NUCLEIC ACID AMPLIFICATION TESTING: THE NEW INFECTIOUS DISEASE TESTING METHOD FOR DONOR BLOOD

Moyne Treat Kornman, MD, German Leparc, MD, and Kaaron Benson, MD

From the Florida Blood Services, Tampa, Fla (MTK, GL) and the Pathology Service at H. Lee Moffitt Cancer Center & Research Institute, Tampa, Fla (KB).

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Introduction

Beginning in the spring of 1999, the American Red Cross and 16 member laboratories of the American's Blood Centers began testing donor blood for the human immunodeficiency virus (HIV) type-1 and the hepatitis C virus with a new research testing method known as nucleic acid amplification testing (NAT). This testing is conducted under well-defined Investigational New Drug research protocols approved by the Food and Drug Administration (FDA). Once licensed by the FDA, NAT will be available for routine screening of all donor blood components for HIV and HCV, depending on the feasibility and effectiveness demonstrated in the Investigational New Drug project.\(^1\) NAT for hepatitis B virus (HBV) is not being implemented at the present time due to anticipated lack of effectiveness when compared to current testing methods.

In recent years, all blood donors have been extensively screened by interview and tested for a number of infectious disease markers. Before 1985, donor units were issued for transfusion only if they were seronegative for syphilis and negative for hepatitis B surface antigen (HBsAg). All donors had to successfully complete a donor interview and were asked to voluntarily exclude themselves if they were in an AIDS risk group. From 1985 through 1989, serologic testing for HIV and human T-lymphotropic virus (HTLV) were added to the testing menu, along with a more sensitive (second-generation) test for HBsAg. The donor interview was expanded to include direct questioning about participation in activities that put donors at risk for HIV or hepatitis infection. Donors were also tested for alanine aminotransferase and hepatitis B core antibody (anti-HBC), which are surrogate markers for possible non-A, non-B hepatitis. Since 1990, the donor interview has been expanded a number of times, and serologic testing for HCV, HIV-2, and HTLV-II has been implemented. Donor testing for HIV p24 antigen began in 1996. A third generation HBsAg test is now used along with the third version, elevated-sensitivity HCV serologic test.

In 1996, the reported risk of transfusion-transmitted HIV was approximately 1 in 493,000 units transfused.\(^2\) This represents a remarkable decrease in risk considering that in the early 1980s, the risk was as high as 1% per unit transfused in some US cities.\(^3\) Schreiber et al\(^2\) estimated the risk of transfusion-transmitted HCV as 1 in 103,000 units transfused.

Infected donors may fail to respond accurately to questions about transmissible disease risk factors at the time of blood donation\(^4\) and may not be detected with current testing methods. The current risks of transfusion-transmitted HIV and HCV, while low, may possibly be further reduced with molecular biologic methods capable of amplifying and detecting viral genome.\(^5\)

Reasons for NAT Implementation

The impetus for initiating NAT of donor blood is multifactorial. (1) Compliance with a European Committee for Proprietary Medicinal Products (CPMP) requires that all plasma derivatives distributed in the European Union after July 1, 1999, be harvested from plasma that has tested negative for HCV by NAT. This mandate arose as a result of HCV infections in some recipients of commercially available immunoglobulin that was prepared after the implementation of donor HCV serologic screening. Because of the removal of anti-HCV-positive units from the plasma pools, the commercially available immunoglobulin preparations were devoid of the protective effects of anti-HCV antibodies and thus were deemed potentially infectious for HCV unless a viral inactivation step was employed in the manufacturing process.\(^6\) As a result of the HCV infections occurring from immunoglobulin preparations that had not undergone viral inactivation, regulatory agencies have mandated that manufacturers include viral inactivation in the production of therapeutic immunoglobulin.\(^7\) As a further safety step, the European CPMP additionally mandated direct HCV testing by NAT and will probably extend this requirement to include genomic testing for HIV and HBV in the future. (2) US FDA policy established under Commissioner Kessler has directed manufacturers and encouraged blood establishments to implement leading-edge technology to decrease or eliminate the "window period" during which a donor is infectious but found nonreactive by currently licensed screening methods. (3) Consumer demand has called for further decreases in transfusion risks by the use of advanced technical means.

