Nucleic Acid Amplification Technology (NAT): Testing Blood Donors

**Introduction:** Although the risk of transmitting infection to transfusion recipients has been drastically reduced due to improved donor selection and sensitive serologic screening assays, currently it is not possible to detect very recently infected donors during the pre-seroconversion "window period" of infection. Rare individuals negative for antibodies to infectious viruses such as hepatitis C (HCV) and the human immunodeficiency virus (HIV) therefore may escape blood donation screening procedures. The current estimated risks for transmission of HCV and HIV by a single antibody negative component are 1/150,000 and 1/676,000 respectively (see Goodnough et al).

Because viremia precedes seroconversion by several days to weeks, tests that detect viral nucleic acids are more sensitive than current screening tests and may reduce the window of infectivity by as much as 60 days for HCV and 11 days for HIV infection. While nucleic acid amplification test technologies (NAT) are available, they have not been used in the United States to screen volunteer blood donors because they have been technically demanding, time consuming and could interfere with timely release of critical blood components. The recent development of commercially-available assays using NAT has made it possible for blood centers to consider applying these tests to blood donor screening.

US blood centers will implement NAT testing of blood donors in April 1999. The testing will be performed under Investigational New Drug (IND) applications submitted to the Food and Drug Administration (FDA) as clinical research studies in collaboration with the manufacturers. The study objectives will be: 1) to determine the specificity and reproducibility of the NAT test systems on pooled samples in high volume blood donor screening laboratories, 2) to assess the impact of NAT testing on blood component availability, and 3) to evaluate the use of NAT tests to improve the safety of the blood supply.

The decision to introduce NAT testing for blood donor screening is driven by the express desire of former FDA Commissioner David Kessler, M.D. to see NAT systems developed that will further increase the safety of the US blood supply.

**NAT Test Performance:** In the past, NAT testing was limited to research and clinical diagnostic laboratories. NAT test systems are time intensive, require restricted laboratory space and highly-trained technical staff, and generally are unsuited to large-scale screening of individual donor samples. US blood programs, including America’s Blood Centers (ABC) and the American Red Cross (ARC), are overcoming the challenges of implementing NAT testing for routine blood donor screening by creating centralized laboratories that meet the test vendors’ requirements for molecular technology. Both ABC and ARC are working with commercial suppliers to develop methods for pooling samples, optimizing the systems for assuring sensitivity and specificity of the tests for pooled samples, and training staff to perform these technique-dependent assays.

Testing of pooled donor samples, although never previously used to screen US blood donors, significantly reduces the number of tests required on a daily basis, the time to perform the testing, and the cost of testing per donation. The
rationale for using pooled testing comes from observations in seroconversion panels that determine the rise of HCV and HIV nucleic acids in plasma. Since this rate is very rapid, the sample dilution inherent in pooling has a minimal impact on the sensitivity to detect window period viremic samples. Pooling samples makes molecular-based testing of blood donors feasible. Pool size and pooling strategy balance assay sensitivity with the practical demands of assuring blood availability.

Algorithms for resolving pools with reactive test results are required to determine the individual donor source of a reactive pool. Retest algorithms vary and are defined in each blood program's IND. Pool sizes selected by US blood programs range from 24 to 128.

US blood centers have adopted two basic pooling and pool resolution strategies. One is a straight pool of 24 in which resolution of a reactive pool requires testing individual samples. In the other strategy, smaller (intermediate) pools are prepared and combined to create a final (master) pool. Resolution of reactive master pools occurs by first testing the intermediate pools and then, if necessary, the individual samples. The pooling process will be performed using automated sample handling systems capable of maintaining positive sample identification, assuring that all samples are represented in the pool and avoiding cross contamination. Two test systems will be used to test US blood donors: the Roche Molecular Systems' COBAS AMPLISCREEN for HCV and HIV and the GenProbe Pooled Plasma HIV-1/HCV Amplified Assay.

The Roche COBAS AMPLISCREEN system is based on five major processes: specimen preparation which uses ultra-centrifugation to concentrate virus particles; reverse transcription of the target RNA to generate complementary DNA (cDNA); polymerase chain reaction (PCR) amplification of target cDNA using virus specific complementary primers; hybridization of the amplified products to oligonucleotide probes specific to the target; and detection of the probe-bound amplified products by colorimetric determination. The reverse transcription and PCR amplification of viral target and internal control target occur simultaneously. Following the PCR amplification, the target-specific probe is hybridized to the amplicon and the detection of amplified DNA is performed using Avidin-Horseradish Peroxidase Conjugate. After the specimen preparation is complete, the remaining processes are fully automated on the COBAS instrument. The entire test process takes approximately 6 hours.

The GenProbe HIV-1/HCV test, which detects HIV-1 and/or HCV RNA in a single tube multiplex format, involves three main steps. Sample preparation uses target capture to isolate multiplex RNA from the plasma. Plasma is treated with detergent to release viral genomic RNA, target-specific oligonucleotides are hybridized to the target, and captured onto magnetic microparticles which are separated from plasma in a magnetic field. In the second step, Transcription Mediated Amplification (TMA), which utilizes two enzymes to produce RNA amplicons via DNA intermediates, is employed to amplify HIV-1/HCV target RNA. Finally, detection is achieved using nucleic acid hybridization. A chemiluminescent probe, complementary to the amplicon, is hybridized to the amplicon and is measured by the GenProbe LEADER HC luminometer. An internal control is added to each reaction to control for all steps of the process. If the HIV-1/HCV test is reactive, discriminatory tests are used to differentiate between the two viruses. These tests use the same process as the multiplex assay. The test process takes approximately 5.5 hours.

Although data from pre-clinical studies are encouraging, specificity, sensitivity, reproducibility and run validity data for these test systems are unknown in the donor screening laboratory setting. The impending NAT studies performed under IND will answer many of the questions regarding test performance and generate data that will provide the information required to evaluate the efficacy of routine use of these tests in increasing the safety of the blood supply.

**Blood Donor Issues:** Blood donors will be informed of the investigational nature of NAT testing during the routine consent process used by blood centers. The Investigational Review Board (IRB) of each institution will determine the specific requirements for the consent of the donor. Donors will be advised that they may be notified of positive or discrepant results, that units with positive results will not be used for transfusion, and they may be asked for additional samples as part of a follow-up study.
Blood Product Release and Impact on Hospitals and Patients: Because the performance characteristics and cycle time requirements of the NAT tests are unknown and blood availability cannot be jeopardized, during the first phase of the study, release of blood components for transfusion will be based on the results of the current FDA required and licensed serological tests. Blood centers will use the data and experiences generated during phase one of the study to optimize strategies for routine NAT testing. Every effort will be made to move to release of all products based on NAT test results as quickly as possible. However, in phase one, it is possible that a NAT-positive component, negative by current screening tests, may be released and transfused. Blood centers will notify and work with hospitals, outpatient treatment facilities and transfusing physicians immediately upon finding positive results.

This investigative research should be discussed with physicians and clinicians ordering blood transfusions. This is especially important because the blood center will request that the attending physician be notified in the rare event that a patient has been transfused with a seronegative component that is reactive by NAT, and physicians, in turn, will need to notify these patients of such an event.

When implemented, NAT will add incrementally to the cost of processing a unit of blood at a blood collection center. Consistent with the rules applying to IND studies, blood collection agencies participating in these investigations will be allowed to recover only the direct costs incurred through their participation.

References