SUMMARY OF BASIS FOR APPROVAL

Reference No.:	103868 (STN)	Product Name:	NGI UltraQual™ HCV RT-PCR assay
Applicant:	National Genetics Institute 2440 S. Sepulveda Blvd., Room 130 Los Angeles, CA 90064	Proper Name:	Hepatitis C Virus Reverse Transcription - Polymerase Chain Reaction

I. Indications for Use

The NGI UltraQual™ Hepatitis C Virus (HCV) 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 HCV 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

NGI's UltraQual™ HCV 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 HCV-specific reactions with distinct primer pairs. Each reaction also includes a primer pair specific for the internal control sequence. The UltraQual™ HCV 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 an HCV-specific digoxigenin-labeled probe. Unbound probe is washed away and an anti-digoxigenin antibody/alkaline phosphatase conjugate is used for colorimetric immunostaining of the HCV-specific PCR product. The presence of a dark, properly shaped and positioned band on the membrane signifies that HCV nucleic acid was present in the specimen. After development of the HCV-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 HCV only when the internal control sequence is detected in both an HCV

primer set 1 and an HCV primer set 2 reaction. Samples are reported as reactive for HCV when either or both HCV-specific primer sets produce HCV specific amplicons.

Assay Controls

In addition to the internal control contained in every sample, each run (60 spaces) includes six positive and at least six negative controls. The positive controls contain known amounts of HCV 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 HCV RNA and a verification of detection of HCV RNA levels approaching the limit of detection, each lot of these reagents must meet the performance requirements of the FDA/CBER HCV 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 UltraQual™ HCV RT-PCR assay was established by an analytical methods validation study. The estimated 95% detection point is the concentration of HCV 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.

Table I summarizes the analytical sensitivity and 50% detection points (the concentration of HCV that is estimated to be detected 50% of the time by the test method), for the 4-reaction and 2-reaction assay formats. All values are expressed in HCV genome copies/mL. The analytical sensitivities for the 4-reaction and 2-reaction formats, both starting with extraction from 1.0 mL of sample, are 17.2 and 26.0 copies/mL, respectively. The 50% detection points were 3.1 and 6.1 copies/mL for the 4-reaction and 2-reaction formats, respectively.

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

Assay	50% Detection Point		Analytical Sensitivity (Estimated 95% Detection Point)	
	(Copies/mL)	(IU/mL)*	(Copies/mL)	(IU/mL)*
1 mL, 4-Reaction	3.1	1.2	17.2	6.9
1 mL, 2-Reaction	6.1	2.4	26.0	10.4

* Conversion factor: 1 International Unit (IU) = 2.5 copies (J.M. Pawlotsky, *et. al.* - Hepatology 2000 Sep;32 (3):654-9). Note: estimates for the conversion factor range as high as 4 copies/IU (NIBSC International Collaborative Study on HCV, J. Saldanha, *et. al*. – Vox Sang 2000; 78(4):217-224.

The primary pools consist of -- units per pool and are tested using 2-reaction format. Thus, the analytical sensitivity for primary pool testing is estimated to be 1,664 copies/mL. Similarly, the analytical sensitivity for master pools, which are tested using 4-reaction format, is 8,806 copies/mL as shown in Table II.

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

Assay	50% Detection Point		Analytical Sensitivity (Estimated 95% Detection Point)	
	(Copies/mL)	(IU/mL)*	(Copies/mL)	(IU/mL)*
Master Pool (Up to 512 samples)	1,588	635	8,806	3,522
Primary Pool (Up to -- samples)	390	156	1,664	666
Individual Samples	6.1	2.4	26.0	10.4

* Conversion factor; 1 International Unit (IU) = 2.5 copies (J.M. Pawlotsky, *et. al.* - Hepatology 2000 Sep;32 (3):654-9). Note: estimates for the conversion factor range as high as 4 copies/IU (NIBSC International Collaborative Study on HCV, J. Saldanha, *et. al.* – Vox Sang 2000; 78(4):217-224.

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™ HCV 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 1 mL, 4-Reaction Assay

Plasma Pool	50% Detection Point (Copies/mL)	95% Confidence Intervals for the Estimated 50% Detection Point (Copies/mL)
1	3.4	2.2 - 5.3
2	3.1	2.0 - 4.9
3	2.9	1.8 - 4.6
Pooled 1, 2 & 3	3.1	2.4 - 4.1

The estimated mean sensitivity for each of the three individual pools is nearly identical (2.9, 3.1 and 3.4 HCV genome copies/mL) and each value is within the estimated 95% confidence intervals for the estimated mean sensitivities of the assay of the pools.

Intermediate precision

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

Intermediate precision was determined by repeated analysis of samples containing 100 HCV copies/mL with the 1 mL, 2-reaction UltraQual™ HCV 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-Reaction Assay

Analyst	Reagent Lots	Number Reactive
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 HCV RNA indicating that varying either reagent lots or analysts did not affect the ability of the assay to detect 100 copies/mL of HCV RNA.

Reproducibility

Reproducibility expresses the precision between laboratories (inter-laboratory).

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 other genomic sequences.

