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Enhancing Blood Transfusion Safety: A Critical Review of ELISA and Nucleic Acid Testing for Detection of Transfusion-Transmitted Infections

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Abstract

Background: Blood transfusion is a vital therapy in modern health care, but the risk of transfusion-transmitted infections (TTIs) is still a huge challenge despite substantial improvements in donor screening. Conventional serological assays can be negative in early infections, and human immunodeficiency virus (HIV), hepatitis B virus (HBV) and hepatitis C virus (HCV) and other emerging pathogens continue to pose a threat to blood safety during the diagnostic window period. Therefore, the use of highly sensitive diagnostic technologies for better transfusion safety has become an objective of global importance. Objective: This review aims at critically discussing the principles, diagnostic performance, advantages and disadvantages, application and implementation of enzyme-linked immunosorbent assay (ELISA) and nucleic acid testing (NAT) in blood donor screening with a focus on the advantages, limitations, and clinical applications of these tests. Key findings: ELISA is the main method of routine blood donor screening due to its high specificity, low cost, scalability, and compatibility with automated laboratory systems. However, NAT allows for a direct detection of viral DNA or RNA, thus significantly shortening the window period for HIV, HBV and HCV and minimizing the potential of transmission of viral infections. Based on comparative analysis, both NAT and ELISA have their distinct merits and drawbacks, with NAT being more sensitive in terms of being able to detect pathogens earlier and perform analysis, and ELISA being more cost-effective and accessible, particularly in low-resource areas. These assays are recommended for simultaneous use, as this combination seems to be most effective for increasing blood safety (currently there is evidence supporting that). Implementation challenges in LMICs are also discussed and some emerging technologies such as artificial intelligence, CRISPR diagnostics, microfluidic platforms, next-generation sequencing and digital blood management systems are highlighted. Conclusion: A combined molecular and serological screening will increase transfusion safety, early detection of pathogens, and public trust in blood transfusion services. Advances in multiplex molecular diagnostics, automation, digital health technologies and global standardization of blood screening practices will continue to shatter the infection transmission risk associated with transfusion and enhance equitable access to safe blood supplies across the world, in the years ahead.

Keywords: Blood transfusion; Enzyme-linked immunosorbent assay (ELISA); Nucleic acid testing (NAT); Blood donor screening; Transfusion-transmitted infections; Blood safety; Diagnostic window period; Molecular diagnostics.

1. Introduction

Blood transfusion is an essential ingredient in modern healthcare, and plays a crucial role in a variety of clinical settings such as trauma, major surgery, obstetric emergencies, oncologic treatments, organ transplantation, and hematological disorders. Although great progress has been achieved in blood transfusion medicine, the problem of transfusion transmitted infections (TTIs) continues to be a major threat to blood safety on a global scale. When infectious agents are transmitted to the recipient from the donated blood, they can cause serious morbidity, chronic disease or death, it is called as TTIs [1]. These risks have been greatly diminished after the implementation of rigorous donor selection criteria and laboratory screening tests, but they remain a challenge to blood transfusion services when they are transmitted during the early stages of disease, especially the diagnostic window period. The World Health Organization (WHO) estimates that there are around 118.5 million blood donations made each year worldwide, but the quantity and quality of blood screening is much different in high-income and low- and middle-income countries. Contrast the developed world, where complete quality management systems have been established and state-of-the-art molecular screening methods are available, with the vast majority of resource-limited settings that still primarily rely on serological assays for financial and infrastructural reasons [2]. This results in differences in the un-scr. risk of TTIs in various regions due to difference in screening practices. The most important ones for routine donor screening, due to their clinical importance and global distribution, include viral diseases such as human immunodeficiency virus (HIV), hepatitis B virus (HBV) and hepatitis C virus (HCV). The safety of transfusion has become even more complex with the emergence of new pathogens such as hepatitis E virus (HEV), West Nile virus (WNV), dengue virus, Zika virus and other locally occurring infectious viruses. The timely, safe and hygienic availability of blood for donated use requires a thorough donor information collection protocol, which involves donor history assessment, physical examination and laboratory testing [3]. Potential transfusion-transmissible diseases (TTDs) are screened prior to release of blood components for clinical use according to international regulation and national blood transfusion services. Traditional screening tests do, however, have their drawbacks. Persons who donate blood during the seronegative window period or persons with low antigen- or antibody levels may not be caught and the residual risk of transmission may be higher. These restrictions have led to constant efforts to make the tests more sensitive and to shorten the time between infection and detection in the laboratory. The use of serological screening tests, such as enzyme-linked immunosorbent assay (ELISA) has been the mainstay of blood donor testing, as these tests are fast, easy to automate, scalable, and cost-effective and have high analytical specificity. The generations of ELISA have greatly enhanced the diagnostic sensitivity and specificity since the viral antigen and antibody can be detected simultaneously [4]. However, the ELISA is dependent on host immune responses or antigen levels, and is susceptible to false-negative results in early infection. Advances in blood donor screening have been brought about by the introduction of nucleic acid testing (NAT), which allows for the direct identification of the viral DNA or RNA before seroconversion has occurred. Molecular amplification methods like the polymerase chain reaction (PCR) and the transcription-mediated amplification (TMA) make HIV, HBV, and HCV diagnostic window periods significantly shorter, minimizing the risk of infectious donations in the blood supply. Some countries have established NAT as part of the normal blood screening process, either as individual patient testing or as pooling strategies, and have seen a reduction in transfusion risks. However, widespread implementation is still hindered by high operating expenses, complex laboratory facilities, and technical skills needed, and quality assurance issues, especially in resource-poor health care systems [5]. Based on these complementary characteristics, it is important to keep in mind that ELISA and NAT are not competing technologies but are complementary to a comprehensive blood safety strategy. This review critically assesses the principles, diagnostic performance, clinical use, strengths and limitations of ELISA- and NAT-based screening strategies for the detection of transfusion transmitted infections. In addition, it reviews current implementation approaches, their effectiveness in various healthcare systems and explores potential barriers to routine implementation and summarizes new technologies that will influence future blood donor screening and transfusion safety.

2. Transfusion-Transmitted Infections (TTIs): Current Landscape

Despite significant progress in donor selection, laboratory testing and quality assurance systems, transfusion-transmitted infections (TTIs) continue to be one of the most important transfusion medicine challenges. Infectious pathogens that are found in donated blood can cause TTIs when the blood or blood products are given to the recipient. There are multiple factors affecting the risk of transmission, such as prevalence of the pathogen in the donor population, when the donor was infected at the time of donation, the pathogen's survival during blood storage, and the sensitivity of screening assays. Effortful screening programs have dramatically decreased the prevalence of TTIs in the developed world, but in many low- and middle-income countries infectious diseases are more prevalent, and access to advanced screening technologies is less [6].

2.1 Major Viral TTIs

Despite their high infectivity, extended latent period and capability of developing chronic disease, viruses continue to be the most common cause of transfusion transmitted infection (TTI). The three most important viral pathogens targeted in blood donor screening programs are human immunodeficiency virus (HIV), hepatitis B virus (HBV), and hepatitis C virus (HCV). HIV is a progressive immune deficiency disease that eventually develops into acquired immune deficiency syndrome (AIDS) if not treated. HBV is one of the most prevalent causes of chronic hepatitis, liver cirrhosis and hepatocellular carcinoma globally and HCV is a leading cause of chronic liver disease and liver-related death. These viruses have a diagnostic window period in early infection, so highly sensitive serological and molecular assays are necessary to reduce the residual transfusion risk. Human T-cell lymphotropic virus types 1 and 2 (HTLV-1/2) are less common in the world and are found in specific areas like parts of Japan, the Caribbean, South America, and Africa. Adult T-cell leukemia/lymphoma (ATL) and HTLV-associated myelopathy (HAM) have been linked to HTLV infection. As a result, some countries have begun to screen all blood donors for HTLV, mainly in high prevalence areas [7].

2.2 Emerging TTIs

Several pathogens have emerged to become transfusion relevant pathogens due to global travel, climate change, urbanisation and changing vector distribution. Hepatitis E virus (HEV) has been drawing increasing interest due to asymptomatic carriers being able to transmit infectious viral particles that can result in serious infection in immunocompromised persons and pregnant women. Likewise, viruses transmitted by mosquitoes, including West Nile virus (WNV), the dengue virus, Zika virus, and chikungunya virus, have been shown to have the potential to be transmitted by transfusion in outbreak settings. Although importantly, the risk of transmission of cytomegalovirus (CMV) has been greatly diminished in many healthcare settings by the use of leukocyte reduction and CMV-seronegative blood components, CMV infection remains clinically important, especially in neonates, transplant patients and the other immunocompromised. The appearance of these pathogens highlights the need for flexible screening methods that can address changing epidemiological threats [8].

