Medical Directors and Charge Technologists of Hospital Transfusion Services – Scientific Document

Nucleic Acid Amplification Testing for HIV

It is the mission of Canadian Blood Services (CBS) to provide the safest possible blood components to blood transfusion recipients. Recently, a new technology has become available for the testing of donated blood. This technology goes under the generic name of nucleic acid amplification testing (NAT) and is based upon the direct amplification and detection of viral nucleic acids rather than antibody production by the immune system of the infected person. [1] In Canada, the type of NAT technology used for blood donor screening is the polymerase chain reaction assay (PCR). CBS implemented NAT for screening of blood and plasma donors for Hepatitis C virus (HCV) in October 1999 and is now ready to implement similar technology for Human Immunodeficiency Virus (HIV).

Scientific background

Although each donated unit of blood is tested for evidence of infection by specific viral agents (HCV, HIV, HTLV, and HBV), there are at least four potential reasons why transmission of these viral agents still occurs. [1,2] The primary reason for continued transmission is that the donor has negative laboratory test results during the early stages of infection, known as the window period. A second factor theoretically contributing to the risk of transfusion transmitted infection is the existence of a chronic carrier state in which a clinically asymptomatic donor will persistently test negative for antibody. Thirdly, a viral agent may have a large enough degree of genetic diversity so that laboratory screening tests fail to identify some infectious donors who harbor a particular atypical genetic variant. A fourth factor theoretically contributing to transfusion transmitted infection is laboratory error in performing screening tests; however, given the low prevalence of infected donors and the high accuracy of automated testing, the occurrence of such errors is thought to be extremely rare.

The major reason for continued very low level transfusion transmission of HIV is from donations made during the window period. [2] Asymptomatic, chronic antibody negative carriers of HIV either do not exist or are exceedingly rare, as evidenced by the results of numerous studies in the past decade.[2,3] The recombinant HIV antigens used in the current versions of licensed HIV antibody tests detect antibody to all known HIV-1 clades and to HIV-2 and only occasionally fail to detect infection with the very rare (especially in North America) Group O variant. [4,5] Given the very low rates of HIV infection in blood donors, the contribution of laboratory testing error to transmitting HIV infection is negligible.

The initial approach used to generate an estimate for the infectious window period preceding HIV seroconversion was based upon an analysis of data collected from transfusion recipients known to have received a unit of blood from donors who later became HIV seropositive. Using HIV antibody assays available in the late 1980s, the infectious window period was calculated to be 42 days. [6] Later studies using samples obtained from high risk cohorts were able to compare the performance of these older HIV antibody screening assays to that of newer HIV antibody tests; these studies produced the current estimate of a 22 day (range 6 to 38 days) infectious window period for currently used HIV antibody screening assays.[7] These findings have been confirmed by a similar analysis conducted using 394 seroconverting subjects from 6 cohort studies; this study established a narrower range (16.5 to 28.3 days) for the window period estimate due to its larger sample size. [8] Additional studies of seroconversion panels obtained from plasma donors have also supported these initial estimates. Whether a donor is actually infectious for the entire length of the mathematically modeled window period has not yet been corroborated by experimental evidence.

These studies further calculated that HIV-1 p24 antigen testing decreases this 22 day window period to approximately 16 days and that single donation HIV-1 PCR assays with sensitivity of approximately 200 - 400 copies/mL decrease the infectious window period to 11 days. [7, 9]

Investigators from the Retrovirus Epidemiology Donor Study (REDS) combined data on HIV incidence with data on the HIV window period to generate an estimate for HIV transfusion risk from window period donations. Prior to the implementation of HIV-1 p24 antigen testing, REDS investigators calculated a per unit HIV risk of 1 in 493,000; this fell to 1 in 676,000 after implementation of HIV-1 p24 antigen testing. [2, 10] In Canada, the risk prior to implementation of HIV-1 p24 antigen testing was estimated to be 1 in 913,000.[11] It is important to remember that these point estimates of risk have wide confidence intervals, thereby making it difficult to accurately estimate the yield to be achieved by introducing more sensitive screening assays such as HIV NAT.

During the HIV RNA positive/HIV-1 p24 antigen negative stage, HIV concentration in plasma ranges from 200 to 10^5 copies per mL and the viral doubling time is estimated to be less than one day. [12] As a consequence of these relatively low viral titers and the dilution factor inherent in pooling, pooled HIV NAT is expected to be less sensitive than single donation HIV NAT in detecting window period infection. [1] This has been confirmed by the transmission of HIV by a window period donor in which pooled HIV NAT testing gave variable results when used retrospectively. [13] It is estimated that pooled HIV NAT will reduce the window period relative to HIV-1 p24 antigen testing by approximately 3 days as compared to 5 days for single donation HIV NAT.

Seroconversion panel data indicate that once HIV-1 p24 antigen is detected, over 10,000 copies of HIV RNA are present; it is therefore expected that pooled HIV NAT will be sensitive enough to detect all HIV-1 p24 antigen positive donor samples. Experience from pooled HIV NAT screening of commercial plasma donors in the US indicates that this has thus far been the case [14]. These data suggest that it should be possible to discontinue HIV-1 p24 antigen testing once enough additional data is accumulated and pooled HIV NAT becomes a licensed blood donor screening assay.

