The Egyptian Journal of Hospital Medicine (April 2025) Vol. 99, Page 1539-1543

Implementing NAT for Screening of Donors' Blood: Benefits versus Cost

Noha bassiouny Hassan*[1], Doaa Mohamed Osama Elwasly[2], Heba Nader El-Sayed[1]
Departments of [1]Clinical Pathology, [2]Internal Medicine and Nephrology, Faculty of Medicine, Ain Shams University
***Corresponding author:** Noha Bassiouny Hassan, **Mobile:** (+20) 01010702010, **E-mail:** nohabassiouny@med.asu.edu.eg

ABSTRACT

Background: One of the major risks of blood/blood products transfusion is transmission of infectious agent (TTIs: Transfusion Transmitted Infections).

Objective: Our study aimed to assess nucleic acid testing (NAT) in the Egyptian Central blood bank of Ain Shams University Hospitals in concordance with a highly sensitive technique (Chemiluminescence) and to evaluate the cost/benefit outcomes for this implementation.

Methods: 6750 blood units from donors were tested for viruses (HCV, HBV and HIV) using two assay systems in parallel (COBAS e 601 ROCHE and NAT Individual testing using Procleix® Ultrio Elite Assay). NAT yield and serology yield were calculated.

Results: Total number of positive results by chemiluminescence were 3% including 2.1% positive for HCV, 0.8% positive for HBV and 0.1% positive for HIV. In parallel, the samples were NAT tested resulting in 78 positive samples of which 46.1% were HCV positive, 53.9% were HBV positive but by chemiluminescence only 39 samples were positive and 3 negative samples "NAT yield" that were positive by HBV core Ab (ELISA) (occult HBV), 2% were positive by chemiluminescence only (Serology yield); 80.4% were positive for HCV Ab, 12 % for HBsAg and 7.5% for HIV Ag-Ab. All HIV positive results by serology were negative by NAT and Western blot technique.

Conclusions: NAT is very sensitive and crucial technique in screening for donors' blood, but in developing countries with limited resources other screening protocols can be adopted to diminish the cost with reaching the maximal safety.

Keywords: TTIs, Blood safety, NAT, Chemiluminescence, Occult, Yield.

INTRODUCTION

Providing safe blood and blood products that satisfy the needs of the receiver is the aim of blood transfusions. The spread of infectious agents is one of the main risks associated with transfusions of blood or blood products (TTIs). The three mains viral TTIs linked to blood transfusion are Human immunodeficiency (HIV), hepatitis C & hepatitis B virus (HCV & HBV) [1]. Therefore, lowering the danger of transfusion-transmitted illnesses, limiting the collection of blood from donors who are more at risk, and reducing resource waste are the first steps in proper recruiting for blood safety. The second is how well the pre-donation interview works to weed out potential donors who act in a dangerous way [2].

Over the past thirty years, many assay types have been created for use in blood screening. The most widely used tests are made to identify antigens, antibodies, or the infectious agent's nucleic acid. When choosing assays, it is important to keep in mind that not all assays are appropriate in every circumstance and that each test has limits. Immunoassays (IAs), Enzyme immunoassays (EIAs), Chemiluminescent immunoassays (CLIAs), Haemagglutination (HA)/ particle agglutination (PA) assays, Rapid/simple single-use assays (rapid tests), and Nucleic acid amplification technology (NAT) assays are the primary assay types used for blood screening [3].

After infection, the different infection indicators show up at different periods. Depending on the infectious agent, screening marker, and screening method, each TTI has one or more window periods, which can range from a few days to months. Even though a newly infected person may be contagious throughout this time, the specific screening signature is not yet detected in them [4]. Trusting the TTI results at a blood bank is essential. While the foundation of enzyme-linked immunosorbent assay blood screening technology is the identification of serological markers, these markers may not appear in the blood for up to three months after an infection, creating a "window period" during which the risk of a TTI is increased. As the immune response develops, nucleic acid, which is a component of the native infectious agent itself, is the first detectable target to appear, followed within a few days by antigen, and then by antibody. The NAT assay shortens this window of time by directly detecting the presence of the viral RNA or DNA [5].

