BioLife Plasma Services L.P.
Division of BAXTER HEALTHCARE CORPORATION

SUMMARY OF BASIS FOR APPROVAL

125100/0/0 (STN)

HIQ-PCR™ HIV-1 RT-PCR assay

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Human Immunodeficiency Virus Type 1 Reverse Transcription - Polymerase Chain Reaction

I. Indications for Use

The HIQ-PCR™ 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.

II. Brief Description of Test

The HIQ-PCR™ HIV-1 RT-PCR assay is an "in-house" test performed only by Baxter Healthcare International; no kit is sold. Each plasma sample is extracted by using the --(b)(4)-- spin column manufactured by -(b)(4)- using the -(b)(4)- procedure. The samples are treated with a --(b)(4)-- --(b)(4)-- to lyse viral particles and the complexes of nucleic acids formed are not degradable by RNase. The complexes are ---(b)(4)--- and -----(b)(4)----- in a ---------(b)(4)----------.

After adjusting the buffering conditions to provide optimum binding of the nucleic acids to a -(b)(4)- matrix, the isolated nucleic acid binds to a --(b)(4)---based membrane in a spin column. Proteins and other potential inhibitors are washed away. The purified nucleic acid is ----------(b)(4)----------.

For the reverse transcription and amplification of viral RNA, a "---------(b)(4)---------" system is used, which combines the reverse transcription and PCR ---(b)(4)---. The RT-activity of rTth polymerase is triggered by the addition of ------(b)(4)-----.

After completion of the reverse transcription of RNA to complementary DNA (cDNA), a buffer change introduces ------(b)(4)------ which trigger the DNA-polymerase activity ---(b)(4)--- enzyme and the PCR takes place. To improve the specificity of the PCR, a "-(b)(4)-" technique is used. The ----------(b)(4)---------- in an ----------(b)(4)---------- with -----(b)(4)-----. This ----(b)(4)--- is also specific for the internal control sequence.

The detection of the amplified material is based on a ----------(b)(4)---------- of the ---(b)(4)--- by denaturing ----------(b)(4)---------- coupled with detection by -----(b)(4)---- -----(b)(4)----.

The assay involves the addition of a -----(b)(4)------ internal control into each sample. This internal control serves to monitor the entire process including extraction, reverse transcription, amplification and detection. A sample may be judged non-reactive for HIV-1 only if the ----------(b)(4)---------- ------(b)(4)------- within the sample is detected. Samples are reported as reactive (positive) for HIV-1 when the HIV-1-specific primer set produces HIV-1 -------(b)(4)--------.

Assay Controls

In addition to the internal control contained in every sample, each run (up to -(b)(4)- samples) includes -(b)(4)- virus-positive and -(b)(4)- virus-negative controls. The positive controls contain known amounts of ---------(b)(4)---------- HIV-1 virus particles and have been calibrated using the ----------(b)(4)----------. These plasma-based positive controls simulate donor/patient specimens and are carried through the entire process in order to evaluate all aspects of the testing including extraction, reverse transcription, amplification and detection.

These controls are included in every test run and provide effective evaluation of reagent stability and efficacy, nucleic acid recovery, reverse transcription, amplification, transfer and detection efficiency. In addition, 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 ----------(b)(4)----------. In addition to meeting all of Baxter's specifications, which include functional testing with in-house panels, when each lot of these reagents is used in the complete HIQ-PCR™ HIV-1 RT-PCR assay to test the FDA/CBER HIV-1 NAT lot release panel, that assay run must meet the performance requirements of the lot release panel.

A Pre-License Inspection of the BioLife Plasma Services facility in ------(b)(4)----- was performed from August 29 to September 1, 2005.

IV. Performance Characteristics

Validations were conducted according to the International Conference on Harmonization- (Q2A, Q2B) Guideline for Validation of Analytical Methods.

A. Analytical Sensitivity

The analytical sensitivity of the HIQ-PCR™ HIV-1 RT-PCR assay was established by an analytical methods validation study. The estimated 95% detection point (LOD) is the concentration of HIV-1 that is estimated to be detected 95% of the time by the test method and is considered to be the analytical sensitivity at the individual sample level. The LOD for the HIQ-PCR™ HIV-1 RT-PCR assay was determined using a dilutional panel prepared from a commercially available clinical specimen for which the HIV-1 RNA concentration had been previously determined in RNA Copies/mL using an FDA approved viral load assay (Table #1).

