Original Article

Evaluation of the Procleix Ultrio Plus ID NAT assay for detection of HIV 1, HBV and HCV in blood donors

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Abstract:

Introduction: The Procleix Ultrio Plusassay is a new-generation qualitative *in vitro* nucleic acid amplification test used to screen for human immunodeficiency virus type 1 (HIV-1) RNA, hepatitis C virus (HCV) RNA and hepatitis B virus (HBV) DNA in blood donors. This study was performed to compare the Procleix Ultrio assay with the new-generation Procleix Ultrio Plus Nucleic Acid Test (NAT) assays. **Materials and Methods:** Ten thousand three hundred and two donor samples were run in parallel for ID NAT using the Procleix Ultrio and the Procleix Ultrio Plus assay. Simultaneously, enzyme-linked immunosorbent assay testing was performed on an EVOLIS Walk away System for HIV, HCV, HBsAg and anti-HBc. Reactive samples were confirmed using polymerase chain reaction. **Results:** In the 10,302 samples tested during the study period, we identified 15 NAT yields, and all these revealed HBV DNA in the discriminatory assays. Eight of these were exclusive yields from the Ultrio Plus assay and the remaining seven cases were determined as HBV NAT yield, both by the Procleix Ultrio as well as the Ultrio Plus assays, i.e. "Combined" yields. No HCV or HIV 1 yields were detected during the study period by either of two assays. **Conclusion:** With an overall yield rate of 1 in 687 and an exclusive yield rate of 1 in 1287, the Procleix Ultrio Plus assay proved to be highly sensitive in detecting occult HBV infections.

Key words:

Discriminatory assay, NAT yield, Procleix Ultrio Plus

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Introduction

Transfusion of blood and blood components is associated with a risk of transfusion-transmissible infections, especially viral infections like Hepatitis B, Hepatitis C and acquired immunodeficiency syndrome.[1-3]

In spite of mandatory testing of donated blood for HBsAg, Hepatitis B still remains the most common transfusion-transmitted infection in India.[4] The spectrum of hepatitis B virus (HBV) test results is varied, with frank positivity for HBsAg enzyme-linked immunosorbent assay (ELISA), anti-HBc and HBV DNA at one end to cases with only anti-HBc positivity on ELISA with or without detectable HBV DNA at the other end. A window period or occult hepatitis B virus infections (OBI) are not infrequent considering the fact that India lies in the intermediate zone for HBV endemicity.[5] Problems with low or fluctuating viral loads also result in diagnostic dilemmas.[6] There is, thus, a growing need for a more sensitive test for detecting these infections.

The Procleix Ultrio Plus Assay (Novartis Diagnostics, Emeryville, CA, USA) is a new-generation qualitative *in vitro* nucleic acid amplification test used to screen for human immunodeficiency virus type 1 (HIV-1) RNA, hepatitis C virus (HCV) RNA and HBV DNA in blood donors.

This study was performed to compare the performance of the Procleix Ultrio Nucleic Acid Test (NAT) assay with the new-generation Procleix Ultrio Plus assays in detection of HIV 1, HBV and HCV in blood donors.

Materials and Methods

The study was performed at the Department of Transfusion Medicine, Indraprastha Apollo Hospitals, New Delhi, India, from April 2012 to April 2013. A total of 10,302 donor samples were run in parallel for ID NAT using the Procleix Ultrio and the Procleix Ultrio Plus assays. Simultaneously, ELISA testing was performed on an EVOLIS Walk away System (Bio-Rad, Hercules, CA, USA) for HIV (Genscreen Ultra, 4th generation kits, Bio-Rad, Hercules, CA, USA), HCV (Qualisa, Qualpro Diagnostics, Goa, India), HBsAg (Monolisa, Bio-Rad, Hercules, CA, USA) and anti-HBc (IgG + IgM, Hepanostika, BioMerieux, Durham, NC). All initial NAT-reactive samples (either by the Ultrio or the Ultrio Plus assays, or by both) that were negative for anti-HIV, anti-HCV and HBsAg by ELISA were retested in triplicate and discriminatory assays for HIV 1, HCV and HBV were performed. Reactive

samples were confirmed using corresponding polymerase chain reaction (PCR) (Cobas Taqmam; Roche Diagnostics, Indianapolis, IN, USA).