Nucleic acid testing (NAT) is a molecular screening technique used to reduce the risk of TTIs in receivers of blood donations. The nucleic acid of the infectious agent may be found on individual donations (ID) or mini-pools (MP), and a new method known as multiNAT can concurrently discover several viral DNA and RNA. Based on the amplification of particular areas of viral ribonucleic acid (RNA) or deoxyribonucleic acid (DNA), the NAT approach is very sensitive and specific for viral nucleic acids. In addition to identifying them sooner than other screening techniques, it also resolves erroneous reactive donations using serological techniques, which is important for donor counseling and notification [6].

This study aimed to assess NAT testing in Central blood bank of Ain Shams University Hospitals in concordance with a highly sensitive technique (Chemiluminescence) and to evaluate the cost/benefit outcomes for this implementation.

Received: 30/10/2024
Accepted: 01/01/2025

SUBJECTS AND METHODS

This cross-sectional study was carried out on 6750 blood donors, attending central blood bank of Ain Shams University Hospitals, during the period from March to June 2024 to assess NAT testing in Central blood bank of Ain Shams University Hospitals in concordance with a highly sensitive technique (Chemiluminescence), all samples were tested by both techniques.

Inclusion criteria: Normal blood donors usually meet the requirements for donor selection outlined in the most recent edition of the Egyptian national guidelines.

Exclusion criteria: Samples with aberrant pigmentation (such as hemolysis, lipemia, or icterus).

Donors' blood samples were collected in two Ethylene Diamine Tetra Acetic Acid (EDTA) tubes (3 ml for each) for both serological (CLIA) and Individualized NAT testing.

Chemiluminescence and NAT testing:

All units were tested by Cobas e601 Roch® for anti-HCV II, HB surface antigen (HBsAg), and HIV COMB Antigen-Antibody (The main and routine testing in addition to Syphilis testing). NAT Individual testing using Procleix® Ultrio Elite Assay kits in the Grifols Procleix Panther System was included in parallel with Chemiluminescence. NAT yield and serology yield were calculated for each parameter. Positive results for HIV were tested by Western blot technique in the central laboratories of the Ministry of Health as a confirmatory method. HBV core Ab (ELISA) technique was performed in samples positive by NAT and negative by CLIA for HBV detection.

The analysis for both tests was performed following the recommendations of the manufacturers. Moreover, positive and negative controls were applied before each run and checked according to the Levey Jennings rules.

Ethical approval: The study was approved by Ain Shams University Faculty of Medicine's Ethical Committee [number FWA 000017585]. Additionally, all enrolled individuals provided written, informed permissions. The study adhered to the Helsinki Declaration throughout its execution.

Statistical analysis

SPSS Statistics Version 20.0 was utilized for the analysis of the data. Mean ± SD, median, and range summarized numerical data, which were compared by Student's t-test. Frequencies and percentages described categorical variables, which were compared by Fisher's exact test. A p-value <0.05 was considered significant.

RESULTS

During the period from January to April 2024; EDTA samples from 6750 donor were tested against HCV antibody, HBsAg, and HIV Ag-AB. Total number of positive results by Chemiluminescence were 208 (3%) samples including 141 samples (2.1%) positive for HCV, 57 (0.8%) positive for HBV and 10 (0.1%) for HIV [Figure 1].

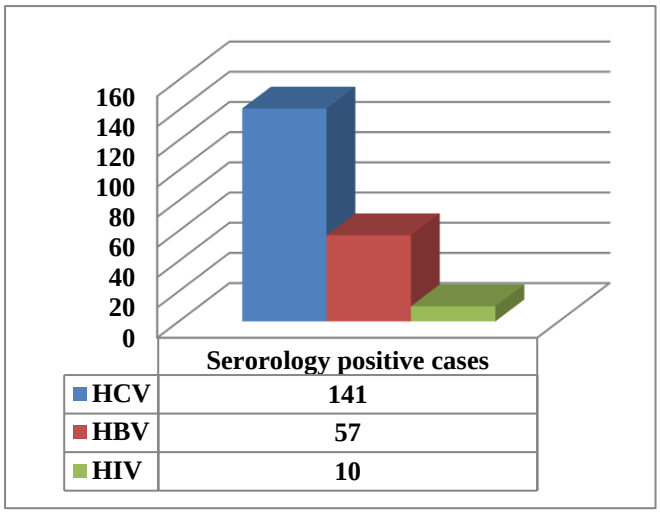

Figure (1): Chemiluminescence positive cases.