The LOD was calculated using the logistic regression model by ----------(b)(4)----------(b)(4)---. This value is expressed in International Units [IU]/mL. Based on an extraction of a -(b)(4)- mL sample, the analytical sensitivity is -(b)(4)- Copies/mL and the conversion factor of 1 IU = 1 +/- 0.5 Copies (NIBSC International Collaborative Study on HIV-1, H. Holmes, et. al.), the analytical sensitivity is -(b)(4)- IU/mL. This corresponds to -(b)(4)- IU/mL in the individual donation when tested in a pool of 512 specimens. These data are summarized in Table #1. This meets the current FDA recommended standard for licensure of 100 IU/mL for the pool and 10,000 IU/mL for the individual donation (http://www.nibsc.ac.uk/partners/SoGAT/2004_Presentations.html).

Table #1: Analytical Sensitivity for HIQ-PCR™ HIV-1 RT-PCR Assay Using a Dilutional Panel from a Clinical Specimen

Panel Members	Clinical Specimen HIV Panel Test Results							
	1 100 IU/mL	2 50 IU/mL	3 25 IU/mL	4 12 IU/mL	5 6 IU/mL	6 3 IU/mL	7 1 IU/mL	8 0 IU/mL
HIQ-PCR™ HIV-1 RT-PCR Assay # reactive/# replicates	24/24	24/24	24/24	23/24	22/24	14/24	6/24	0/24

Members of an FDA/CBER HIV-1 RNA Panel were processed using the HIQ-PCR™ HIV-1 RT-PCR assay. As shown in Table #2, this assay detected 100% of all positive members ranging from 100-500 Copies/mL. The HIQ-PCR™ HIV-1 RT-PCR assay detected 8 of 9 replicates at 50 Copies/mL and 3 of 9 replicates at 10 Copies/mL. All negative samples were non-reactive. These data support the analytical sensitivity that was determined above.

Table #2: Analytical Sensitivity for HIQ-PCR™ HIV-1 RT-PCR Assay Using a FDA/CBER HIV-1 RNA Panel

Panel Members	B9 500 Copies/mL	B5 100 Copies/mL	B6 50 Copies/mL	B2 10 Copies/mL	B4 0 Copies/mL
HIQ-PCR™ HIV-1 RT-PCR Assay # reactive/# replicates	9/9	9/9	8/9	3/9	0/9

* Conversion Factor: 1 Copy = 0.5 IU (International Collaborative Study on HIV-1, H. Holmes, et. al,)

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: intra-assay precision, inter-assay precision and reproducibility.

Intra-Assay Precision (Repeatability)

Intra-assay precision expresses the precision under the same operating conditions over a short interval of time. Intra-assay precision is also termed repeatability. Intra-assay precision of the HIQ-PCR™ HIV-1 RT-PCR assay was determined by -(b)(4)- different operators performing -(b)(4)- assays on -(b)(4)- different virus-positive preparations (-----(b)(4)------). Of these preparations, each of the -(b)(4)- operators subjected -(b)(4)- identical vials to HIQ-PCR, including extraction, reverse transcription, amplification, and detection. The results of intra-assay variability are shown in Table #3.

Table #3: Assessment of Intra-Assay Precision (Repeatability)

[----------(b)(4)----------]

The results demonstrate 100% intra-assay repeatability at different levels of virus concentration.

Inter-Assay Precision (Intermediate Precision)
Inter-assay precision expresses intra-laboratory variations: different days, different analysts, etc. Inter-assay precision is also termed intermediate precision.

Inter-assay precision was determined by repeated analysis of virus-spiked samples that were subjected to the whole analytical procedure, including all steps from extraction to detection. Data for each nominal value are the result of -(b)(4)- repetitions tested on -(b)(4)- different days by multiple analysts. The results are summarized in Table #4.

Table #4: Inter-Assay Precision (Intermediate Precision)

Virus Concentration (IU/mL)	HIV-1 Detected (Positive Tests)	n
-(b)(4)-	-(b)(4)-	-(b)(4)-
-(b)(4)-	-(b)(4)-	-(b)(4)-

The results demonstrate 100% inter-assay repeatability at different levels of virus concentration.

Reproducibility
Reproducibility expresses the precision between laboratories (inter-laboratory). Reproducibility is not applicable since this assay is performed only at Baxter. However, Baxter participates in regular proficiency testing programs (----------(b)(4)----------).

