SUMMARY OF BASIS FOR APPROVAL

Reference No.:	103902 (STN)	Product Name:	NGI UltraQual™ HIV-1 RT-PCR assay
Applicant:	National Genetics Institute 2440 S. Sepulveda Blvd., Room 130 Los Angeles, CA 90064	Proper Name:	Human Immunodeficiency Virus Type 1 Reverse Transcription - Polymerase Chain Reaction

I. Indications for Use

The NGI UltraQual™ Human Immunodeficiency Virus, Type 1 (HIV-1) Reverse Transcription (RT) Polymerase Chain Reaction (PCR) assay, when used in combination with FDA approved pooling and resolution algorithms, is indicated for the qualitative detection of HIV-1 ribonucleic acid (RNA) in pools of human Source Plasma comprised of equal aliquots of not more than 512 individual plasma samples. This method may be used as an alternative to licensed HIV-1 p24 antigen tests for screening Source Plasma.

II. Brief Description of Test

NGI's UltraQual™ HIV-1 RT-PCR assay is an "in-house" test performed only by National Genetics Institute; no kit is sold. The assay involves extraction of nucleic acids by the addition of a mixture of guanidine isothiocyanate, phenol, chloroform, EDTA, DTT and an internal control synthetic RNA. The internal control RNA added to each sample serves as a control for the entire process including extraction, reverse transcription, amplification and detection. A non-reactive result cannot be released for a particular sample without successful detection of the internal control RNA within that sample. Following extraction, nucleic acids are further purified and concentrated by ethanol precipitation. Purified RNA is then reverse-transcribed using random hexadeoxyribonucleotides to prime complementary DNA (cDNA) synthesis prior to performing PCR. Aliquots of cDNA are then amplified by PCR in two types of HIV-1-specific reactions with distinct primer pairs. Each reaction also includes a primer pair specific for the internal control sequence. The UltraQual™ HIV-1 RT-PCR assay can be performed on plasma that has been subjected to a centrifugation step to concentrate viral particles prior to extraction or on plasma that has not been concentrated. Amplification reactions can be performed once per assay or in duplicate to achieve better sensitivity (see section IV below).

Following PCR amplification, specimens are electrophoresed on agarose gels, vacuum blotted and UV cross-linked onto nylon membranes. The membranes are then probed with HIV-specific digoxigenin-labeled probes. Unbound probe is washed away and an anti-digoxigenin antibody/alkaline phosphatase conjugate is used for colorimetric immunostaining of the HIV-specific PCR product. The presence of a dark, properly shaped and positioned band on the membrane signifies that HIV-1 nucleic acid was present in the specimen. After development of the HIV-probed membrane the results are recorded electronically by scanning the membrane and storing the image file. After scanning, the membrane is re-hybridized with an internal control-specific probe that binds to each location where material from a successful nucleic acid extraction/RT-PCR has been transferred.

The quality of the nucleic acid extraction and reverse transcription procedures as well as each of the independent amplification reactions and transfer procedures is assessed by

detection of the amplified internal control sequence. A sample may be judged non-reactive for HIV-1 only when the internal control sequence is detected in both an HIV-1 primer set 1 and an HIV-1 primer set 2 reaction. Samples are reported as reactive for HIV-1when either or both HIV-1-specific primer sets produce HIV-1-specific amplicons.

Assay Controls

In addition to the internal control contained in every sample, each run (60 spaces) includes a six positive and at least six negative controls. The positive controls contain known amounts of HIV-1 infected plasma calibrated using internationally recognized sources for reference material such as the National Institute for Biological Standards and Control (NIBSC) or the World Health Organization (WHO). These plasma-based positive controls simulate donor/patient specimens and are carried through the entire process, therefore monitoring all aspects of the testing including ultracentrifugation, extraction, reverse transcription, amplification and detection. These controls are included in every test run and thus provide effective monitoring of reagent stability and efficacy, nucleic acid recovery, reverse transcription, amplification, transfer and detection efficiency. Additionally, negative controls are co-analyzed to monitor for nucleic acid contamination.

III. Manufacturing and Controls

Acceptance criteria and specifications have been established for all reagents and controls. Several reagents have been identified as crucial and requiring an increased level of scrutiny prior to release. These include reverse transcriptase, Taq DNA polymerase, deoxynucleotide triphosphates, primers and probes. In addition to meeting all of NGI's specifications, which include functional testing with in-house panels containing varying levels of HIV-1 RNA and a verification of detection of HIV-1 RNA levels approaching the limit of detection, each lot of these reagents must meet the performance requirements of the FDA/CBER HIV-1 NAT lot release panel.

