Brown F, Vyas G (eds): Advances in Transfusion Safety. Dev Biol.
Basel, Karger, 1999, vol 102, pp 109-111

Large Scale PCR Screening of Pooled Plasma Samples for HIV-1 and HCV

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National Genetics Institute (NGI) and Alpha Therapeutic Corporation (ATC) have developed a highly sensitive and efficient system for the large scale screening of Source Plasma donations for HCV and HIV-1 RNA using NGI's highly sensitive UltraQual™ reverse transcriptase (RT) polymerase chain reaction (PCR) assays. The PCR testing programme is an integral part of our multilevel safety system for Source Plasma donations which includes not only PCR testing of pooled donations but also the IPPIA (International Plasma Products Industry Association) voluntary standards for the use of plasma only from qualified donors, mandatory inventory hold and lookback.

The NGI UltraQual™ assays utilize NGI's proprietary technology to achieve a high throughput PCR test which is sensitive and accurate. We have conducted validation studies of the HCV and HIV-1 assays following the International Conference on Harmonization Guidelines for the Validation of Analytical Methods. The estimated mean sensitivity (the concentration of virus genome at which 50% of the tests would be positive) and the estimated 95% detection point (the concentration of virus genomes at which 95% of the tests would be positive) were determined. The estimated mean sensitivity for the HIV-1 test is 1.4 HIV-1 genome copies/mL with an estimated 95% detection point of 5 HIV-1 genome copies/mL. The estimated mean sensitivity for the HCV test is 3.1 HCV genome copies/mL with an estimated 95% detection point of 20 HCV genome copies/mL.

The Source Plasma donations are processed by robotic sample processors at ATC's Central Testing Laboratory to prepare pools of up to 512 samples. The pooling algorithm uses a three-dimensional mapping transformation that places an aliquot of each sample into a unique coordinate within an 8 row by 8 column by 8 layer virtual cube. An aliquot of each sample is pipetted into a unique combination of one row, one column and one layer primary pool, such that the identity of each of the original 512 samples is specified by its row, column and layer coordinates. A master pool is prepared with aliquots from each of the primary pools. The master pool is tested at NGI using the UltraQual™ assay. If the master pool is negative, all samples are assigned a negative test result. If the master pool is positive, the 24 primary pools

(8 row, 8 columns and 8 layers) are each tested. The intersection of the positive row, positive column and positive layer primary pools identifies the suspect positive donation. An independent sample of each suspect positive is individually tested to confirm that each suspect positive identified is a true positive. The overall sensitivity of the pooling process, using a 512 sample pool, is 512 times the 95% detection point for each test i.e. 10,240 HCV genomes in the original donation sample or 2,560 HIV-1 genomes in the original sample.

We have conducted stability studies which show that HCV and HIV-1 are stable for up to 45 days of storage at -15°C, up to seven days at -5°C or +8°C, up to 10 hours at +24°C and after up to five freeze-thaw cycles.

We conducted prospective clinical trials to demonstrate the safety and effectiveness of this screening programme and the NGI UltraQual™ assay in the detection of HCV and HIV-1 in Source Plasma donations. The clinical trial was conducted in accordance with applicable Federal regulations under the oversight of an independent Institutional Review Board. All donations were tested for HCV and HIV-1 following the pooled testing algorithm. All donations identified as positive were identified, and the donor was contacted, counselled and asked to enroll in the clinical follow-up study. All enrolled donors were followed until seroconversion for an independent HCV or HIV-1 marker of infection or for six months. Study sites provided approximately 340,000 donations over approximately a four-month period.

Ten HIV-1 and 85 HCV window period donations (PCR positive, antibody negative) were identified during the clinical trial period, respectively. The NGI UltraQual™ PCR test, used to test pooled samples, significantly reduced the window period for HIV-1 relative to detection by either HIV-1 p24 antigen or HIV-1 antibody and for HCV relative to detection by HCV antibody.

We conducted a supplemental sensitivity study using HIV-1 or HCV suspect positive donations from seroconversion panels, suspect positive samples and other sources to increase the number of HCV and HIV-1 window period units. All samples were tested by the NGI SuperQuant™ quantitative RT-PCR test for the suspect virus. If negative, the sample was re-tested undiluted with the NGI UltraQual™ test. If positive by either the SuperQuant™ or the UltraQual™ assays, the sample was re-tested after diluting 1:512 in negative pooled plasma. Samples were also tested for their respective viral serological markers without dilution.

The results of the supplemental sensitivity study were combined with the results from the prospective clinical study to provide adequate numbers for the estimation of the clinical sensitivity of the tests. The NGI UltraQual™ HCV test of pooled Source Plasma donations showed significantly greater sensitivity than the HCV antibody test on undiluted samples. The NGI UltraQual™ HIV-1 test of pooled Source Plasma donations showed significantly greater sensitivity than the HIV-1 p24 antigen test, the HIV-1 antibody test or the combination of HIV-1 p24 antigen and HIV-1 antibody test on undiluted samples. There were no samples that were HIV-1 p24 antigen positive undiluted and HIV-1 PCR negative on dilution.

The amount of virus removed during the clinical trial period was estimated by using the NGI SuperQuant™ assay of the positive samples. Six HIV-1 RNA positive, HIV Ab and p24 Ag negative units, containing 1.2×10^8 HIV-1 genome copies and 75 HCV RNA positive, HCV Ab negative units with normal ALT levels, containing 8.5×10^{11} HCV genome copies were removed from plasma manufacturing pools. While the HIV-1 units would have been detected and removed by the inven-

tory hold, lookback and qualified donor standards, a substantial amount of the HCV would not have been removed.

The HCV positive donors in the window period who were detected before seroconversion to any other HCV marker were counselled and referred for earlier treatment. Early detection and referral for treatment of infected individuals may have beneficial effects on the outcome of their HCV infection, although the results of such early treatment are as yet unknown. The testing of pooled samples in pools of up to 512 samples for HCV and HIV-1 using the NGI UltraQual™ RT PCR assays is a safe, effective and highly sensitive method for the detection, identification and removal of window period HIV-1 and HCV donations of Source Plasma.

Acknowledgements

We would like to acknowledge the cooperation of our colleagues William S. Craig, Lorraine B. Peddada, Charles Frisbie and Lolita Mercado at Alpha Therapeutic Corporation and Andrew Conrad, Peter Schmid and Jeffrey Albrecht, at the National Genetics Institute.

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