2.3 Bacterial, Parasitic, and Fungal Infections

While the focus is on viruses, bacterial and parasitic pathogens, and occasionally fungal, are also affecting blood safety, and they are not the most talked about. For a long time, Treponema pallidum, the causative bacteria of syphilis, has been used as an important indicator of donor eligibility and blood quality. The most common cause of transfusion-associated septic reactions, especially for platelet concentrates stored at room temperature, is bacterial contamination. Infection with parasites is a major issue in endemic areas of the world, such as Plasmodium spp. causing malaria, and in North America, Babesia spp. is a growing cause of transfusion-transmitted parasitosis. The risk of transfusion in Latin America and in countries not endemic to the disease with migration is posed by the etiological agent of Chagas disease, Trypanosoma cruzi. In addition, some of the other pathogens found in the region that could pose a threat to transfusion safety do so only in certain epidemiological situations, such as Leishmania species and some opportunistic fungi. In fact, monitoring and implementing a risk-based screening policy is crucial to guarantee microbiological safety of blood globally [9].

Table 1. Common transfusion-transmitted pathogens, their prevalence, routes of transmission, and routine screening methods.

Pathogen	Global prevalence/risk	Transfusion transmission	Routine screening method
Human immunodeficiency virus (HIV-1/2)	Worldwide	Cellular blood components and plasma	ELISA (antigen/antibody), NAT [10]
Hepatitis B virus (HBV)	High global burden, especially Asia and Africa	Whole blood, plasma, cellular components	HBsAg ELISA, anti-HBc (where applicable), NAT
Hepatitis C virus (HCV)	Worldwide	Blood and blood components	Anti-HCV ELISA, NAT [11]
Human T-cell lymphotropic virus (HTLV-1/2)	Endemic in selected regions	Cellular blood components	Antibody ELISA/CLIA with confirmatory testing
Hepatitis E virus (HEV)	Increasing in Europe and Asia	Plasma and blood components	NAT (selected countries)
West Nile virus (WNV)	Seasonal outbreaks	Blood components	NAT during outbreak seasons [12]
Dengue virus	Tropical and subtropical regions	Blood components	NAT, NS1 antigen, serology (selected settings)
Zika virus	Endemic and epidemic regions	Blood components	NAT where indicated
Chikungunya virus	Tropical regions	Rare but documented	NAT during outbreaks
Cytomegalovirus (CMV)	Worldwide	Leukocyte-containing blood products	CMV serology, leukoreduction, CMV-negative units [13]
Treponema pallidum (Syphilis)	Worldwide	Rare with modern blood storage	Treponemal/non-treponemal serological tests
Plasmodium spp. (Malaria)	Malaria-endemic countries	Red blood cell transfusion	Donor deferral, microscopy, rapid diagnostic tests, NAT (limited use)
Babesia spp.	Mainly North America	Red blood cell transfusion	NAT and antibody testing in endemic regions [14]
Trypanosoma cruzi	Latin America; imported cases elsewhere	Blood transfusion	Serological screening, NAT (limited use)

3. Blood Donor Screening Strategies

Security of blood transfusion depends upon a complete donor screening strategy that consists of donor selection, clinical evaluation, and laboratory tests that seek to minimize the threat of transfusion transmitted infections (TTIs). Today, multiple layers are used in blood transfusion services with every screening as an independent protection against blood transfusion infected with infection. This well established process starts prior to blood donation and extends through to laboratory confirmation that donated blood is suitable for clinical use.

3.1 Donor Selection

Donor selection is the front line in fighting against TTIs. The eligibility criteria for blood donors are determined as per the National and International criteria laid down by the World Health Organisation (WHO), American Association of Blood Banks (AABB) and the National Regulatory authorities. The criteria for selection normally are age, body weight, hemoglobin concentration, blood pressure, pulse rate, medical fitness and donation interval. Those who have had a history of high-risk activities, recent exposure to potentially infectious diseases, recent blood transfusion, organ transplant or travel to areas where certain diseases are endemic may be deferred temporarily or permanently. The selection of appropriate donors greatly minimizes the risk of collecting blood from people during the time when they are likely to be infected and also ensures safety of the donor [15].

3.2 Medical History Assessment

A standard donor health questionnaire is part of the pre-donation screening process. A questionnaire is designed to uncover predispositions for transfusion transmitted infections from a behavioral, occupational, travel and medical perspective. Previous history of infectious diseases, fever and illness in last two weeks, use of medication, surgical problems, tattooing or body piercing, use of IV drugs, high risk sexual activity, pregnancy history, and recent vaccinations are all questioned. Confidential self-reporting together with medical assessment by professionals ensures that the risk assessment of a donor is as accurate as possible, and adds to laboratory testing to detect any possible infections that might not be identified by diagnostic assays [16].

3.3 Laboratory Screening Workflow

After eligibility screening, the blood is required to be tested in the lab to detect major TTIs before blood components can be issued for clinical use. Despite the development of other serological tests, enzyme-linked immunosorbent assay (ELISA) continues to be the mainstay of routine testing, due to its very high sensitivity, specificity, cost-effectiveness, and ability to be tested in a high throughput format. To further lower the risk of transmitting HIV, hepatitis B virus (HBV) and hepatitis C virus (HCV), blood centers have begun to use nucleic acid testing (NAT) as another screening method to identify nucleic acids of these viruses in the diagnostic window period. Confirmatory testing of reactive samples and strict adherence to SOP are a part of quality control in laboratory practice. Non-reactive blood units are approved for clinical use, while reactive or indeterminate units are sent back to the blood bank and donors are advised based on institutional or regulatory requirements. Today's blood safety programmes are based on the combination of donor assessment and additional serological and molecular testing [17].