NAT yields

All cases that were negative for anti-HIV, anti-HCV and HBsAg by ELISA but positive in the ID NAT test were earmarked as NAT yields. Depending on which ID NAT assay (Procleix Ultrio/Ultrio Plus) was positive, these yields were further categorized into:

1. Exclusive ULTRIO PLUS yield.
2. Combined (ULTRIO + ULTRIO PLUS) yield.
3. Exclusive ULTRIO yield.

Results

A total of 10,302 samples were tested during the study period, from which 15 NAT yields were identified. Eight of these were Exclusive ULTRIO PLUS yields, while the remaining seven were identified both by the Procleix Ultrio as well as the Ultrio Plus NAT assays and were therefore labeled as "Combined (ULTRIO + ULTRIO PLUS) NAT yields". Subsequent discriminatory NAT assays revealed HBV DNA in all the 15 cases.

HBV DNA was also detected by PCR in six of the eight Exclusive ULTRIO PLUS yields, while anti-HBc antibodies were found to be reactive in seven of these eight cases.

All the seven "Combined" yields were reactive for anti-HBc antibodies, and five of them were confirmed to be bearing HBV DNA by PCR.

The overall yield rate observed was 1 in 687 and the exclusive Ultrio Plus yield rate was 1 in 1287.

No exclusive ULTRIO yields, HCV or HIV 1 yields were detected during the study period.

Discussion

Although advanced testing techniques are now becoming available and are being adopted at many centers, the risk of contracting transfusion-transmitted infections after transfusion of blood or components still persists. Hepatitis B is an important transfusion-transmitted infection, the diagnosis of which largely relies on the detection of HBsAg.[7]

India is in the intermediate zone of HBV endemicity, with HBsAg prevalence among the general population ranging from 2% to 8%.[5,8]

OBI is defined *as the presence of HBV DNA in blood or liver tissues without detectable hepatitis B surface antigen (HBsAg), with or without anti-HBV antibodies*.[1] OBI can be further classified as "*seropositive*," indicating patients who are positive for antibodies to hepatitis B core antigen (anti-HBc), with or without concomitant antibody to hepatitis B surface antigen (anti-HBs), or "*seronegative*," to denote those who are negative for both anti-HBc and anti-HBs.[9]

All the seven combined HBV yields and seven of eight exclusive Ultrio Plus HBV yields in our study were reactive for anti-HBcAb by ELISA, indicative of seropositive OBI. One case, where both HBsAg and anti-Hbc Ab were not detected by ELISA but HBV DNA was detected by NAT, could either represent a "First Window Period" HBV infection, i.e. immediate post exposure period when HBsAg has not yet appeared or "Chronic occult persistent" HBV infection that occurs in the late stages of HBV infection after disappearance of HBsAg and very low or undetectable titers of anti-HBc antibodies in the donor.

The main characteristic of occult infection is the low level of HBV DNA detected in the blood and in the liver tissue.[10] In four of the 15 yields identified in our study, HBV DNA was not detected by PCR but was detected by discriminatory NAT assays. This is indicative of the fluctuating viral loads of HBV in these cases and hence justifies the need of sensitive tests for its detection.

Conclusion

With an overall yield rate of 1 in 687 and an exclusive Ultrio Plus yield rate of 1 in 1287, the Procleix Ultrio Plus assay proved to be highly sensitive in detecting OBIs. Its incorporation in the blood screening protocol in place of the Procleix Ultrio assay may go a long way in interdicting HBV infections, the burden of which is still very high in India.

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