Prior to implementation of the HIV-1 p24 antigen assay, the REDS incidence/window period model was used to project the assay's yield by multiplying the point estimate for the HIV incidence rate by the fraction of a year that the HIV-1 p24 antigen assay would detect donors in

the viremic, pre-seroconversion window period. [10] An estimate of a 6 day shortening of the theoretical 22 day HIV infectious window period was used to calculate that one HIV-1 p24 antigen positive, HIV antibody negative unit would be found per 1.5 million donations screened. [2,10] In the first three and one half years of testing in the US, the HIV-1 p24 antigen yield has been 1 per 9 million donations or only 16% of the prediction. [15] Not a single HIV-1 p24 antigen positive, HIV antibody negative donation has been detected in Canada. This discrepancy between estimated and observed yield suggests that the assumption that donors are likely to donate during the interval of antigenemia prior to seroconversion may not be true.

Based upon current HIV incidence rates in the US and a 10 day shortening of the theoretical 22 day antibody negative window period by pooled HIV NAT, it had been estimated that such testing will detect HIV infectious donors at a rate of 1 in 3.8 million HIV antibody negative, p24 antigen negative donations in the US. This predicted yield has been observed in the first 12-18 months of pooled HIV NAT in the US. Given the fact that the incidence of HIV in blood donors in the US is higher than in Canada,, it is expected that the yield from pooled HIV NAT in Canada will be even lower. [16]

In Canada, the risk of transfusion transmitted HIV infection following implementation of HIV NAT has been calculated to decrease to 1 in 1.3 to 1.6 million donations; this estimate assumes that HIV transmission can occur during the entire theoretical early window period. A recent transmission experiment in chimpanzees has shown that a negative plasma HIV RNA PCR result may indicate lack of infectivity in the early theoretical infectious window period, possibly indicating that HIV transmission risk is even lower than has been calculated. [17]

Implementation

The implementation program for HIV NAT will be very similar to that previously used for HCV NAT. Roche Diagnostics Canada has filed an Application for Investigational Testing and CBS has applied for a Licence Amendment with Health Canada to use the AmpliScreen 2.0 HIV PCR assay, manufactured by Roche Molecular Systems, to screen blood donations for HIV nucleic acid. CBS will begin HIV NAT with approval granted

under this regulatory mechanism. Since such testing will begin prior to the assay's licensure, Health Canada regulations will not permit blood components to be labeled as tested for HIV NAT.

After the CBS NAT program is fully operational, all units subsequently collected by CBS will be tested by HIV NAT. However, at the outset of implementation of HIV NAT, hospitals will have a mixed inventory of HIV NAT tested and untested blood components. Because the current risk of HIV transmission from HIV antibody negative units is so low, HIV NAT untested units in hospital inventory can continue to be safely transfused during the early weeks of HIV NAT implementation. CBS will begin a program to rapidly supply hospitals with units of FFP, cryosupernatant and cryoprecipitate that are HIV NAT tested. Within 120 days (or sooner) of implementation of HIV NAT, all HIV NAT untested units of FFP, cryosupernatant and cryoprecipitate will be retrieved from inventory and replaced by product collected from HIV NAT tested donors.

Due to limitations in available technology, HIV NAT will be performed on pools of 24 samples, rather than on each donated unit individually. In addition, due to the complexity of testing and the need for specialized equipment, testing will be centralized in four CBS laboratories located in Halifax, Ottawa, Toronto, and Vancouver. Samples from other collection areas will be shipped to one of these laboratories. Because of the additional testing, the turnaround time for HIV NAT may be 1-2 hours longer than for current blood donor screening tests. Despite this increased turnaround time, CBS plans to obtain HIV NAT results on all units prior to their release into hospital inventory. However, in rare instances, it may be necessary to release HIV antibody negative blood components into inventory prior to the completion of HIV NAT. A contingency plan has been implemented to handle this circumstance, which is expected to occur only in situations of extreme blood shortage. For example, in the first 15 months after the implementation of HCV NAT, CBS released HCV NAT untested allogeneic platelets on 16 occasions. HIV NAT untested units released to inventory will be labeled with an “UNTESTED” sticker and a NAT Pre-Release tag to indicate that such testing has not yet been completed.

If a blood component is released to a hospital prior to obtaining HIV NAT results and if these HIV NAT results are later found to be positive, CBS will inform the hospital promptly so that the unit can be removed from inventory. On those very rare occasions when HIV NAT positive blood components have already been transfused, it is important to remember that such components would have been transfused under current testing protocols which do not include HIV NAT. In such a case, it is recommended that the recipient's physician be promptly informed and that the affected patient be referred to an infectious disease specialist for monitoring, counseling, and consideration of therapeutic intervention. Early notification of possible HIV infection could be beneficial to the recipient in that early treatment of HIV infection can be initiated and counseling can be provided to prevent secondary HIV transmission.

It is recommended that hospital transfusion services discuss the implementation of HIV NAT with physicians at their hospital who order blood transfusions so that they understand the benefits of HIV NAT, the logistics of test implementation, and the possibility of the need for emergency release procedures for units that have not yet undergone HIV NAT.

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NAT Questions and Answers

Same as Hospital Administrator and Clinical Physicians