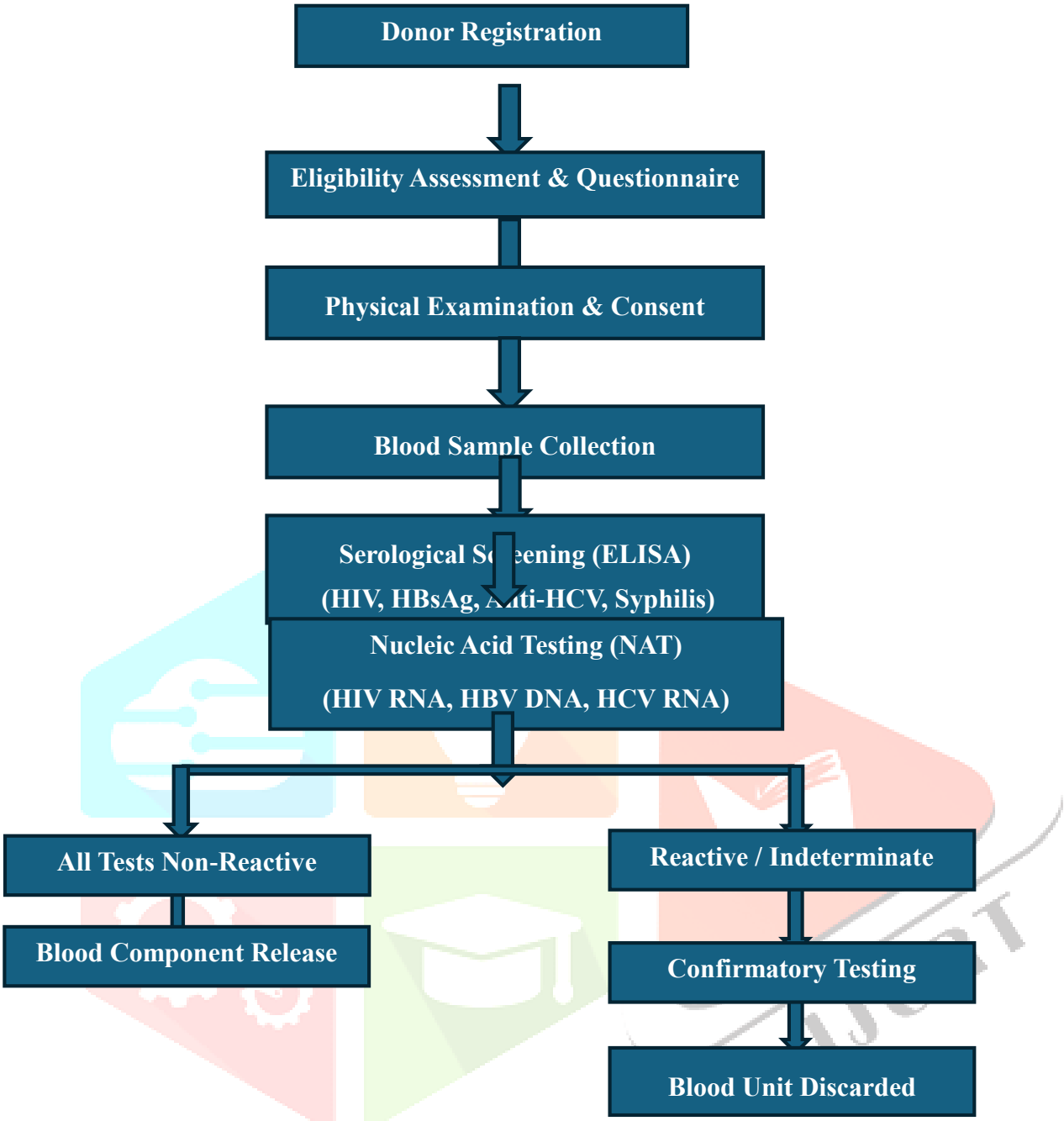

Figure 1. General workflow of blood donor screening in transfusion services

4. ELISA-Based Screening

One of the most used serological methods for the screening of transfusion-transmitted infections (TTIs) in blood donors is the enzyme-linked immunosorbent assay (ELISA). In the 1970s, ELISA was introduced and has been used as the basis of routine blood donor tests because of its high analytical sensitivity, specificity and its ability to be easily scaled up and interfaced with automated laboratory systems. The method can be used for the qualitative or quantitative identification of the antigens or antibodies of a pathogen, e.g., in the serum or plasma of a donor, and is widely used to screen for infections like human immunodeficiency virus (HIV), hepatitis B virus (HBV), hepatitis C virus (HCV), human T-cell lymphotropic virus (HTLV), and Treponema pallidum (syphilis). Nucleic acid testing (NAT) has contributed to superior pathogen detection in the early phase, but ELISA is essential due to its cost-effectiveness, feasibility and its ability to process a huge number of samples in blood centers around the world.

4.1 Principle of ELISA

The principle of ELISA is the very specific reaction between antigen and antibody. It is based on the use of enzyme labelled antibodies or antigens to produce a measurable colourimetric/fluorescent/chemiluminescent response which is proportional to the concentration of the target analyte. In the assay, one of the antigen or antibody is attached to the surface of a microtiter plate. Once the patient (or donor) sample is introduced, certain antigen-antibody complexes will be generated if the target molecule is present. After incubation, the excess unbound components are washed away to reduce background interference and increase the specificity of the assay. Detection system is based on enzymes like horseradish peroxidase (HRP) or alkaline phosphatase (ALP) attached to antibodies/antigens [18]. The enzyme causes a chemical reaction after the addition of a suitable chromogenic substrate resulting in the formation of a coloured product. The intensity of the colour is spectrophotometrically measured and it is directly proportional to the amount of antigen or antibody present in the sample, usually at wavelengths from 450 nm to 620 nm. Some modern ELISAs can also use fluorogenic or chemiluminescent substrates to enhance analytical sensitivity and extend the range of detection. ELISA is used to detect viral antigens (eg hepatitis B surface antigen [HBsAg] or HIV p24 antigen) in the blood of blood donors, or host antibodies against infectious agents. The fourth generation assays have been able to detect HIV antigen p24 as well as anti-HIV antibody, which has significantly improved the diagnostic performance of these assays, compared with the previous generations, thus reducing the serological window period. The same improvements have been seen for HBV and HCV, with improved antigen detection and optimal recombinant antigen technologies. There are various factors that can affect the performance of ELISA, such as the quality of the antigen, the affinity of the antibody, the design of the assay, the incubation conditions, the efficiency of washing, and the quality control measures. Under laboratory conditions, ELISA is a very accurate analytical method, but its usefulness in detecting the infection is restricted in the early stages of infection when there are not measurable levels of antibodies or detectable amounts of antigen in the bloodstream. This is why nucleic acid testing is often used in combination with ELISA in modern blood testing algorithms to ensure the utmost safety of blood transfusion [19].

4.2 Types of ELISA

There are a number of formats for the ELISA that have been developed to suit a variety of diagnostic uses. Each format has the same immunological principle, but it is configured differently and is detected in various ways, has different analytical properties and is used in different types of laboratory applications.

Direct ELISA

In the simplest assay format, the target antigen is attached directly onto the microplate surface, and is then detected by a single primary antibody that has been conjugated to an enzyme (Direct ELISA). The antibody procedures require only one antibody, which limits the assay time and decreases the number of incubation and washing steps, therefore making the procedure relatively quick and simple. Direct ELISA, on the other hand, is less commonly used in routine blood donor testing because of its poorer analytical sensitivity, which is because it does not involve signal amplification [20].

Indirect ELISA

In indirect ELISA, an antibody which recognizes the target antigen is used first and then an enzyme labelled antibody that recognizes the first antibody is used. This is a dual antibody that can give higher sensitivity than direct ELISA. Indirect ELISA is widely used for the detection of pathogen-specific antibodies present in donor serum such as anti-HIV, anti-HCV and anti-HTLV antibodies. The assay is economical as one enzyme-labeled secondary antibody can be used with many different primary antibodies, but there is the potential for non-specific binding of secondary antibody, which can sometimes result in a higher background reactivity [21].

Sandwich ELISA

Sandwich ELISA is regarded as one of the most sensitive and specific ELISA formats. A capture antibody fixed to the microplate is used to bind the target antigen, which is then detected by a second antibody that binds to the target antigen at a different epitope with an enzyme. This double recognition system significantly improves the analytical specificity and decreases interference from non specific proteins in clinical samples. Sandwich ELISA is used extensively for the detection of viral antigen, such as HBsAg and HIV p24 antigen and hence is very useful in blood donor screening program [22].

Competitive ELISA

Competitive ELISA is used for the detection of small antigens or analytes with one antigenic determinant or epitope. This assay involves the presence of the antigen in the donor sample competing with a labelled reference antigen in a limited binding site. Therefore, the amount of signal is inversely proportional to the concentration of target analyte. While competitive ELISA is very useful in the determination of hormones, toxins and some therapeutic drugs, it is not widely used for routine screening for transfusion transmitted infections (TTIs), as indirect and sandwich ELISA formats are usually more effective in the detection of infectious diseases. The choice of the form of the ELISA to be used in a diagnostic application depends on the diagnostic goal, the nature of the target antigen, the assay sensitivity desired, the laboratory instrumentation available, and cost. The serological tests most commonly used in blood transfusion services are fourth generation, sandwich and indirect ELISAs, which offer an ideal combination of analytical sensitivity, specificity, automation and high throughput processing of samples. However, even with the constant advances in technology there is always a residual risk of infection during the serological window period that cannot be fully excluded with ELISA. This restriction has led to the use of nucleic acid testing as an ancillary testing method for many national blood transfusion programs [23].

