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Assessment of nucleic acid amplification test in blood donors in Darbhanga medical college and hospital

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Abstract
A nucleic acid test (NAT) or nucleic acid amplification test (NAAT) is a technique utilized to detect a particular nucleic acid, virus, or bacteria which acts as a pathogen in blood, tissue, urine, etc. The NAT system differs from other tests in that it detects genetic materials rather than antigens or antibodies. Detection of genetic materials allows an early diagnosis of a disease because the detection of antigens requires time for antigens to appear in the bloodstream. Hence based on above findings the present study was planned to compare the sensitivity of NAT by using individual test and serological assays by using enzyme immunoassay.
The study was planned in the Department of pathology, Darbhanga Medical College and Hospital, Darbhanga, Bihar from Jan 2018 to Oct 2018. Total 4500 patients have donated the blood and Out of that 60 patients were enrolled in the present study. The blood samples were evaluated for the Nucleic Acid Test (NAT). Approval of the institutional ethical committee was taken prior to conduct of the study. ELISA and NAT reports of HIV, HBV and HCV were collected in a specially designed proforma from the blood bank records. Both the tests are routinely done on the donor blood samples.
The data generated from the study presented important data about nuclear acid test (NAT) testing of blood donors. Effectiveness of nucleic acid testing for blood donors screening is a debating area in transfusion medicine. Wide-national study is required to assess the safety and cost-effectiveness of using traditional and NAT testing to screen blood donors.

Keywords: nucleic acid test, Nat, blood donors, HIV

Introduction
A nucleic acid test (NAT) or nucleic acid amplification test (NAAT) is a technique utilized to detect a particular nucleic acid, virus, or bacteria which acts as a pathogen in blood, tissue, urine, etc. The NAT system differs from other tests in that it detects genetic materials rather than antigens or antibodies. Detection of genetic materials allows an early diagnosis of a disease because the detection of antigens requires time for antigens to appear in the bloodstream [1]. Since the amount of a certain genetic material is usually very small, NAT includes an amplification step of the genetic material. There are several ways of amplification including polymerase chain reaction (PCR), strand displacement assay (SDA), or transcription mediated assay (TMA) [2]. Virtually all nucleic acid amplification methods and detection technologies utilize the specificity of Watson-Crick base pairing; single-stranded probe or primer molecules capture DNA or RNA target molecules of complementary strands. Therefore, the design of probe strands is highly significant to raise the sensitivity and specificity of the detection. However, the mutants which forms the genetic basis for a variety of human diseases are usually slightly different from the normal nucleic acids. Often, they are only different in a single base, e.g., insertions, deletions, and single-nucleotide polymorphisms (SNPs). In this case, imperfect probe-target binding can easily occur which results in false-positive outcomes. Much research has been dedicated to achieving single-base specificity.

There are several different kinds of nucleic-acid amplification tests. However, all NAATs are based on the same principles. First, scientists have to figure out the sequence of the nucleic acids they want to identify and make probes that will attach to them. Then, the NAT uses a series of repeated chemical reactions to make numerous copies of the DNA or RNA that doctors are trying to detect. These reactions selectively amplify the signal of the interesting nucleic acids in the test sample so that they are easier to identify.
The process of amplifying bacterial or viral nucleic acids is not in itself the STD test. Instead, once the amount of DNA or RNA has been increased in the sample using PCR or LCR, more conventional tests are used to detect it. These tests usually involve some form of nucleic acid hybridization. In those tests, the sample is probed with an artificially produced complementary strand of DNA or RNA that has been labeled in some way that makes it easy to detect. It may help to picture it as a glow in the dark tag that only sticks to one very specific piece of identifying information.
Nucleic-acid amplification tests are incredibly useful for STD testing. They allow doctors to detect an STD pathogen even when only a very small number of organisms are present. It is this sort of technology that has made it possible to do urine testing for STDs that were previously only detectable by swab.
Furthermore, since nucleic-acid amplification tests are incredibly sensitive to even small amounts of viral DNA, they

are very important for screening the blood supply. These tests make it possible to detect tiny amounts of HIV and other blood-borne pathogens that might otherwise be missed.

There are also non-amplified nucleic acid tests available for certain STDs, such as gonorrhea and chlamydia. Non-amplified nucleic acid hybridization tests are more likely to be used when large amounts of bacterial or viral DNA (or RNA) would be expected to be present, such as in a urethral swab or in a bacterial culture sample. In such circumstances, no amplification is necessary. In these samples, if DNA or RNA is present, it should be present in detectable amounts. Nucleic-acid amplification tests are incredibly sensitive methods of detecting whether a bacteria or virus is present in a biological sample. When it comes to detecting genital herpes in a sore from a person who has symptoms, these tests serve as a viable alternative to a viral culture. Viral cultures can be difficult for some laboratories to perform. Unlike herpes blood tests, a NAT still involves the direct determination of whether a virus is present in the sample rather than looking for anti-herpes antibodies.

Nucleic-acid amplification has also allowed for an expansion of chlamydia and gonorrhea screening around the country. Now such screening can now be done on urine samples instead of requiring a urethral or cervicovaginal swab. It has thus become easy to test large numbers of young men and women for these STDs in a variety of both clinical and non-clinical settings. Collecting urine requires no medical expertise. People are also more likely to be willing to pee in a cup than undergo a genital swab. Researchers have also used nucleic-acid amplification tests to get more information about the extent of the problem of asymptomatic STDs in the United States. Large-scale NAT-based screening programs have been implemented in the military, in urban teenagers, in men who have sex with men, and in other high-risk and low-risk groups. These tests allow for the detection of STDs in the small urine or blood samples that are often taken as part of large research studies on population health [3-5].