Those samples that were positive by NAT and negative by CLIA were tested by HBV core Ab (ELISA) and gave positive results, which meant that the three cases were occult HBV, as they were positive by nucleic acid testing and core Ab and negative for HBsAg. No HIV cases were detected by NAT testing [Figure 2].

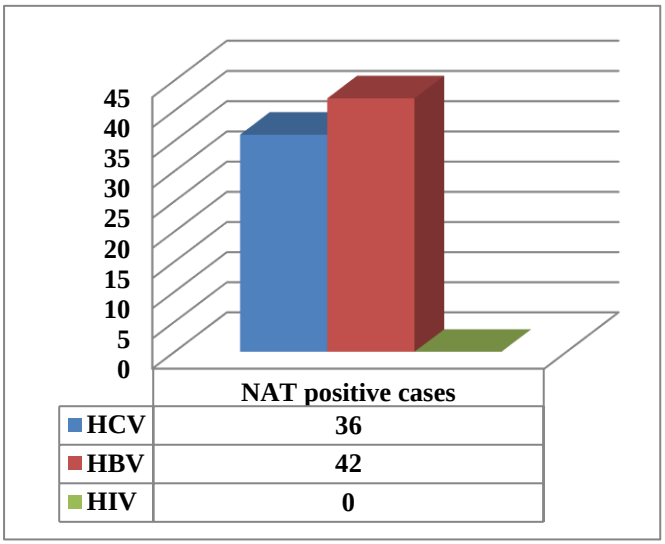

Figure (2): NAT positive cases.

In parallel with the Chemiluminescence, the samples were NAT tested resulting in 78 positive samples most of them were commonly positive with Chemiluminecence. Out of the 78 positive samples; 36 (46.1%) were positive for HCV, all of them were positive by Chemiluminescence. Forty-two (53.9%) were HBV positive but by chemiluminescence only 39 samples were positive and 3 negative samples "NAT yield" [Table 1 and figure 3].

Table (1): Number of positive cases by chemiluminescence and NAT testing

	HCV	HBV	HIV	Total number	Percentage (%) of positive cases/ total number of donors
Serology positive cases	141	57	10	208	3%
NAT positive cases	36	42	0	78	1.2%
Serology Yield	107	16	10	133	2%
NAT Yield	0	3	0	3	0.04%

One hundred thirty-three (2%) out of 6750 donors were positive by chemiluminescence only (Serology yield); 107 (80.4%) were positive for HCV Ab, 16 (12 %) for HBsAg and 10 (7.5%) for HIV Ag-Ab [Table 1 and Figure 3].