NAT Technical Principles

Genomic screening for infectious agents using NAT can be performed with several nucleic acid amplification techniques. Polymerase chain reaction, ligase chain reaction, nucleic acid sequence-based amplification, and transcription-mediated amplification are genomic amplification techniques that use different approaches to achieve the in vitro amplification of nucleic acids.\(^7\) Nucleic acid sequence-based amplification and transcription-mediated amplification are used to amplify RNA targets (eg, HCV and HIV), whereas polymerase chain reaction and ligase chain reaction need DNA or cDNA sequences as targets and therefore require a reverse transcription step for the amplification of RNA viruses.

All of these techniques directly detect microorganisms in donor blood by amplifying the nucleic acid sequences specific to the microorganism. Use of these techniques provides a much higher level of sensitivity and specificity than routine testing methods currently provide (enzyme immunoassay [EIA]). Despite the current diligent EIA screening of donor blood for the detection of antigens (HBsAg, HIV p24 antigen) and antibodies (anti-HIV/1/2, anti-HBC, anti-HCV), there is still a residual risk of posttransfusion infection from HIV or hepatitis viruses acquired from donors donating in the early window (or latent) period of infection.\(^7\) The power of NAT is its ability to detect the presence of infection by directly testing for viral genomic nucleic acids rather than by indirectly testing for the presence of antibodies. Lee and Allain\(^7\) report that the efficacy of such screening depends on the prevalence of the infection in the population and the duration of the window period. In most Western countries, HCV shows a higher prevalence and longer window period (80 days) than HBV (56 days) and HIV (16 days). This explains why NAT detection of HCV is the primary focus of implementation of this approach to blood screening.

Under the method developed by Gen-Probe, Inc (San Diego, Calif), the NAT procedure for HIV-1 and HCV in donor blood will involve three main steps: sample preparation, HIV-
and HCV RNA target amplification, and detection of the amplified products (amplicons). During sample preparation, pooled plasma samples from donors are treated with a detergent to solubilize the viral envelope, denature proteins, and release viral genomic RNA. Oligonucleotides homologous to highly conserved regions of the HCV genome and HIV polymerase are hybridized to the RNA targets of HCV or HIV. These hybridized targets are then adsorbed onto magnetic microparticles and separated out of the plasma by a magnetic field. Transcription-mediated amplification, which uses a reverse transcriptase and an RNA polymerase for the amplification process, is used to amplify the HIV-1 and HCV targets. Detection is then performed using nucleic acid hybridization of the amplicon with its complimentary chemiluminescent single-stranded nucleic acid probe. A luminometer is used to detect the presence of chemiluminescent signals produced by the hybridized probes.

This routine multiplex assay detects the presence of HIV or HCV genomes, but it cannot differentiate between the two. Therefore, discriminatory assays are performed on the samples found to be reactive in the multiplex test in order to determine if they are positive for HIV, HCV, or both. These discriminatory assays use the same basic procedure as the multiplex assay. However, HIV-specific and HCV-specific probe reagents are used separately rather than jointly as in the multiplex probe reagent.

An alternative approach was developed by Roche Molecular Systems, Inc (Pleasanton, Calif). The COBAS AmpliScreen HCV Test is based on five major processes: (1) specimen preparation, (2) reverse transcription of the target RNA to generate complementary DNA (cDNA), (3) polymerase chain reaction amplification of target cDNA using HCV-specific complementary primers, (4) hybridization of the amplified products to oligonucleotide probes specific to the target(s), and (5) detection of the probe-bound amplified products by colorimetric determination.

The COBAS AmpliScreen HCV Test is used with two specimen-processing procedures — the Multiprep procedure, which is used for the testing of 24-specimen primary plasma pools and for follow-up testing of six-specimen secondary plasma pools, and the Standard procedure, which is used for testing of individual specimens in order to identify the positive specimen(s) in the primary and secondary pools. Unlike the Gen-Probe transcription-mediated amplification assay, HIV and HCV primers are used separately in the COBAS AmpliScreen HCV Test.