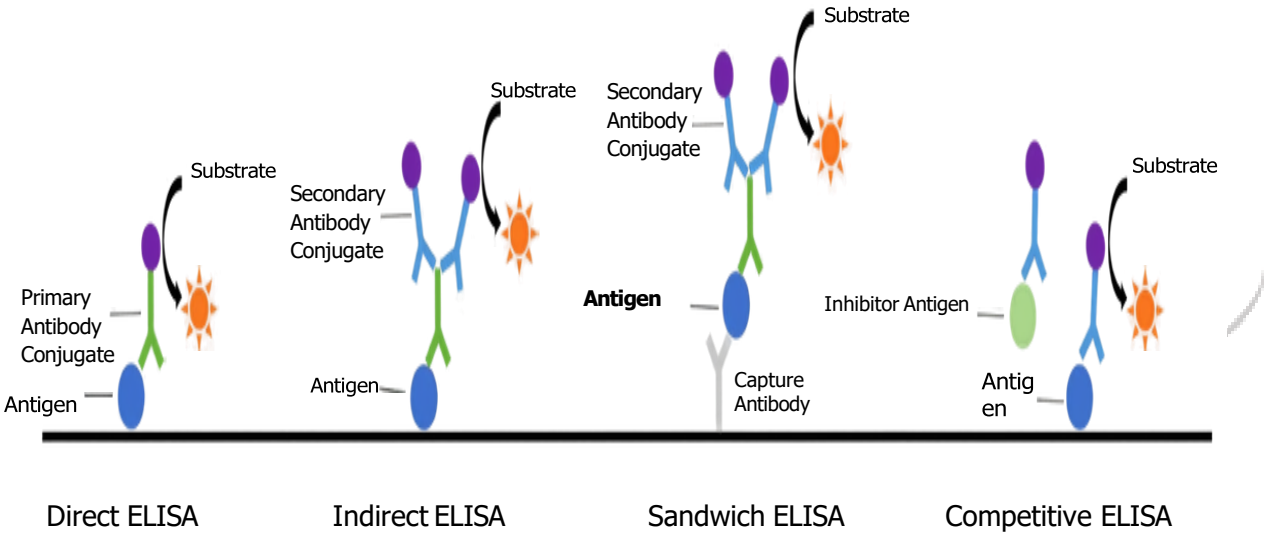

Figure 2. Schematic representation of the four major ELISA formats used in immunodiagnostics: (A) Direct ELISA, (B) Indirect ELISA, (C) Sandwich ELISA, and (D) Competitive ELISA. Each format differs in antibody configuration, detection strategy, and analytical application, with indirect and sandwich ELISA being the most commonly employed for screening transfusion-transmitted infections in blood donor testing.

4.3 ELISA in Blood Banks

ELISA has been the preferred method for serological screening in blood banks and transfusion services because it is reliable, automated and can be readily scaled up to an automated laboratory platform. Major transfusion transmitted infections (TTIs) are tested for before blood components are released for clinical use by most national blood transfusion programs, with ELISA being used to detect them. ELISA can be used to identify specific antigens and antibodies of pathogens, which helps to reduce the risk of infectious blood being introduced into the blood transfusion chain and plays a role in ensuring the safety of the transfusion chain. ELISA is one of the main uses in the diagnosis of human immunodeficiency virus (HIV) infection. New generation ELISA includes HIV-1/2 antibody and HIV-1/2 antigen assays, allowing the detection of HIV-1/2 antibodies and antigen (p24) simultaneously, which means earlier detection of HIV than antibody-based assays, and thus shorter serological window period. Due to their high sensitivity and specificity, these assays have become the most common screening assay used in blood banks. However, the use of hepatitis B virus (HBV) ELISA is primarily to detect hepatitis B surface antigen (HBsAg) which is the earliest regularly detectable serological marker of active infection. Some blood transfusion services test for other markers, including antibodies to hepatitis B core antigen

(anti-HBc) to detect occult HBV infection – especially in areas with a high level of HBV endemicity. However, early detection of HBV infection is difficult as the viral DNA can be detected before the appearance of HBsAg. ELISA also is widely used to detect antibodies against hepatitis C virus (anti-HCV). The current recombinant antigen based assays are highly accurate and significantly have reduced the risk of HCV transmission through blood transfusion. Anti-HCV ELISA, however, does need time after infection to produce antibodies, and because of this, anti-HCV cannot be used to detect donations collected in the early part of infection, so it is important that anti-HCV and nucleic acid testing (NAT) are used in concert. ELISA assays are also used to help screen for bacterial infections like syphilis, which is caused by the bacterium Treponema pallidum. Because of their high level of analytical performance and the increased automation and reproducibility, treponemal ELISA is becoming the preferred blood center test method for diagnosing seroconversion and replacing the traditional non-treponemal screening tests in many centers. Incorporation of automated, ELISA assay blood bank analyzers allows the processing of hundreds of thousands of donor samples per day with the same assay run parameters, under standard conditions, and with reduced operator-dependent variability and adherence to quality regulatory parameters. Thus, ELISA remains the main serological screening tool in developed and resource-limited health care facilities [24].

4.4 Advantages of ELISA

ELISA is used as the basis for routine blood donor screening in most countries of the world because it has several advantages. Cost effectiveness is one of its greatest attributes. ELISA is less expensive than the molecular diagnostic tests, and is suitable for blood transfusion services with limited financial resources. This economic benefit is especially significant in low and middle income countries where rolling out universal molecular testing might not be possible. It also has a high throughput. Modern automated ELISA systems can readily be used to test many samples from donors in the same run with very little manual intervention, thus enabling the efficient screening of a high number of donations in a short amount of time. ELISA is quite easy to be automated. The automation of pipetting, incubation, washing and optical reading will greatly improve the reproducibility of experiments, minimise human error and boost laboratory productivity. Further, integration with the laboratory information systems (LIS) allows for the interpretation, documentation, and traceability of results to be further made easier. Moreover, ELISA has a well-established international protocols and regulatory acceptance. Efforts for standardized operating procedures, commercial quality controlled assay kits, external quality assessment programs have led to consistent analytical performance throughout blood centers around the world. Continued improvements in the design of the assays (recombinant antigens, monoclonal antibodies) have further improved the sensitivity and specificity of the diagnostic tests. These benefits combined make ELISA an essential blood donor screening tool and an essential first line diagnostic tool in the fight against transfusion transmitted infections [25].

4.5 Limitations of ELISA

Even though its use is widespread, ELISA still has a number of inherent limitations that means that the risk of transfusion transmitted infections cannot be completely eliminated by its use. The most serious drawback is the diagnostic window period when the infected person could be negative due to an undetectable antibody concentration or lack of antigen in the bloodstream. The leading source of residual transfusion risk is the donations collected during this phase which, despite negative serological tests, are still infectious. Nonspecific antibody binding, autoimmune diseases, pregnancy, recent vaccinations, and technical errors can also cause false-positive results in ELISA. Confirmatory testing reduces the number of unnecessary donor deferrals but has the drawback of widening the workload of laboratories and possibly the number of healthy people being denied the opportunity to donate blood. On the contrary, false-negative results could be obtained if the antibody titres are low, the antigen level is below the detection limit of the assay, or if mutations in the virus change the antigenic epitopes that diagnostic antibodies recognize. In immunocompromised people, the ability to mount a normal antibody response may also be delayed or impaired, thereby decreasing the sensitivity of the assays. A second limitation is cross-reactivity, where anti-microbial antibodies or antibody to endogenous proteins cross-react with assay antigens, resulting in a non-specific positive signal. Improvements in recombinant antigen technology have significantly minimised cross reactivity but it is still a known analytical problem for some infectious diseases. In addition, ELISA only looks for immunological markers and not the pathogen itself. Thus, it is not reliably able to detect infections at the earliest stage of viral replication, before seroconversion. This is why nucleic acid testing is increasingly starting to be used as a complementary

testing method by many national blood transfusion programs to enhance the early detection of pathogens and further decrease the residual transfusion risk [26].

Table 2. Advantages, limitations, and major clinical applications of ELISA in blood donor screening.

Feature	Description
Principle	Detection of pathogen-specific antigens and/or antibodies using enzyme-linked immunological reactions.
Major applications	Screening for HIV-1/2, HBsAg, anti-HCV, HTLV-1/2, Treponema pallidum (syphilis), and other transfusion-transmitted infections.
Advantages	High sensitivity and specificity; relatively low cost; high-throughput testing; rapid processing of large sample numbers; compatibility with automation; standardized commercial kits; well-established regulatory acceptance; minimal technical complexity compared with molecular assays [27].
Limitations	Diagnostic window period; inability to detect very early infections before seroconversion; false-positive and false-negative reactions; potential cross-reactivity; dependence on adequate antigen or antibody concentrations; requires confirmatory testing for reactive samples [28].
Clinical significance	Serves as the primary serological screening method in blood banks and substantially reduces the risk of transfusion-transmitted infections when combined with donor selection and quality assurance measures.
Current role	First-line screening assay worldwide; increasingly complemented by nucleic acid testing (NAT) to improve early detection of HIV, HBV, and HCV infections [29].

5. Nucleic Acid Testing (NAT)

Nucleic acid testing (NAT) has transformed the blood donor screening process and is used to detect the presence of viral nucleic acids before the appearance of detectable antigens or antibodies. NAT can detect and identify pathogen-specific DNA or RNA, whereas serological assays like enzyme-linked immunosorbent assay (ELISA) rely on the host immune response, significantly cutting the diagnostic window period and residual risk of transfusion-transmitted infections (TTIs). NAT has been adopted in blood transfusion services since the end of the 1990s and has been part of donor screening in most developed nations, and is increasingly being implemented in low- and middle-income countries. The technology has been shown to have contributed greatly to the detection of human immunodeficiency virus (HIV), hepatitis B virus (HBV), and hepatitis C virus (HCV) which has significantly improved transfusion safety.