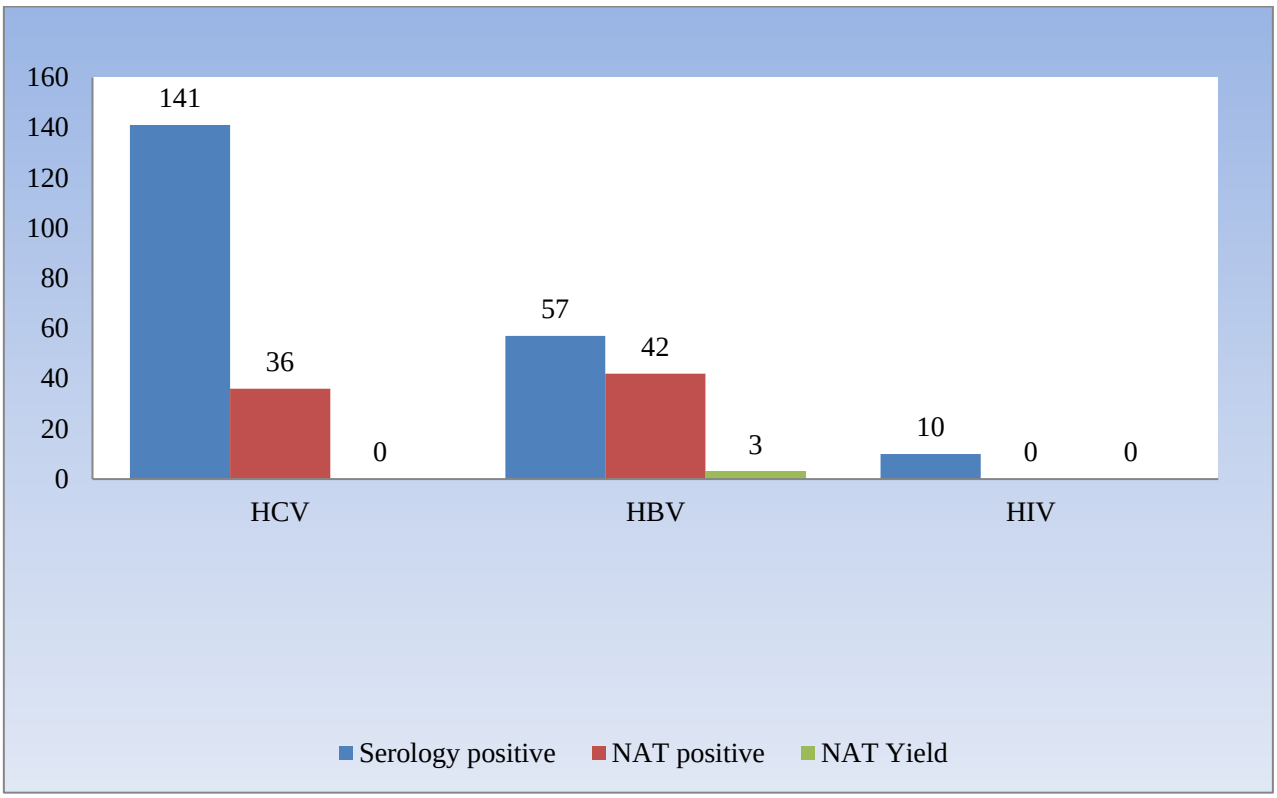

Figure (3): Serology positive, NAT positive and NAT yield numbers.

All HIV positive results by serology were negative by NAT and Western blot technique (confirmatory method for HIV testing) donating that these results were false positive. There were many samples, which were reactive with serology but non-reactive with NAT (Serology yield). These could be false positive due to the high sensitivity of chemiluminescence technology, treated cases or spontaneous clearance of infections.

DISCUSSION

The process of identifying certain TTI markers in donated blood by laboratory screening guarantees that the blood is safe for usage in clinical or industrial settings [3]. Although the detection of serological markers is the foundation of serological tests like ELISA and chemiluminescent blood screening technology, these markers may remain undetected in blood for up to three months following an infection (the window period), during which time the risk of TTI is elevated. The NAT assay was discovered to shorten this window period by detecting viral DNA or RNA, thereby lowering the risk of infection [7].

In our study, individualized NAT was applied simultaneously with chemiluminescent on 6750 donor samples for screening of TTI. It has been disputed in other studies whether pooling samples lowers NAT sensitivity when the amount of each individual sample in a pool decreases. As a result, a pool's sensitivity decreases with the number of samples it contains. Also, the viral burden is relatively low during the window phase and the reproduction rate of HBV is very low, with a typical doubling time of 2–6 days [8]. According to a research comparing the sensitivity of pooled and personalized NAT, individualized NAT is the best technique for TTI screening because pooled NAT missed 67% of samples with low viral loads [9].

