of modern testing techniques and rigorous donor selection are the only remaining alternatives for developing nations to increase the blood safety.

5. Limitations

First, a confirmatory test was not performed and donors were notified only on the basis of repeat reactive screening results. Secondly, donor follow-up was not performed in our facility because the reactive donors were referred to respective departments where counselling, confirmatory testing, and management were done. (Unfortunately, results and data of reactive donors after referral from transfusion facility were not available with us.) Moreover, our study population was comprised of mostly replacement donors that may not reflect the situation for voluntary nonremunerated blood donor population.

6. Conclusion

Our study showed that the PUE has enhanced sensitivity (as measured in terms of NAT yields) compared to PUA for HBV and HCV. Since most of the yield cases were of HBV, introduction of PUE ID-NAT screening in India will reduce transmission of HBV. Full automation, reduced workspace, and infrastructure requirements with increased sensitivity and HIV-2 detection are additional advantages of the newer system.

Conflict of Interests

Mr. Abhishek Kumar Maurya is an employee of Hemogenomics Pvt. Ltd., company which markets PROCLEIX systems in India.

### Table 3: Sensitivity of different nucleic acid testing assays used.

| Viral marker | Procleix Ultro assay [11] | Procleix Ultro Elite assay [12] | Quantitative PCR (equipment used) |
|--------------|---------------------------|-------------------------------|----------------------------------|
| HIV-1        | 20.72 copies/mL           | 18.01IU/mL                    | NA                               |
| HIV-2        | NA                        | 10.41IU/mL                    | Not tested                       |
| HCV          | 2.78 IU/mL                | 3.0 IU/mL                     | 18 IU/mL (Roche Cobas TaqMan Test) [13] |
| HBV          | 7.46 IU/mL                | 4.3 IU/mL                     | 6.40 IU/mL (Abbott real time PCR m2000) [14] |

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Authors’ Contribution

Conception, design, and interpretation were performed by Dr. Rahul Chaurasia. Drafting of paper was done by Dr. Dipranjan Rout and Dr. Shamsuz Zaman. Acquisition of data was done by Mr. Abhishek Kumar Maurya. Critical revision and final approval of the paper were done by Dr. Kabita Chatterjee and Dr. Hem Chandra Pandey.

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