5.1 Principle of NAT

The ability of NAT to identify the presence of pathogen-specific nucleic acids in samples of donor blood is the basis for the entire method. The method can identify the genetic material of a virus (DNA or RNA) even when present in very low concentrations, and involves enzymatically amplifying target genetic sequences, making it possible to identify an infected donation even at the earliest stages of infection. The viral RNA of viruses like HIV and HCV is first transcribed into complementary DNA (cDNA) and then amplified. In case of DNA virus (e.g. HBV) the step of reverse-transcription can be skipped and the virus can be amplified directly. The amplification process is divided into three phases: obtaining the nucleic acids from the donor plasma or serum, amplifying specific genomic targets by the action of enzymes and detecting the amplified products through fluorescent probes or other systems capable of generating signals. The exceptional analytical sensitivity of NAT allows for the detection of only a few copies of the viral nucleic acids present in milliliters of blood, thus greatly reducing the potential risk of transfusion transmitted infections. NAT can detect the infection, not the immune response, so it can detect the

infection at the point when the host's immune system hasn't yet produced antibodies, and the conventional serological assays will be negative [30].

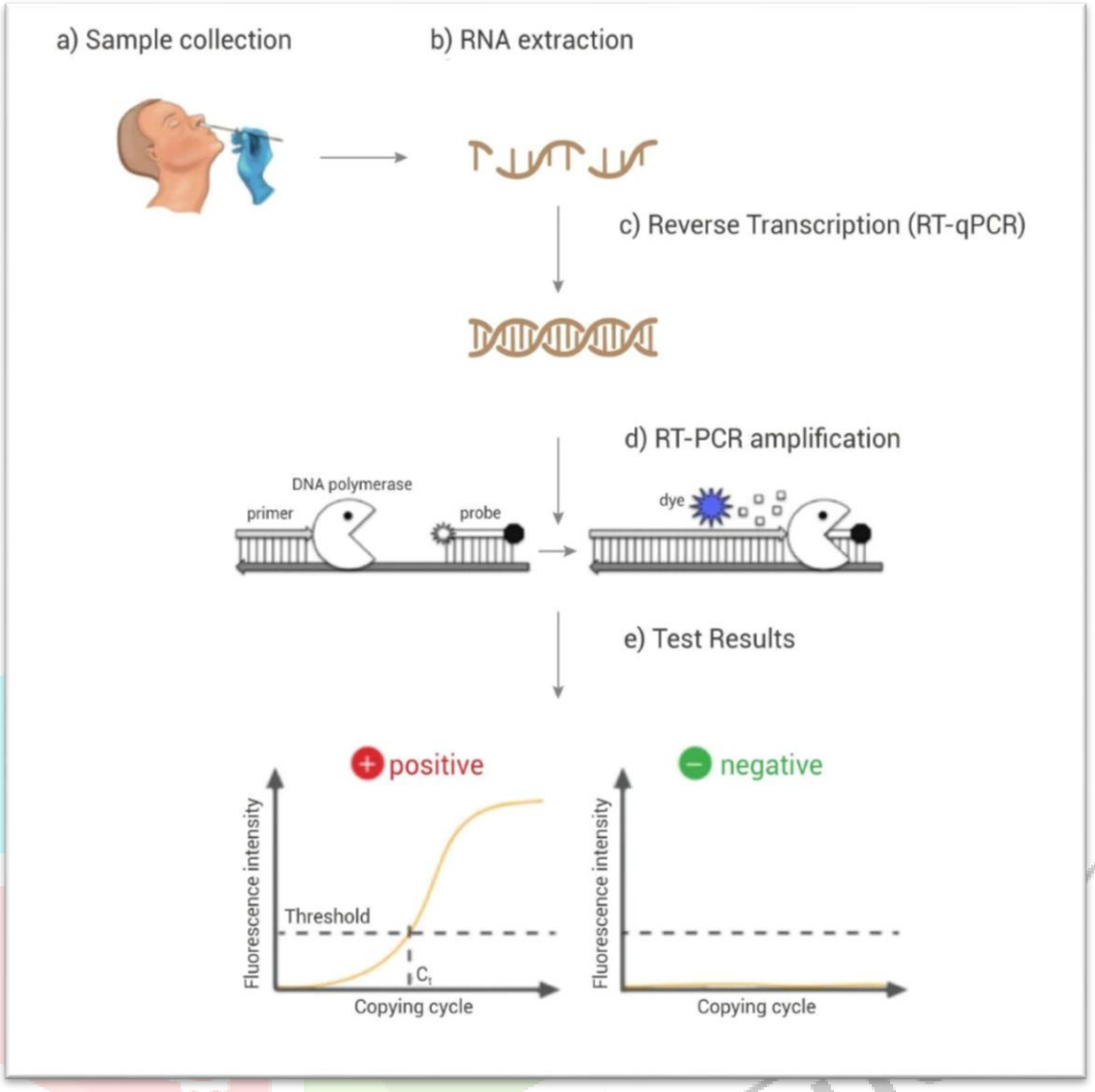

Figure 3. Principle of nucleic acid testing (NAT). Viral DNA or RNA is extracted from donor plasma, amplified using molecular techniques such as PCR, real-time PCR, or transcription-mediated amplification (TMA), and detected to identify infected blood donations before seroconversion.

5.2 Types of NAT

The current blood donor screening technologies employ several types of molecular amplification techniques that have different analytical features and prerequisites.

Polymerase Chain Reaction (PCR)

The most popular method of nucleic acid amplification is conventional polymerase chain reaction (PCR). The principle of PCR is to use repeated cycles of denaturation and annealing of a specific sequence to the primers followed by elongation by the DNA polymerase to amplify the target sequence in an exponential fashion. For RNA viruses, reverse transcription PCR (RT-PCR) is used, which involves converting the RNA to complementary DNA (cDNA) for amplification. With great analytical sensitivity and specificity, PCR has been adopted as the gold standard for clinical laboratories to detect the presence of viral nucleic acid [31].

Real-Time PCR

Real Time PCR (also known as quantitative PCR or qPCR) is a combination of nucleic acid amplification with the simultaneous real-time fluorescence detection of target nucleic acids. During each cycle of a reaction, the fluorescent dyes/sequence specific probes emit signals in proportion to the quantity of the amplified product. Real-time PCR has many advantages over conventional PCR, such as a rapid

turnaround time, reduced contamination, greater throughput and greater automation, which makes it an ideal choice for screening blood donors on a regular basis [32] .

Transcription-Mediated Amplification (TMA)

Transcription-mediated amplification (TMA) is an isothermal nucleic acid amplification technique that amplifies RNA targets without thermal cycling. It uses the reverse transcriptase and RNA polymerase enzymes to produce RNA amplicons continuously at constant temperature. Because of its ability to detect very low amounts of viruses and its ability to be processed quickly, TMA is also used in commercial blood screening platforms and highly sensitive in detecting RNA viruses like HIV and HCV [33].

Multiplex NAT

Multiple pathogens can be amplified and detected in a single reaction. HIV RNA, HBV DNA and HCV RNA can be tested at the same time on the same sample with a commercial multiplex assay, enhancing the laboratory efficiency and reducing the consumption of reagents and testing expenses. Multiplex molecular diagnostics are still advancing, allowing for detection of more pathogens, thus contributing to all-encompassing blood safety programs [34].

5.3 NAT in Blood Donor Screening

One of the primary goals of NAT in blood donor screening is to identify viral infections prior to the appearance of serological markers. HIV RNA, HBV DNA and HCV RNA are frequently detected using NAT, and these are the most important viral risks to blood safety. NATs detect viral RNA several days before the appearance of p24 antigen, or anti-HIV antibodies, thus significantly reducing the diagnostic window period for HIV. Likewise, HBV NAT will detect viral DNA prior to the appearance of detectable hepatitis B surface antigen (HBsAg) and will aid in the detection of occult hepatitis B infection, which is a major source of residual transfusion risk. The ability to detect HCV RNA enables identification of HCV infected donors at the early viremic phase, long before anti-HCV antibodies can be detected. The use of NAT in blood donor screening algorithms has led to a marked decrease in the transmission of these viruses in the blood and it is now routine in many of the national blood transfusion services. However, NAT is usually used in addition to serological screening to achieve the highest level of diagnostic certainty [35].