Logistic Barriers to NAT Implementation

NAT of donor blood is still in the early stages of development. A cost-effective, automated system does not yet exist to perform the assays in toto, so the initial testing is performed on pools of donor blood rather than individual units, using both part-manual and semiautomated systems. Lee and Allain list several economic and technical limitations of NAT that impose great burdens on blood centers: (1) NAT requires staff that is trained in molecular biological techniques and also requires equipment that is unfamiliar in the blood bank laboratory setting, (2) prevention of cross-contamination of amplicons among samples requires that reagent preparation, sample handling, genomic amplification, and detection be performed in separate rooms, thus imposing space constraints, (3) commercially available NAT assays require more than 12 hours to perform, a schedule that places a significant burden on routine blood screening and product release, and (4) the cost of each commercial NAT test is approximately 10 times that of the most expensive EIA test.

Several vexing issues exist concerning NAT of donor blood. The testing of pools of plasma will be economically practical; however, the act of pooling itself produces some technical concerns. For example, HCV circulates at concentrations of only $10^4$ to $10^5$ genome equivalents per milliliter. Although some observers believe that viruses can be concentrated using ultracentrifugation prior to NAT testing, several viruses, including HCV, may not concentrate in the pellet after centrifugation. In addition, HCV can bind to lipids present in the plasma, which then migrate to the surface during centrifugation and are discarded during decanting and washing steps. Laboratories may be able to overcome this problem by testing small pool sizes. Pools of fewer than 50 samples do not require a concentration step and do not impair NAT sensitivity as long as the use of highly sensitive amplification techniques are used.

Also inherent to the use of pooled specimens is the inadvertent pooling of endogenous inhibitors, which could potentially lead to false-negative results. Little is known about such inhibitors, but their prevalence is estimated to be as high as 1%. An additional problem with the use of pooled samples that warrants further study is the interpretation and disposition of pools that are positive on initial testing but are negative on secondary testing of smaller pools. This problem also applies to pools that are positive on initial and secondary testing but are negative when tested on an individual basis. Although this presents a great logistic and economic burden on the blood centers, it is part of the evaluation protocol set up in the Investigational New Drug design. Approximately 50% of the samples found to be NAT-positive are expected to be false positive (ie, no positive individual donation can be identified).

Also related to the problem of discrepant test results is the proper dispensing of the units within a positive primary pool. Some of the blood-collecting agencies may not have test results available within 24 to 36 hours following the blood collection. While the investigation of NAT technology is underway, the FDA has agreed to allow the issue and transfusion of units before obtaining NAT results, provided the current licensed testing continues without interruption. Under those circumstances, some institutions may receive NAT-untested units. Physicians may want to explain to their patients that the blood they will receive may or may not have completely finished the nucleic acid testing. Physicians may discuss the theoretical implications of NAT on the safety of the blood, but they must continue to identify the risks of transfusion-transmitted diseases as a result of the current licensed testing methods. Patients should understand that until the reliability of NAT is established, the current risks associated with transfusion-transmitted diseases will remain unchanged.

Potential Effectiveness of NAT

NAT could theoretically close the window period of HIV from the current 16 days to approximately 10 days as well as the window period of HCV from the current 70 to 80 days to approximately 10 to 30 days. This would reduce the risk of transfusion-transmitted HCV from approximately 1 in 100,000 units transfused to 1 in 500,000-1,000,000 units transfused. Interesting preliminary data have been reported by blood centers in Germany. Of 1,134,102 blood donor units tested for HBV, HCV, and HIV by NAT, 24 were NAT positive and seronegative (2 for HBV, 22 for HCV, and 0 for HIV). However, regulatory restrictions do not allow claims of increased safety to be made while NAT is under the Investigational New Drug research protocol. The FDA has informally stated that it will allow a blood establishment to inform its customers of its participation in a NAT research study to determine the value of these tests as donor screening tools.

To date, there are no conclusive data to support the use of NAT as a means of increasing the safety of the blood supply. However, in order to pursue its stated policy of seeking innovative means to increase the safety of the blood supply and to allow compliance with the European requirements, the FDA has cooperated with blood bank facilities and encouraged their participation in the early clinical trials of this powerful and promising technology for the screening of donor blood.

References