IV. Performance Characteristics

Validations were conducted according to the International Conference on Harmonization-3 Guideline for Validation of Analytical Methods.

A. Analytical Sensitivity

The analytical sensitivity of the NGI HIV-1 RT-PCR assay was established by an analytical methods validation study, conducted according to the ICH-3 guideline. The analytical sensitivity of the NGI HIV-1 RT-PCR assays was defined as the viral RNA concentration at which the presence of viral RNA can be detected at least 95% of the time. Table I summarizes the analytical sensitivity for two test formats. In addition, the table also provides a 50% detection point (the RNA level at which the samples could be detected as reactive 50% of the time). All analytical sensitivity values are expressed in HIV-1 genome copies/mL. The analytical sensitivity of the NGI HIV-1 RT-PCR assay for the individual units, primary pools and master pools of plasma are presented in Table II.

Table I: Summary of Sensitivity Study Results for the 2 mL, 4-Reaction and 1 mL, 2-Reaction NGI UltraQual™ HIV-1 RT-PCR Assays

Assay	50% Detection Point		Analytical Sensitivity (Estimated 95% Detection Point)	
	(RNA Copies/mL)	(IU/mL)*	(RNA Copies/mL)	(IU/mL)*
2-mL, 4-Reaction	1.4	1.4	4	4
1-mL, 2-Reaction	2.3	2.3	12.7	12.7

* Conversion factor: 1 International Unit (IU) = 1 ± 0.5 copies
(NIBSC International Collaborative Study on HIV-1, H. Holmes, *et. al.*)

Table II. Sensitivity of the NGI UltraQual™ HIV-1 RT-PCR Assays for HIV-1 in Master Pools, Primary Pools and Individual Samples

Pool	50% Detection Point		Analytical Sensitivity (Estimated 95% Detection Point)	
	(RNA Copies/mL)	(IU/mL)*	(RNA Copies/mL)	(IU/mL)*
Master Pool (Up to 512 samples)	717	717	2,048	2,048
Primary Pool (Up to 64 samples)	147	147	813	813
Individual Samples	2.3	2.3	12.7	12.7

* Conversion factor: 1 International Unit (IU) = 1 ± 0.5 copies
(NIBSC International Collaborative Study on HIV-1, H. Holmes, *et. al.*)

As revealed in Table I, the analytical sensitivity for 2 mL, 4-reaction and 1 mL, 2-reaction formats are 4 and 12.7 copies/mL, respectively. The analytical sensitivity for master pool testing (2 mL, 4-reaction testing format) is 2,048 copies/mL (2,048 IU/mL) while primary pool testing (1 mL, 2-reaction testing format) is 813 copies/mL (813 IU/mL).

The NGI UltraQual™ HIV-1 RT-PCR test detects all known HIV-1 groups, including 9 out of 9 group O samples tested, and one transcript of each of HIV-1 group M subgroups A, B, C, D, E, F, G, H, I, and J. The NGI UltraQual™ HIV-1 RT-PCR test does not detect HIV-2.

B. Precision

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision may be performed at three levels: repeatability, intermediate precision and reproducibility.

Repeatability

Repeatability expresses the precision under the same operating conditions over a short interval of time. Repeatability is also termed intra-assay precision.

Repeatability of the NGI UltraQual™ HIV-1 RT-PCR assay was determined by separate analyses of the sensitivity data obtained using each of three plasma pools.

Table III. Summary of Repeatability Study Results for the 2 mL, 4-Reaction Assay

Plasma Pool	50% Detection Point (RNA Copies/mL)	95% Confidence Intervals for the Estimated 50% Detection Point (RNA Copies/mL)
1	1.6	1.0 - 2.4
2	1.1	0.8 - 1.5
3	1.7	1.2 - 2.4
Pooled 1, 2 & 3	1.4	1.2 - 1.8

The estimated 50% detection points for each of the three plasma pools used for dilutions are nearly identical (1.1, 1.6 and 1.7 HIV-1 RNA copies/mL) and the 95% confidence intervals for each value overlap with each other as well as with that for the estimated 50% detection points of the assay of the pools.

Intermediate precision

Intermediate precision expresses intra-laboratory variation: different days, different analysts, different critical reagents, etc.

Intermediate precision was determined by repeated analysis of samples containing 100 HIV-1 copies/mL with the 1 mL, 2-reaction UltraQual™ HIV-1 RT-PCR assay. The assays were performed 30 times by two or more analysts utilizing two different lots of one or more selected critical reagents.

Table IV. Summary of Intermediate Precision Study Results for the 1 mL, 2-reacton Assay

Analyst	Reagent Lots	Reactivity
1	1	30/30=100%
1	2	30/30=100%
2	1	30/30=100%
2	2	30/30=100%

All samples were found reactive for HIV-1 indicating that varying either reagent lot or analysts did not affect the ability of the assay to detect 100 copies/mL of HIV-1 RNA.