5.4 Minipool NAT (MP-NAT)

In minipool NAT, plasma samples from a number of donors are pooled together into a single specimen for molecular testing. If the pooled sample is reactive the individual donations are sent for further testing to determine which sample is the infected donor. The main benefit of MP-NAT is the lower testing costs, as multiple donations can be tested together with fewer reagents or consumables. This method is especially appealing for blood centers that are serving high numbers of donors, with limited budget. MP-NAT also has relatively high laboratory throughput and is easy to integrate in the routine workflow. During the very early stage of infection, however, when viral loads are very low, dilution of viral nucleic acids during the pooling of specimens results in a decrease in the analytical sensitivity. Thus, infection with a low viremia level can sometimes go undiagnosed and give a slightly higher residual risk than that of individual donation testing [36].

5.5 Individual Donation NAT (ID-NAT)

Individual donation NAT performs individual donor screening without prior pooling; it offers the most analytical sensitivity for routine blood donor screening available today. The primary benefit of ID-NAT is that it is able to detect extremely low concentrations of viral nucleic acid, significantly reducing the diagnostic window period and enhancing the ability to detect occult infections. The use of ID-NAT is the preferred molecular screening strategy in several developed countries, due to its high competence in diagnostics and its role in achieving maximal blood safety. However, pooled testing is significantly less expensive than ID-NAT as each donation needs a separate molecular test. This also requires that a higher amount of reagent is used, that more time is spent in the laboratory, that the laboratory is more complex in terms of automation, and that more skilled workers are required to operate it. This means that it will be difficult to implement for resource poor blood transfusion services [37].

5.6 Diagnostic Performance

Because of its pathogen-specific nucleic acid detection capability, NAT is a highly effective analytical method. Analytical sensitivity is usually higher than for serologic methods, with the ability to detect only a few copies of viral genomes per milliliter of plasma. This significantly reduces the risk of transfusion transmitted infections for HIV, HBV and HCV and shortens the infectivity period for these viruses by NAT. Specificity is also very high due to the specific primer and probe design for conserved regions of the genome that are amplified. Commercial NAT systems have internal controls and tight quality control to reduce contamination and false-positive amplification. Moreover, multiplex platforms enable the simultaneous detection of various pathogens without a negative effect on the analytical performance. The detection limit of NAT may vary depending on the design of the assay, extraction efficiency, target pathogen, and amplification platform. Generally, individual donation NAT offers lower limits of detection than pooled testing since the viral nucleic acids are not diluted before the amplification process [38].

5.7 Limitations

Although the NAT is a more powerful diagnostic tool, it also has a number of drawbacks that prevent its widespread use. High cost, whether because of the use of expensive instrumentation, proprietary reagents, maintenance contracts, or quality assurance programs are the biggest hurdle. Regular adoption is often restricted in low resource health care systems due to these financial needs. In addition to this, advanced laboratory facilities are required for NAT, such as specific molecular diagnostic facilities with contamination controlled work spaces, continuous power supply, reagent storage at controlled temperature and complex automation. There is a need for highly skilled laboratory personnel to perform nucleic acid extraction, molecular amplification, instrument calibration, QC and interpretation of results in order to successfully implement it. Ongoing training and competency checking are important to ensure the accuracy of diagnostics. Lastly, equipment maintenance and quality assurance is a continuous problem during operation. To ensure accurate molecular reading results, molecular analyzers must be periodically calibrated, preventatively serviced, software updated and subjected to external proficiency testing programs. Equipment failures or lab operations problems can affect the accuracy of the assay and lead to higher expenses. As a result, NAT has become the benchmark for detecting transfusion-transmitted viral infections, but it is not yet widely implemented due to limited resources, expertise, and infrastructure in the health care system [39].

Table 3. Comparison of major nucleic acid testing (NAT) platforms used for blood donor screening.

NAT Platform	Principle	Target Nucleic Acid	Major Applications	Advantages	Limitations
Conventional PCR	Thermal cycling amplification of DNA using sequence-specific primers	DNA (or cDNA generated from RNA)	Detection of HBV DNA; research and confirmatory testing	High analytical sensitivity and specificity; well-established methodology	Longer turnaround time; post-PCR processing increases contamination risk; lower throughput [40]
Reverse Transcription PCR (RT-PCR)	Viral RNA is reverse-transcribed into complementary DNA (cDNA) followed by PCR amplification	RNA viruses (HIV RNA, HCV RNA)	Routine detection of RNA viruses in blood donor screening	Detects infections before seroconversion; excellent sensitivity	Requires reverse transcription step; relatively expensive instrumentation
Real-Time PCR (qPCR)	Fluorescent probes monitor amplification in real time during	DNA and RNA (after reverse	Quantitative and qualitative detection of	Rapid turnaround; closed-tube system	High equipment and reagent costs; requires [41] technical

	PCR cycling	transcription)	HIV, HBV, and HCV	minimizes contamination; automation compatible; quantitative analysis	expertise
Transcription-Mediated Amplification (TMA)	Isothermal amplification using reverse transcriptase and RNA polymerase	Primarily RNA targets (also adapted for DNA targets)	Commercial blood donor screening for HIV, HCV, and HBV	Extremely high analytical sensitivity; rapid amplification without thermal cycling; high throughput	Proprietary technology; specialized instruments and reagents required
Multiplex NAT	Simultaneous amplification of multiple pathogen-specific targets in a single reaction	DNA and RNA	Concurrent detection of HIV RNA, HBV DNA, and HCV RNA	Reduces reagent consumption; improves laboratory efficiency; high-throughput testing	Complex assay optimization; potential primer/probe interference; higher initial setup cost [42]
Minipool NAT (MP-NAT)	Molecular testing performed on pooled donor plasma samples	DNA and RNA	Routine blood donor screening in high-volume blood centers	Lower cost per donation; efficient large-scale screening	Reduced sensitivity due to sample dilution; low-level infections may be missed
Individual Donation NAT (ID-NAT)	Molecular testing performed on each donor sample individually	DNA and RNA	High-sensitivity blood donor screening	Highest analytical sensitivity; superior detection of early and occult infections; shortest diagnostic window period	Highest operational cost; increased reagent consumption; greater laboratory workload [43]

6. ELISA versus NAT: A Critical Comparison

The implementation of NAT has greatly improved blood donor screening by allowing earlier detection of transfusion transmitted infections (TTIs). But, although NAT is more sensitive, it has not been used as a replacement for enzyme-linked immunosorbent assay (ELISA) in the routine blood transfusion environment. Rather, both technologies complement each other and together help to maximize blood safety. These differences in their detection mechanisms, analytical capabilities, operating conditions and cost-effectiveness dictates their functions in the present day blood screening program. The biggest difference between ELISA and NAT is the target of the test. ELISA is a serological test which can detect pathogen-specific antigens or host-generated antibodies, while NAT is a direct detection assay for pathogen nucleic acids (DNA or RNA). NATs are able to detect infections at a much earlier point than the presence of detectable antigen/antibody concentrations, since viral replication occurs prior to the development of antigen/antibody. As a result, the diagnostic delay for the major blood borne viruses (HIV, hepatitis B virus (HBV) and hepatitis C virus (HCV)) is significantly shortened by NAT, thus lowering the risk of transfusion transmitted infections (TTIs). In terms of analytical sensitivity, NAT is always superior to ELISA because it can detect very low levels of viral nucleic acids. The individual