Each nation should create a screening approach for TTI that is appropriate to its unique situation. This will be determined by the incidence and prevalence of infection, the capability and infrastructure of blood transfusion services, the costs of screening, and the available resources. Therefore, the objective of our study was to evaluate the importance of implementing NAT testing in central blood bank of Ain Shams University in concordance with chemiluminescence and to evaluate the cost benefit outcomes especially with limited resources.

In our study using chemiluminescent technique, 208/6750 samples were reactive; 0.8% for HBsAg while 2.1% and 0.1% for HCV Ab and HIV Ab respectively. On the other hand, a study done by **Ebeid *et al.*** [10] found that the prevalence of HBsAg, HCV Ab and HIV Ag-Ab were 1.4%, 2.2% and 0.4% respectively. The slight difference between the two studies could be explained by lower number of screened donors and use of different serological technique (ELISA with repetition of reactive samples) in this study. The seroprevalance of HCV in our study using the serological method was 2.1 % (141/6750) that was significantly greater than the infection incidence of 8/2037 (0.5%) reported by **Shah *et al.*** [11] who gathered 2037 blood samples from donors and used the ELISA and fast screening tests to identify HCV infection. For blood transfusion screening, **Ibrahim *et al.*** [12] found that ELISA is more sensitive and specific than chemiluminescence. Nonetheless, they recommended that PCR should be employed as a confirmatory approach due to the grey zone findings.

In our study, NAT was reactive in 78/6750 samples all of them were the same reactivity by chemiluminescence except for three HBV results (NAT yield) that were confirmed by positive HBV core antibody done by ELISA technique. **Ankit *et al.*** [7], stated that the NAT yield was 11/50930, 10 of them were HBV and 1 HIV with no NAT yield with HCV. These results are nearly similar to our results as our NAT yield was 3/6750, where three of them were HBV with no HIV or HCV yield.

In our study the serological yield (positive results by chemiluminescence and negative by NAT) was relatively high 19.7% (133/6750), especially with HCV infection 107/6750 (1.5%). These were approved to be either false positive results owing to the high sensitivity of chemiluminescence technology or due to spontaneous clearance of infections as the WHO organization reported that, in the absence of therapy, about 30% (15–45%) of HCV-infected individuals spontaneously recover the virus within six months of infection. Also, the new era of HCV treatment with Sovaldi may be a cause of presence of HCV antibody with no viremia, although those cases must be excluded from donation, some donors hide the information not to be excluded from donation as unfortunately most of the Egyptian donors are family replacement donations, therefore using a rapid test before donation process will appeal those cases. In our study, HIV (Ag+Ab) screening by chemiluminescence yielded 10 positive cases shown to be false positive results (100 %) as it was confirmed negative by western blot assay and by NAT, so using NAT in those cases could decline the wastage of those components. In our opinion developing countries can use the Western blot technique to substitute the NAT in all positive results given by the chemiluminescence to avoid this wastage.

Regarding NAT testing of HBV, 3 occult samples (3/6750) were found in which NAT positive and negative chemiluminescence. Total HB core antibody was done only for those samples that yielded reactive results as NAT, which is similar to a study done by **Correa *et al.*** [13] with 6 occult cases by NAT (6/45332) with similar results by anti- HB core and recommend adopting anti-HB core test in serological triage to avoid transfusion of blood components with occult hepatitis. They reported that using an analytical sensitive NAT assay is very crucial as the window period of HBV differs according to genotype. In a study done by **Tsoi *et al.*** [14], they found that higher than 10-fold improvement in analytical sensitivity in identifying

HBV genotype B and C strains explains the reported greater than 2-fold enhanced window period NAT yield with the ultrio plus test. Further studies should be done on the association of HB genotype with occult NAT detection comparing different analytical NAT assays in different main Egyptian blood banks.