C. Assay Specificity
Assay specificity measures the ability of the assay to detect the RNA sequence of interest in the presence of other genomic sequences. The assay specificity of the HIQ-PCR™ HIV RT-PCR assay is based on the ----------(b)(4)---------- and the ----------(b)(4)----------. The identity of the amplified product is confirmed by the ----------(b)(4)----------. The assay specificity was evaluated by analyzing ---------(b)(4)--------- previously found to be non-reactive for HIV-1 RNA containing -(b)(4)- IU/mL or more of ----------(b)(4)---------- in the HIQ-PCR™ HIV-1 RT-PCR assay. A positive signal was detected only in the presence of HIV-1. No other microorganism tested gave rise to a positive signal.

D. Analytical Specificity
Analytical specificity was evaluated by analyzing -(b)(4)- HIV-1 plasma pools that were composed of donations determined to be non-reactive by HIV antibody screening with assays licensed by the EU and/or FDA. All analytical specificity samples tested non-reactive for HIV-1 RNA.

E. Interference

An interference study was designed to evaluate whether any substance likely to be present in the plasma samples might affect the detection of HIV-1 RNA with this assay. The study involved the testing of contrived samples containing a low level of HIV-1 virus (495 IU/mL) in the presence of the substances listed below:

- ---------(b)(4)----------
- ---------(b)(4)----------
- ---------(b)(4)----------
- ---------(b)(4)---------
- ---------(b)(4)-----
- ---------(b)(4)--
- ---------(b)(4)----------
- ---------(b)(4)----------
- ---------(b)(4)----------
- ---------(b)(4)----------

Each sample with a potentially interfering substance was found reactive in the HIQ-PCR™ HIV-1 RT-PCR assays, indicating that the substances listed do not interfere with the ability of the HIQ-PCR™ HIV-1 RT-PCR assay to detect HIV-1 RNA.

F. Subtype Detection

The ability of the HIQ-PCR™ HIV-1 RT-PCR primers and probes to detect various HIV-1 subtypes was assessed by testing available and certified HIV-1 subtype samples at low concentration (e.g. ----------(b)(4)----------). Results demonstrate that HIQ-PCR™ HIV-1 RT-PCR reliably detects HIV-1 M subtypes at low concentrations (Table #5). HIQ-PCR™ HIV-1 RT-PCR does not detect HIV-1 Group O RNA and HIV-2 RNA.

Table #5: HIV Subtypes Evaluated for Reactivity with the HIQ-PCR™ HIV RT-PCR Assay

Subtype	Number of Samples
A	4
B	4
C	3
D	6
E	3
F	5
G	2
H	1
AE	1
AG-GH	1

V. Summary of Clinical Data

A clinical study was conducted to evaluate the sensitivity and specificity of the HIQ-PCR™ HIV-1 RT-PCR assay used to detect HIV-1 RNA in pooled Source Plasma samples.

This clinical study was also intended to evaluate the pooling algorithm and resolution testing algorithm (hereafter referred to as the resolution testing algorithm). A total of 1,700,000 donations from all plasma donors from 31 donor centers were screened during a clinical study from April 2001 through June 2002.

These donations were first pooled into primary pools (-(b)(4)- units per primary pool), which were subsequently pooled to form master pools (512 units per master pool). No false reactive results were obtained for the master pools. The clinical specificity of the HIQ-PCR™ HIV-1 RT-PCR assay for the testing of 1.7 million donations was 100%.

No HIV-1 PCR reactive units/anti-HIV-1 EIA non-reactive were detected in the testing of 1.7 million donations in the time frame of the clinical study. Shortly after conclusion of the study however, a HIV-1 reactive unit that was anti-HIV-1 EIA non-reactive was detected which, in the absence of HIV-1 RT-PCR testing, would have been used in the production of plasma derivatives. This donor's infection with HIV-1 was confirmed upon follow up testing that showed subsequent seroconversion. The HIQ-PCR™ HIV-1 RT-PCR detected the presence of HIV-1 in a positive sample pool 7 days prior to detection by the HIV-1 p24 antigen assay and 14 days prior to detection by anti-HIV-1/2 EIA.

In the clinical trial protocol, all 50 master pools pooled including 30 spiked positive and 20 negative samples were correctly identified (= 90% detection rate with 95% confidence).

In the time frame of the clinical protocol and several supporting studies, a total of 71 confirmed positive clinical samples were tested and detected at the masterpool dilution of 1:512. The data show that the HIQ-PCR™ HIV-1 RT-PCR test on pools of not more than 512 samples is safe and effective when used in combination with the FDA approved pooling algorithm and resolution testing algorithm.

VI. Benefit Analysis

The HIQ-PCR™ 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 HIQ-PCR™ HIV-1 RT-PCR assay, when used in combination with FDA-approved pool size, pooling and resolution algorithms, is a safe and effective donor screening procedure for HIV-1 RNA in pools of not more than 512 samples.