donation NAT (ID-NAT) has proven to be most useful for identifying infection with low levels of the virus and occult HBV infection, which may not be detected serologically. However, there has been significant progress in fourth generation ELISA recently, resulting in better diagnostic sensitivity by the simultaneous detection of HIV p24 antigen and anti-HIV antibodies. These advances have reduced the differences in performance but NAT continues to be the recommended method for early detection of infections during viral replication. In both methods, good analytical specificity is attained if validated commercial assays and standard laboratory methods are employed. Recombinant antigens and monoclonals have greatly improved the specificity of the ELISA, minimizing non-specific reactions and cross reactivity [44]. Similarly, NAT uses specially formulated primer and fluorescent probes that are designed to amplify highly conserved sections of the genome with little background amplification. But false positive NAT results can be obtained in the laboratory during the molecular amplification process, highlighting need for strict quality control and contamination control procedures. A major benefit of NAT is the reduction of the diagnostic window period. NAT is able to detect viral nucleic acids earlier than serological markers in HIV, HBV, and HCV infections, 10 days or even weeks before the markers are present. In countries where NAT is now routinely used for donor screening, the residual risk of transfusion transmitted infections (TPI) has been greatly diminished. ELISA is very reliable once seroconversion has occurred, but can only be used when there is enough antigen or antibody and cannot completely rule out the risk of early infection. There is also a difference in the two techniques based on the operational needs. Overall, ELISA is well-suited for high volume blood centers due to its advantages of shorter routine laboratory workflows, simple assays and easy interpretation. Today, the automated ELISA analyzers can process hundreds of donor samples without much manual participation by the operator. While modern NATs are becoming increasingly automated, the process of molecular testing has specific requirements for nucleic acid extraction, contamination monitoring, nucleic acid amplifications, and specialized instrumentation, making it more complex than traditional NATs. Cost continues to be an important factor in deciding screening strategies. In terms of instrumentation, reagents, maintenance and personnel training, ELISA is significantly less expensive than NAT. In many low and middle income countries where there is limited health care available, therefore, ELISA is the main initial screening tool. NATs, on the other hand, need significant capital investment, such as advanced molecular analyzers, dedicated laboratory facilities, ongoing quality assurance and well-trained staff. While the future medical value of NAT may make the investment worthwhile because of the prevention of transfusion-associated infections, it is difficult for many health care systems to implement. The two methods are also differentiated by the required infrastructure and human resources for the labs. ELISA can be performed in the conventional clinical laboratories that have automated immunoassay analyzers and basic biosafety measures. For NAT, on the other hand, a dedicated molecular diagnostic laboratory that has physically separated pre- and post-amplification areas, contamination-controlled environments, uninterrupted power supply, temperature-controlled reagent storage, and experienced molecular biologists or trained laboratory staff that can perform nucleic acid extraction, amplification, quality assurance and instrument maintenance are required. These two methods can be automated and implemented in high throughput screening, but have varying operational efficiency. The automated ELISA systems can be used to screen a large number of donor samples in a day at a comparatively low operating cost, especially in the national blood transfusion services. Newer NAT platforms now have high throughputs, created by robot sample preparation, multiplex amplification, and integrated workflow management. However, NAT is resource consuming due to its technical complexity and costs. Both methods have their own limitations in terms of false positive and false negative results. ELISA can give false-positive results due to cross-reactive antibodies, autoimmune reactions, pregnancy, or non-specific immune interactions. On the other hand, false negative ELISA tests can be obtained in the serological window period and immunocompromised persons with delayed antibody production [45]. There are significant advantages in reducing false-negative results with NAT, but occasional false-positive results can also occur with NAT due to contamination in the laboratory or the amplification of contaminating PCR products. Thus, confirmatory testing and effective quality assurance are still necessary, no matter what screening method is used. ELISA and NAT are complementary, not competing, from a clinical point of view. ELISA is able to detect immune responses and/or viral antigen presence in donors, whereas NAT is able to detect viremia in the earliest stages of infection. This complementary relationship has inspired most developed blood transfusion services to adopt combined screening protocols, where ELISA is combined with NAT for the most effective diagnostic sensitivity and the lowest transfusion risk remaining. Although NAT is superior to ELISA in analytical performance, there are several reasons why it cannot replace ELISA. Firstly, NAT is much more expensive and technically difficult and is not economically feasible to implement

universally in many countries. Second, NAT does not detect all the transfusion-transmissible pathogens (TTPs) serological markers that are utilized in blood donor screening, including antibodies against Treponema pallidum and some other viruses. Third, serological assays also give important information on immune status and exposure which cannot always be achieved by molecular testing. Thus, the use of ELISA is still essential for routine blood donor screening program [46].

Table 4. Comparative analysis of ELISA and nucleic acid testing (NAT) in blood donor screening [47].

Parameter	ELISA	NAT
Principle	Detection of pathogen-specific antigens and/or antibodies	Detection of pathogen-specific DNA or RNA by nucleic acid amplification
Primary target	Antigens and antibodies	Viral nucleic acids (DNA/RNA)
Diagnostic window period	Longer; dependent on seroconversion or antigen appearance	Shorter; detects infection before serological markers become detectable
Analytical sensitivity	High (particularly fourth-generation assays)	Very high; superior for early infection detection
Analytical specificity	High	Very high
Turnaround time	Rapid and suitable for routine screening	Moderate; includes extraction, amplification, and detection steps
Cost	Relatively low	High
Laboratory infrastructure	Standard clinical laboratory	Dedicated molecular diagnostic laboratory
Automation	Highly automated and widely available	Highly automated but technologically complex
Throughput	Excellent for large-scale screening	High, although more resource-intensive
False-positive results	Possible due to cross-reactivity or nonspecific antibody reactions	Rare; usually associated with contamination or amplification artifacts
False-negative results	Possible during the serological window period or delayed antibody response	Less frequent; may occur with extremely low viral loads or technical failure
Skilled personnel	Moderate technical expertise	Advanced molecular diagnostic expertise required
Suitable healthcare settings	Universal application, particularly resource-limited countries	Primarily well-resourced laboratories; expanding to developing countries
Current role	First-line serological screening	Complementary molecular screening to reduce residual transfusion risk

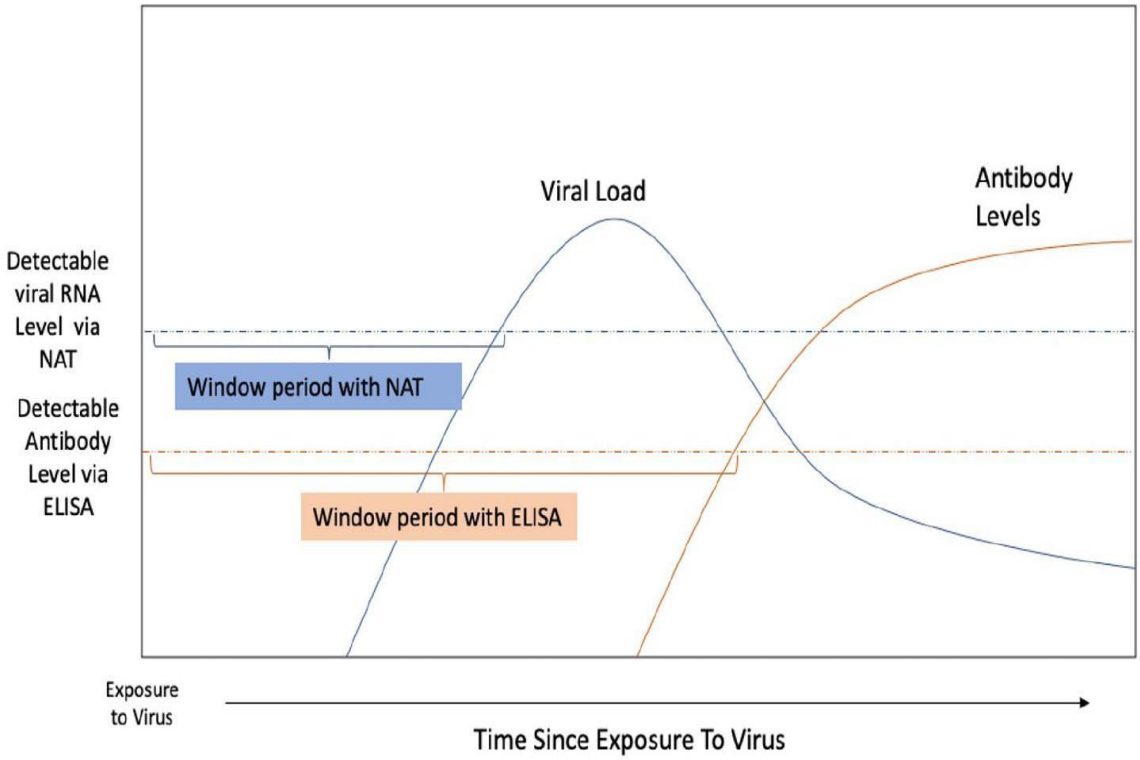

Figure 4. Comparison of the diagnostic window periods for HIV, HBV, and HCV using conventional serological screening (ELISA) and nucleic acid testing (NAT). NAT substantially shortens the infectious window period, enabling earlier detection of viral infections and reducing the residual risk of transfusion-transmitted infections.