Hence based on above findings the present study was planned to compare the sensitivity of NAT by using individual test and serological assays by using enzyme immunoassay.

Methodology

The study was planned in the Department of pathology, Darbhanga Medical College and Hospital, Darbhanga, Bihar from Jan 2018 to Oct 2018. Total 4500 patients have donated the blood and Out of that 60 patients were enrolled in the present study. The blood samples were evaluated for the Nucleic Acid Test (NAT). Approval of the institutional ethical committee was taken prior to conduct of the study. ELISA and NAT reports ofHIV, HBV and HCV were collected in a specialy designed proforma from the blood bank records. Both the tests are routinely done on the donor blood samples.

Following was the inclusion and exclusion criteria for the present study:

Inclusion Criteria: Based on the NACO guidelines all healthy donors donating blood in our blood bank and in the voluntary donation camps conducted by the blood bank.

Exclusion Criteria: Based on the NACO guidelines donors not eligible to donate blood were excluded from the study like-

1. Age group <18 and >60 years.
2. Known cases of HIV, HCV, HBV, syphilis and malaria positive patients.
3. Donors suffering from acute illness, malignancies, cardiac diseases.
4. Females who are pregnant, breast feeding, and during periods.

Results & Discussion

The data from the 60 patients were collected and presented as below. The data were discussed with the already reported literature.

Table 1: Total Positive Cases

	Elisa & Nat	Nat	Elisa	Total
Total Positive Cases	49	6	5	60

ELISA- Enzyme Linked Immuno Sorbant Assay, NAT – Nucleic Acid Testing

Table 2: Distribution of Cases as Per H

	Elisa & Nat	Nat	Elisa	Total
HIV	6	1	2	9
HBV	40	4	1	45
HCV	3	1	2	6
Total	49	6	5	60

HIV- Human Immunodeficiency Virus, HBV- Hepatitis B Virus, HCV- Hepatitis C Virus

NAT data from India are a cause for great concern particularly in the way we are now managing our donors as well as depending only on the 3rd generation serology for the majority of the blood banks in India.

In India under Sec 3(b) of drug and cosmetics act human blood is considered as drug and is regulated by the rules of the act. The inspection and licensing of blood banks in India is covered under this act of the Ministry of Health and Family Welfare. The rules of this act stipulate that before transfusion, mandatory testing should be done of each sample of donor blood for hepatitis B (HBV), HIV, hepatitis C virus (HCV), syphilis and malaria [6].

To overcome this residual risk and to provide an additional layer of safety, evaluation of donor blood by NAT was started in early 1990's in developed countries and was introduced in developing countries in late 1990's. According to Ministry of Health and Family Welfare data [last updated on 2015] in India, there are 2760 licensed blood banks which includes both government and private institutions. According to the study by Ghosh K and Mishra K in 2017 around 58 blood banks have implemented NAT test to screen the donor blood which accounts to 2.1% of the blood banks [6].

NAT detects viral ribonucleic acid (RNA) or deoxyribonucleic acid (DNA) by the amplification method. Early in the course of an infection, NAT detects low levels of viral genetic material present in the blood. NAT is thus able to detect viruses during the 'window period' allowing earlier detection of infection and further decreasing the possibility of transmission via blood transfusion. NAT also detects mutants and occult cases [7-8].

Prevalence of TTI's among donors is considered as surrogate

marker of infections in the general population [9]. In majority of the studies discussed above including our study it was observed that NAT yield was very high for Hep B suggesting high number of occult infection cases and cases in window period in the general population thus suggesting stringent measures has to be taken to screen actual number of Hep B cases in the society, to assess the immunization status and about considering public policy measures to immunize all for Hep B as the donors alone doesn't mirror the exact prevalence since donors below 18, above 60, with other co-morbid conditions are excluded from screening and also female donors are very less.

In countries like India being highly populated and with high incidence rates of TTI's, significant number of donors in window periods can be picked up by NAT. However it cannot alone be used as a screening test, as at times viral load may be of undetectable levels by NAT but antibody response may still be present. And cases of HIV-2 can be missed. Thus highly sensitive serological assays are also required for the safety of the blood for transfusion.

Finally and not in the least, the blood donor interview and evaluation for the positive risk of transmission of viruses should be done in more user-friendly circumstances. Blood camps in India are often very cramped and crowded hence is not suitable for proper donor counseling. Extensive adult vaccination with Hep B virus vaccine has also been suggested [10] to reduce the Hep B viral load in the community. This may be a practical solution in the long run when all vaccinated children becomes adult and enters as voluntary blood donors, but all adults who are healthy cannot be persuaded to take Hep B vaccination at present, and its financial implication could be stupendous.

For low resource countries several challenges, for example, high cost of NAT, requirement of qualified technical staffs, laboratory facilities, reagent procurement, and maintenance of delicate equipment conspires against NAT testing as is used in developed countries. In such a situation, the 4th generation serological test which combines both antigen and antibody in an ELISA format may be a good beginning along with other facets of improving the blood safety [11].

Conclusion

The data generated from the study presented important data about nuclear acid test (NAT) testing of blood donors. Effectiveness of nucleic acid testing for blood donors screening is a debating area in transfusion medicine. Wide-national study is required to assess the safety and cost-effectiveness of using traditional and NAT testing to screen blood donors.

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