Reproducibility

Reproducibility expresses the inter-laboratory precision.

Reproducibility is not applicable since this assay is performed only at NGI. However, NGI participates in international collaborative studies with NIBSC and CLB among others, to establish the values for international standards.

C. Assay Specificity

Assay specificity measures the ability of the assay to detect the RNA sequence of interest in the presence of genomic sequences of other microorganisms.

The assay specificity of the NGI UltraQual™ HIV-1 RT-PCR assay is based on the specificity of the primers and probes used. The identity of the amplified product is confirmed by the ability of sequence-specific probes to bind to the amplified material and by the electrophoretic mobility of the amplicon detected. The assay specificity was

evaluated by analyzing plasma pool samples containing 1,000 copies/mL of HAV RNA, HBV DNA, HCV RNA, EBV DNA or CMV DNA in the NGI UltraQual™ HIV-1 RT-PCR assay six times utilizing the 2 mL, 4-reaction assay.

The results for all assay specificity samples were non-reactive for HIV-1 and reactive for the added virus by the corresponding PCR test.

D. Analytical Specificity

Analytical specificity was evaluated by analyzing 100 HIV-1 non-reactive plasma pools utilizing the 2 mL, 4-reaction assay. All specificity samples were non-reactive for HIV-1 RNA.

E. Interference

An interference study was designed to evaluate whether there was any substance present in the plasma samples that might affect the detection of HIV-1 RNA with this assay. The study involved the testing of 12 contrived samples containing 100 HIV-1 copies/mL in each of the following matrices. All specimens were detected as reactive with the NGI UltraQual™ HIV-1 RT-PCR assay, utilizing the 1 mL, 2-reaction assay configuration.

- hemolyzed plasma
- lipemic plasma
- icteric plasma
- bacteremic plasma
- acid citrate plasma
- sodium citrate plasma
- citrate/phosphate/dextrose plasma
- plasma containing HAV
- plasma containing HBV
- plasma containing HCV
- plasma containing CMV
- plasma containing EBV
- plasma containing nucleoside analogues
- plasma containing anti-HIV-1 antibodies

V. Summary of Clinical Data

Clinical sensitivity and specificity were determined for the NGI UltraQual™ HIV-1 RT-PCR assay and the master pool/primary pool/individual donation resolution testing algorithm (hereafter referred to as the resolution algorithm) by screening 342,729 donations from all plasma donors from 33 donor centers during a three-and-one-half-month clinical study.

The clinical specificity of the NGI UltraQual™ HIV-1 RT-PCR assay was 100%, with no false positive pools confirmed by additional testing. The specificity exceeds that of licensed p24 antigen tests used for blood and plasma donor screening.

The rate of HIV-1 RT-PCR reactive donations in the clinical study was 0.0053% (18 donations detected from a total of 10 donors). The rate of HIV-1 RT-PCR reactive, HIV-1 p24 antigen reactive and HIV-1/2 antibody non-reactive window-period donations was 0.0029% (10 donations detected from 4 donors). The rate of HIV-1 RT-PCR reactive, HIV-1/2 antibody non-reactive and HIV-1 p24 antigen non-reactive donations was 0.0018% (6 donations detected from the same 4 donors). The rate of HIV-1 RT-PCR reactive, HIV-1 p24 antigen and antibody non-reactive donors was estimated to be 0.0083% (4 of approximately 48,000 donors).

Thus, during the clinical study, the use of the NGI UltraQual™ HIV-1 RT-PCR assay and resolution algorithm resulted in the identification of six HIV-1 reactive units from four donors that were HIV-1 p24 antigen and HIV-1 antibody non-reactive. These six units were prevented from entering manufacturing pools as a result of HIV-1 RT-PCR testing. These data indicate that conducting nucleic acid testing using the NGI UltraQual™ HIV-1

RT-PCR assay provided an additional level of safety for the manufacturing of plasma derivatives.

There was no instance in the screening of 342,729 plasma donations in which a sample from an HIV-1-positive donation was reactive by HIV-1 p24 antigen testing and non-reactive by HIV-1 RT-PCR even though HIV-1 RT-PCR was performed on pools representing up to 512 samples and HIV-1 p24 antigen testing was performed on undiluted samples.