7. Clinical and Public Health Impact

The combined use of serological screening and nucleic acid testing (NAT) has significantly enhanced the safety of blood transfusion by minimizing major transfusion transmitted infections (TTIs) transmission. The blood safety process has evolved from a largely reactive approach to a proactive, evidence-based approach over the last 20 years, thanks to developments in donor selection, laboratory diagnostics, and quality assurance. When used together, enzyme-linked immunosorbent assay (ELISA) and NAT has helped to reduce the number of transfusion-associated HIV, hepatitis B virus (HBV) and hepatitis C virus (HCV) infections, and has led to better patient outcomes. Perhaps the greatest success of contemporary blood screening programmes has been the dramatic decline in HIV infection in blood transfusion [48]. Transfusion-associated HIV was a significant public health problem before the advent of sophisticated serological and molecular screening methods. When fourth-generation ELISA assays were introduced and subsequently regular NAT, the window period has been dramatically curtailed, and the risk of HIV-positive donations contaminating the blood supply has been significantly reduced. These same enhancements have been seen for the detection of HBV and HCV; molecular detection of viral DNA or RNA allows detection of infected donors prior to the appearance of conventional serological markers. These innovations have contributed greatly to reducing the risk of virus transmission left behind and to increasing the confidence of blood transfusion services. In the United States and in Europe the benefits to the public health of NAT have also been well documented and are now part of national blood safety programs. With the use of stringent donor selection, the use of ELISA based serological testing, NAT, and the use of comprehensive quality management systems, these areas have reported extremely low rates of transfusion-transmitted HIV, HBV, and HCV. Ongoing system monitoring and mandatory reporting further support these programmes by providing a way to detect any new infectious threats rapidly and continually improving transfusion safety. Expansion of voluntary blood donation, uniform blood testing protocols and step by step inclusion of NAT in blood institutes of tertiary care centres and regional blood banks has significantly improved the blood safety situation in India and many other LMICs. However, the variety of implementation capacity is still present, due to financial constraints, infrastructure differences, and differences in laboratory capacity. Most blood centers still use ELISA in its basic form because it is inexpensive and easy to use; NAT is now being used at many high-volume centers to increase the sensitivity of the test and where this is cost effective. However, with all these advancements, there is still

a chance for residual risk to occur. Potential challenges remain with infections that occur at very early viremia, rare variants of viruses, laboratory error, and emerging viruses that are not part of common screening panels. The transfusion safety is not only dependent upon the use of sensitive diagnostic assays, but also relies on donor education, donor deferral policies, quality assurance programs and continuous epidemiological surveillance. The health economics aspects of NAT differ from region to region based on the prevalence of the disease, the numbers of blood donors and the capacity of the healthcare system. While ELISA may be more expensive, the prevention of transfusion-associated infections will save health care costs for chronic viral hepatitis, HIV treatment, in-hospital care, and complications of these infections in the long run. Hence, combining ELISA with NAT offers the most clinical value both in terms of diagnostic accuracy and patient safety and the economic sustainability. Investment in advanced screening technologies, laboratory facilities and national blood safety policies will remain crucial for the realization of universally safe blood transfusion services [49].

8. Challenges in Low- and Middle-Income Countries

However, although there have been significant advances in blood donor screening, achieving universal blood safety is still a huge challenge in many low- and middle-income countries (LMICs). Nucleic acid testing (NAT) is a widely used method for screening blood for infectious diseases in most blood transfusion services, but widespread adoption of NAT is inhibited by financial, technical and regulatory considerations. These barriers help to create the inequity in transfusion safety between resource-rich and resource-limited health care systems. The cost of implementing and sustaining more complicated screening programs is one of the biggest problems. Routine implementation of NAT is difficult for many public healthcare institutions, as it is expensive to buy the molecular analyzers, maintenance contracts and external quality assessment programs required. The infrequent expenses associated with consumables and instrument maintenance can also be higher than the budgets of regional blood centers. Therefore, most LMICs still use ELISA mainly due to its performance/affordability. Other major challenges are lack of laboratory facilities. The molecular diagnostics also demand a specific laboratory space and separate space for pre- and post-amplification, constant electrical power, climate-controlled storage conditions, biosafety and secure information management system. Poor laboratory conditions, frequent power failures and lack of maintenance in many areas make it difficult to successfully operate NAT platforms. Ensuring quality assurance is also a crucial aspect, but difficult to accomplish. Standardize the operating procedure, implement internal quality controls, external proficiency testing, equipment calibration, and audit regularly to ensure consistent assay performance. Financial constraints and lack of technical expertise are both obstacles to the establishment of quality management systems at many blood centers in resource-limited areas. Another major concern is the supply chain for the diagnostic reagents and consumables. Routine donor screening could be affected by delays in procurement, import restrictions, transportation and interruptions in test kit availability, all of which might impact the continuity of blood services. Higher reliance on outside molecular reagents adds to operational expense and vulnerability to disruption of the global supply chain. Another major constraint is the availability of appropriately-trained staff. While both ELISA and NAT require skilled laboratory personnel, molecular testing also requires specific skills in molecular nucleic acid extraction, amplification, contamination monitoring, instrument upkeep and data analysis. Ongoing training and assessment of competency are thus vital to ensure acceptable performance in the laboratory [50].

9. Emerging Technologies and Future Perspectives

Advances in diagnostic technologies are changing the nature of blood donor screening by increasing the analytical sensitivity, turnaround time and laboratory efficiency. Even though enzyme-linked immunosorbent assay (ELISA) and nucleic acid testing (NAT) are the current gold standards for the detection of transfusion-transmitted infections (TTIs), there are several emerging blood safety technologies that will be further enhancing blood safety. A growing number of uses of artificial intelligence (AI) and machine learning (ML) are being investigated for donor risk assessment, analysis of laboratory results, quality assurance, and forecasting of infections. These technologies can help with decisions based on data, laboratory workflow optimization and enhance the efficiency of blood transfusion services. The use of digital blood banks, incorporating laboratory information systems (LIS), electronic donor records and real-time inventory management has improved blood traceability, efficiency of blood use, and monitoring of new infectious outbreaks [51]. Likewise, decentralized, molecular diagnostics, which detect nucleic acids at the point of care, increase access to cutting-edge blood

screening in resource-constrained areas. The new platforms being developed like microfluidic NAT or the lab-on-a-chip devices combine sample preparation, nucleic acid amplification, and detection in a compact device that uses less reagents and short processing time. CRISPR diagnostic platforms have been shown to have a high specificity and sensitivity for the rapid detection of pathogens and next generation sequencing (NGS) has been shown to offer a comprehensive characterization of pathogens – known and novel – for epidemiological surveillance and outbreak monitoring. New blood testing in the future is also likely to be enhanced by multiplex molecular assays that can detect multiple pathogens in the same test, as well as by growing automation and robotics in blood testing to boost throughput and minimize human error. Furthermore, blockchain technology provides digital traceability of blood throughout the supply chain, promoting transparency and compliance with regulations. Together these innovations will be expected to supplement current ELISA- and NAT-based screening programs and help to improve the safety, efficiency and sustainability of blood transfusion services.

10. Conclusion

Maintenance of the microbiological safety of blood transfusion continues to be an important goal of transfusion medicine in modern practice. The sensitivity and diagnostic accuracy of ELISA, the cost-effectiveness, and the ability to test large numbers of blood donors in the field make it an ideal tool for routine blood donor screening. NATs, however, positively affect the safety of the blood by allowing the detection of viral nucleic acids before the diagnostic window (DTW) and the residual risk of transfusion-transmitted infections (TTI). Although NAT is more analytically sensitive, it cannot completely replace ELISA due to its higher cost, requirement for infrastructure, and technical complexity. Rather, the evidence at hand agrees that the most effective mode of blood safety is the combined use of both ELISA and NAT, which encompass the benefits of both serological and molecular diagnostics. Expansion of NAT and further investment in laboratory infrastructure, quality assurance and staff training, coupled by more widespread implementation of NAT, especially in LMICs, will be key to future developments in transfusion medicine. Recent advances in emerging technologies, such as artificial intelligence, diagnostics using CRISPR, microfluidic platforms, next generation sequencing, multiplex molecular assays, and digital blood management systems, are projected to further improve diagnostic performance, automation and blood traceability. As they become part of regular blood donor checks, they will help to make transfusion more safe, fair and sustainable for all.

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