CONCLUSION

We concluded that although the NAT is very sensitive and crucial technique in screening for donors' blood, but in developing countries with limited resources other screening protocols can be adopted to diminish the cost with reaching the maximal safety. Chemiluminescence is a highly sensitive technique in screening for blood donors as we found no false negative results. Only cases with NAT yield were occult HBV, which can be avoided by implementing the HBV core Ab. Also for the HIV, the usage of Western blot technique can substitute the NAT in developing countries with limited resources in all positive results given by the chemiluminescence to avoid the wastage of donated blood. For the HCV, there were many samples, which were reactive with serology but non-reactive with NAT (Serology yield). These could be false positive due to the high sensitivity of chemiluminescence technology, treated cases or spontaneous clearance of infections. Even though these cases have no viremia but they are still rejected to donate blood by all the international guidelines, so we recommend that more studies should be done to evaluate the safety of this group to donate blood.

Acknowledgment: The authors thank Dr. Wael Mahmoud, the Central Blood Bank manager of Ain Shams University's Faculty of Medicine, for his artwork.

No funding.
No conflict of interest.

REFERENCES

1. **Rizal D (2017):** Transfusion Transmitted Infections in Blood Donors. Journal Fikrah Jilid., 8: 49-61.
2. **Kleinman S, Busch M, Korelitz J *et al.* (1997):** The incidence/window period model and its use to assess the risk of transfusion-transmitted human immunodeficiency virus and hepatitis C virus infection. Transfusion Medicine Review, 11 (3): 155–172.
3. **WHO (2010):** Screening Donated Blood for Transfusion-Transmissible Infections, WHO recommendation. https://www.who.int/publications/i/item/9789241547888
4. **van Hulst M, Sagoe K, Vermande J *et al.* (2008):** Cost-effectiveness of HIV screening of blood donations in Accra (Ghana). Value Health, 11 (5): 809-19.
5. **Shyamala V (2014):** 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., 8: 13-18.
6. **Yang M, Li L, Hung Y *et al.* (2010):** The efficacy of individual-donation and minipool testing to detect low-level hepatitis B virus DNA in Taiwan. Transfusion, 50: 65-74
7. **Ankit M, Sanjana D, Latha J (2017):** A Study of Centralized Individual Donor Nucleic Acid Testing for Transfusion Transmitted Infections to Improve Blood Safety in Karnataka, India. Global Journal of Transfusion Medicine, 2 (1): 24. DOI:10.4103/GJTM.GJTM_8_17
8. **Roth W, Busch M, Schuller A *et al.* (2012):** International survey on NAT testing of blood donations: Expanding implementation and yield from 1999 to 2009. VoxSang., 102: 82-90.
9. **Chigurupati P, Murthy K (2015):** Automated nucleic acid amplification testing in blood banks: An additional layer of blood safety. Asian Journal of Transfusion Science, 9 (1): 9-12.
10. **Ekram Y, Hoda A, Nancy H (2019):** Role of Nucleic Acid Test (NAT) in Detection of Transfusion Transmitted Viruses in Comparison to Other Methods. The Egyptian Journal of Hospital Medicine, 76 (2): 3542-3549.
11. **Shah G, Gurung K, Baral B *et al.* (2018):** Hepatitis B and C Virus Infections among Blood Donors in Blood Transfusion Center, Pokhara, Nepal: Seroprevalence and its Associated Risk Factors. Nepal Journal of Biotechnology, 6 (1): 33-38.
12. **Ibrahim A, Abo-El-Azaem N, Mohamed M *et al.* (2018):** Evaluation of some available HCV antibody detection tests (ELISA, Chemiluminescence, Immune Assay) and RT-PCR assay in the diagnosis of Hepatitis C virus infection. The Egyptian Journal of Hospital Medicine, 72 (7): 4874- 4879.
13. **Corrêa A, Lamarão L, Vieira P *et al.* (2018):** Prevalence, incidence and residual risk of transfusion-transmitted HBV infection before and after the implementation of HBV-NAT in northern Brazil. PLoS One, 13 (12): e0208414. doi: 10.1371/journal.pone.0208414.
14. **Tsoi W, Lelie N, Lin C (2013):** Enhanced detection of hepatitis B virus in Hong Kong blood donors after introduction of a more sensitive transcription-mediated amplification assay. Transfusion, 53: 2477–88.