Nucleic Acid Amplification Test (NAT), an extra layer of safety being practiced at Surat Raktadan Kendra and Research Centre

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Background

- Nucleic acid Amplification testing (NAT) is able to detect viruses during the ‘window period’, which is not possible by ELISA.
- This allows earlier detection of infection and further decreases the possibility of transmission via transfusion.
- NAT is more crucial for those recipients who need regular blood transfusions like Thalassemia and Sickle cell anemia patients.

Semi automated system for Minipool of 10 units

- In April 2013, we started donor mini-pool nucleic acid testing (MP-NAT) at our blood bank with commercially available viral detection kits.

- This test detects HIV-1, HBV and HCV in sample of blood donors. We assessed the impact of NAT in preventing transfusion associated transmission of viruses among the seronegative donors in and around Surat.

Methods

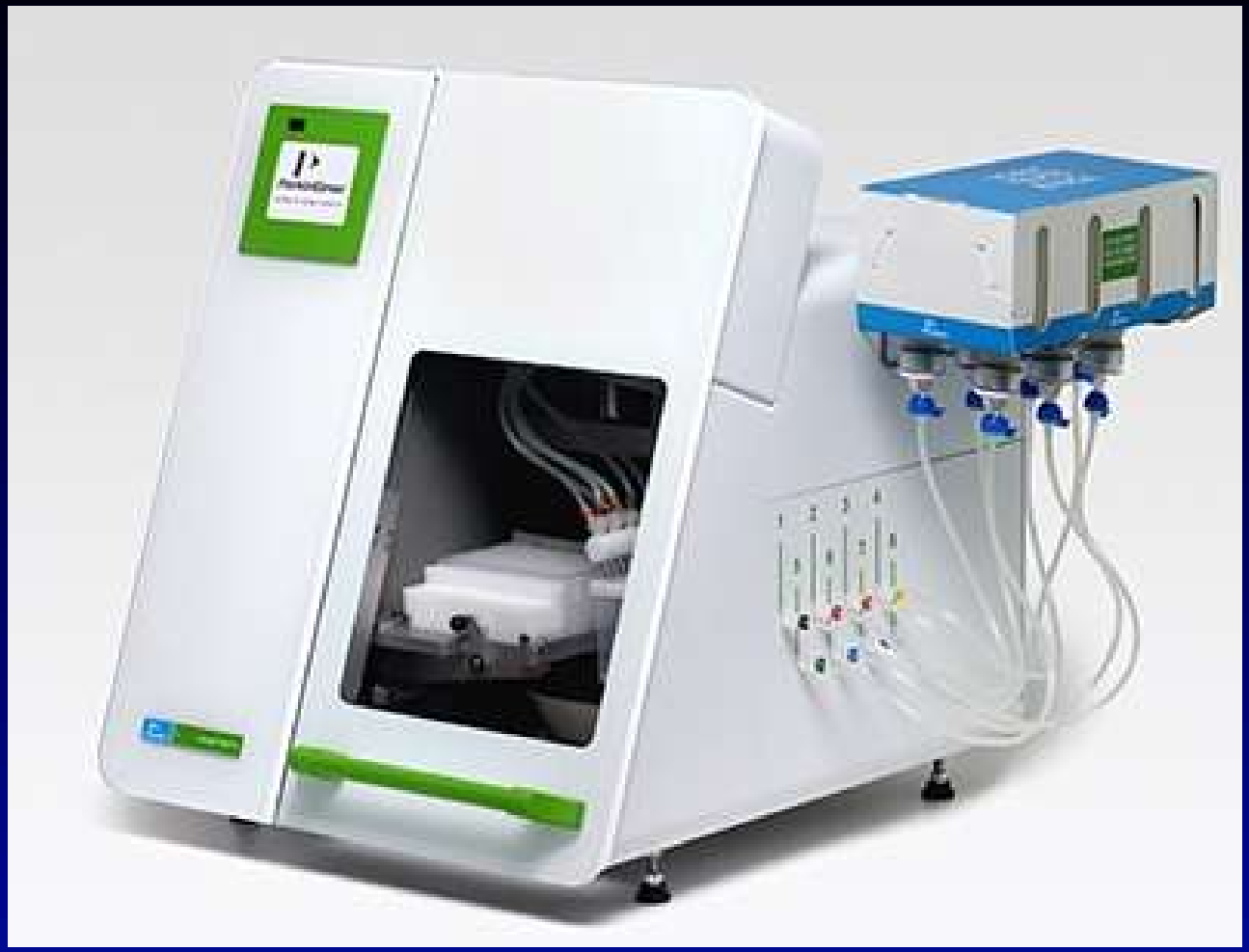
The 3 main steps of our semi automated NAT system are;

- Pooling of Seronegative plasma samples
- Viral DNA/RNA Extraction
- RT-PCR and Detection system

Step-1: Pooling

- Seronegative plasma samples were pooled to a maximum of ten and positive results were resolved through individual NAT testing.

- Pools were then subjected to Viral DNA / RNA Extraction.