Clinical sensitivity during the early stages of HIV-1 infection and the preseroconversion window period was also determined by testing a larger sample set composed of the samples from the clinical study, samples obtained from retrospective testing of frozen plasma samples in ATC (Alpha Therapeutic Corporation) inventory from donors subsequently identified as HIV-1 infected, and samples from commercially available HIV-1 seroconversion panels. The sample set for evaluation of clinical sensitivity in early HIV-1 infection consisted of 288 samples from 67 donors with early HIV-1 infection that were reactive by at least one HIV-1 assay (HIV-1 RT-PCR, HIV-1 p24 antigen, or HIV-1/2 antibody). Of these 288 samples, 185 were HIV-1/2 antibody non-reactive and hence could be used to assess sensitivity in the preseroconversion window period.

The clinical sensitivity of pooled HIV-1 RT-PCR testing in this larger early HIV-1 infection sample set was 84.7% (244 of 288 HIV-1-positive samples detected; 95% CI: 80.5 - 88.9%), whereas that of the HIV-1 p24 antigen assay was 51.7% (149 of 288 HIV-1-positive samples detected; 95% CI: 45.9 - 57.4%). All 149 HIV-1-positive samples detected by HIV-1 p24 antigen testing were also detected by HIV-1 RT-PCR testing; not one HIV-1 p24 antigen reactive sample from an HIV-1-positive donor was missed by pooled HIV-1 RT-PCR. The sensitivity of pooled HIV-1 RT-PCR testing in the preseroconversion window period sample set was 80.5% (149 of 185 HIV-1 positive samples detected; 95% CI: 74.8 - 86.2%), whereas that of the HIV-1 p24 antigen assay was 52.4% (97 of 185 HIV-1-positive samples detected; 95% CI: 45.2 - 59.6%). The increased sensitivity of the NGI UltraQual™ HIV-1 RT-PCR assay and resolution algorithm compared to HIV-1 p24 antigen testing of individual donations was statistically significant in both the preseroconversion window period and the larger early HIV-1 infection sample sets ($p < 0.001$ in both circumstances). These results indicate that screening of plasma pools with the NGI UltraQual™ HIV-1 RT-PCR assay and resolution algorithm is more sensitive than screening of individual donations by the HIV-1 p24 antigen assay in early HIV-1 infection.

Another measure of the clinical sensitivity of pooled HIV-1 RT-PCR is its performance in reducing the length of the HIV-1 infectious window period relative to HIV-1 p24 antigen testing. Data from 43 donors indicate that the NGI UltraQual™ HIV-1 RT-PCR assay performed on pooled plasma samples reduced the HIV-1 infectious window period relative to HIV-1 p24 antigen testing by a minimum of 4.1 ± 3.1 days.

HIV-1 RT-PCR clinical sensitivity was directly related to HIV-1 RNA copy number. In the clinical study, 100% of 130 master pools (or diluted samples designed to simulate master pools) with calculated HIV-1 RNA concentrations of $\geq$5 copies/mL were reactive by HIV-1 RT-PCR. These clinical sensitivity data were consistent with the results of an analytic sensitivity study, which demonstrated an assay analytical sensitivity of 5 RNA copies/mL at 95% detectability for the 2-mL, 4-reaction testing format. Data from the analytical sensitivity study were subsequently re-analyzed using the Probit method resulting in a revised estimate of 4 copies/mL for the 95% detection cutoff (section IV).

These combined data indicate that the margin of safety of Source Plasma for further manufacture will be increased by the use of the NGI UltraQual™ HIV-1 RT-PCR assay and resolution algorithm. In addition, the data also support that discontinuation of the HIV-

1 p24 antigen assay after implementation of NGI UltraQual™ HIV-1 RT-PCR testing of plasma pools will not compromise safety of Source Plasma, since HIV-1 p24 antigen testing did not detect any HIV-1 positive sample that was not detected by the NGI UltraQual™ HIV-1 RT-PCR assay and resolution algorithm.

The data show that the substitution of the NGI UltraQual™ HIV-1 RT-PCR assay on pools of not more than 512 samples of Source Plasma donations for the HIV-1 p24 antigen test is a safe and effective procedure. The data further show that the NGI UltraQual™ HIV-1 RT-PCR test is at least as sensitive as the HIV-1 p24 antigen test of individual samples.

VI. Benefit Analysis

The NGI UltraQual™ Human Immunodeficiency Virus Type 1 (HIV-1) Reverse Transcription (RT) Polymerase Chain Reaction (PCR) assay is an *in vitro* nucleic acid amplification test (NAT) for the detection of HIV-1 ribonucleic acid (RNA) in pooled human Source Plasma. The NGI UltraQual™ HIV-1 RT-PCR assay, when used in combination with FDA approved pool size, pooling and resolution algorithms and confirmation procedures, is a safe and effective donor screening procedure for HIV-1.

LICENSING REVIEW COMMITTEE

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