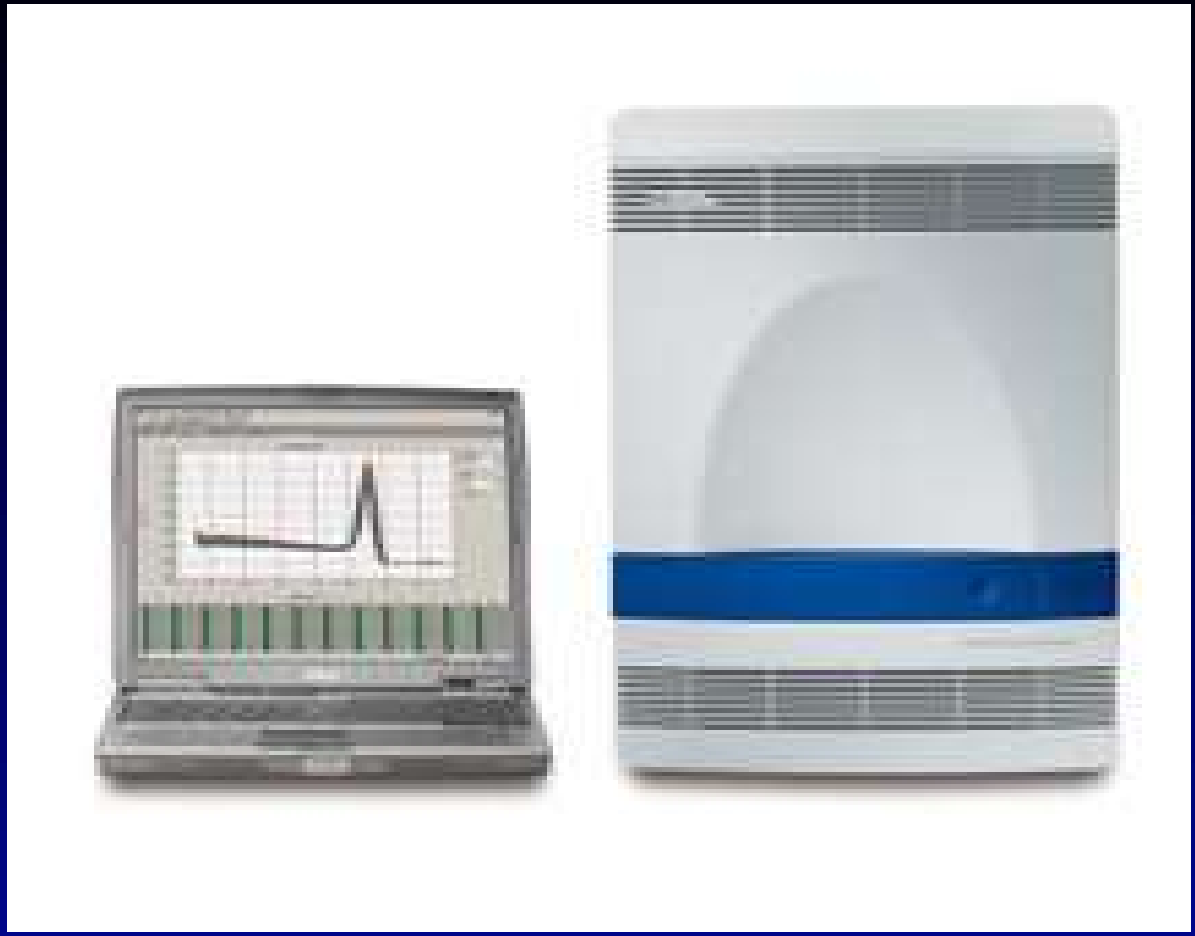


Chemagic Prepito-D

Step-2: Extraction of Viral DNA/RNA

- Pools were first subjected to pre-treatment and further extraction was done using Chemagic Prepito® -D automated extractor (Perkin Elmer, Germany), using reagents of the Prepito Viral DNA/RNA 1k-Kit.

- At the end of the run, extracted viral DNA/RNAs were collected and further subjected to RT-PCR.



ABI-7500 RT-PCR System

Step-3: RT-PCR system

- Amplification and detection of target sequence was done on the ABI PRISM 7500 RT-PCR instrument.

- We used commercially available viral detection kits (RealStar® HIV/HCV/HBV RT-PCR Kit-1.0, Altona Diagnostics GmbH, Hamburg).

Detection system

- All the RT-PCR kits were validated using WHO standards.
- An internal control (IC) was incorporated in the assay to monitor the extraction, target amplification, and detection processes.
- Positive signals were detected in FAM channel while Internal Control signals captured in JOE channel.

Aims and Objectives

- Detection of the Human immunodeficiency virus (HIV), Hepatitis C virus (HCV) and Hepatitis B (HBV) virus using a semi automated nucleic acid testing method to catch infections at early stage (window period).

- Availability of NAT tested blood units for every recipient at an affordable price.

Results

Table-1: ELISA Reactivity among 1,03,752 blood units

Infections	No. of positive units	Percentage
HIV	76	**0.06%**
HCV	100	**0.08%**
HBV	658	**0.58%**

Table-2: NAT yield

Total Units Tested	Total NAT positive units	Total NAT yield
1,03,752	61	**1 in 1700**

Table-3: NAT yield for three viral infections

Total Units Tested	Infections	No. of NAT positive units	Percentage	Yield
1,03,752	HIV	06	0.005%	**1 in 17,292**
	HCV	02	0.002%	**1 in 51,876**
	HBV	53	0.051%	**1 in 1,957**

Conclusion

- Our semi automated NAT system detected 61 potentially infectious HIV, HCV and HBV cases out of 1,03,752 seronegative units which may otherwise infect 183 individuals.

- Total NAT yield for all three viruses was 1 in 1,700.

- NAT Cost per unit – 400 Rs only.


Thank You