The assay specificity of the NGI UltraQual™ HCV 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, HIV RNA, EBV DNA or CMV DNA in the NGI UltraQual™ HCV RT-PCR assay six times utilizing the 1 mL sample, 4-reaction test.

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

D. Analytical Specificity

Analytical specificity was evaluated by analyzing 100 HCV-non-reactive plasma pools utilizing the 1 mL, 4-reaction assay. All analytical specificity samples tested non-reactive for HCV 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 HCV RNA with this assay. The study involved the testing of contrived samples containing 100 HCV copies/mL in the following matrices using 1 mL, 2-reaction assay configuration; 12 assays were performed for each category of matrix.

- 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 HIV
- plasma containing CMV
- plasma containing EBV
- plasma containing nucleoside analogues
- plasma containing anti-HCV antibodies

Each interference sample was found reactive in all 12 NGI UltraQual™ HCV RT-PCR assays, utilizing the 1 mL, 2-reaction assay configuration indicating that the substances listed do not significantly interfere with the ability of the UltraQual™ HCV RT-PCR assay to detect HCV RNA.

F. Genotype Detection

The ability of the UltraQual™ HCV RT-PCR primers and probes to detect various HCV genotypes was assessed by testing *in vitro* RNA transcripts of cloned HCV genomes using a quantitative method that employs the same primers and probes as the UltraQual™ method. RNA transcripts were independently quantified by spectroscopy then diluted to three different concentrations for quantitative PCR. RNA was measured three times at each of the three concentrations, the results for each genotype were plotted against each other and linear regression analyses were performed.

Table V. Rho values from HCV Genotype Detection Efficiency Plots

Genotype	HCV 1a	HCV 1b	HCV 2a	HCV 2b	HCV 3a	HCV 4a	HCV 5a	HCV6a
HCV 1a	1.000	0.859	0.977	0.995	0.991	0.998	0.998	0.988
HCV 1b		1.000	0.896	0.906	0.920	0.887	0.892	0.909
HCV 2a			1.000	1.000	0.998	1.000	1.000	1.000
HCV 2b				1.000	0.999	0.999	1.000	1.000
HCV 3a					1.000	0.997	0.998	1.000
HCV 4a						1.000	1.000	0.999
HCV 5a							1.000	0.999
HCV 6a								1.000

The *r*-values obtained from the data for each HCV genotype plotted against the other HCV genotypes indicate no significant difference in detection efficiency for any particular HCV sequence analyzed.

V. Summary of Clinical Data

A clinical study was conducted to evaluate the sensitivity and specificity of the NGI UltraQual™ HCV RT-PCR assay used for detecting HCV 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 342,729 donations from all plasma donors from 33 donor centers were screened during a three and one-half month clinical study. These donations were first pooled into primary pools (-- units per primary pool), which were then pooled to form master pools (512 units per master pool).

The clinical specificity of the NGI UltraQual™ HCV RT-PCR assay for the testing of 342,729 donations, excluding 15 donations that were not tested for anti-HCV EIA, was 100%.

The use of the NGI UltraQual™ HCV RT-PCR assay and resolution algorithm resulted in the identification of 85 HCV-reactive units that were anti-HCV EIA non-reactive. Of these, 75 had normal ALT values and, in the absence of HCV RT-PCR testing, would have gone into the production of plasma derivatives.

The 85 HCV RT-PCR reactive, anti-HCV EIA non-reactive donations came from 22 donors. Of these, 13 donors were enrolled into the follow-up study and all subsequently developed HCV antibody. The NGI UltraQual™ HCV RT-PCR assay and resolution testing algorithm detected HCV infection in these donors earlier than did anti-HCV EIA testing with a calculated shortening of the preseroconversion HCV infectious window period by a mean of 57.0 days $\pm$ 14.1 days.

The rate of HCV RT-PCR reactive donations in the clinical trial was 0.031% (105 of 342,729). For donations that tested anti-HCV EIA non-reactive from both repeat and new donors, the HCV RT-PCR reactive rate, i.e. the rate of HCV window-period donations, was 0.025% (85/342,652). For anti-HCV EIA non-reactive donations from repeat donors this rate was 0.026% (81/316,039) and for donations from new donors, this rate was 0.015% (4/26,613). The rate of HCV RT-PCR reactive, anti-HCV EIA non-reactive donors was estimated to be 0.046% (22 of approximately 48,000 donors).

The clinical sensitivity of the NGI UltraQual™ HCV RT-PCR assay and resolution testing algorithm on pooled plasma donor samples was 70.7% (95% CI: 63.3-78.1%; 104 of 147 samples detected) assuming that all anti-HCV EIA reactive donations indicated HCV infection. This clinical sensitivity of HCV RT-PCR exceeds that of the anti-HCV EIA assay using the same sample set (42.2% with 95% CI: 34.2-50.2%; 62 of 147 samples detected).

The sensitivity of the NGI UltraQual™ HCV RT-PCR assay and resolution testing algorithm in detecting HCV infection during the preseroconversion window-period was 90.2 % (95% CI 87.0-93.4%; detection of 296 of 328 preseroconversion window-period samples) when compared to individual sample HCV RT-PCR testing. HCV RT-PCR sensitivity was directly related to HCV concentration (RNA copy number). In the clinical trial protocol, 100% of 289 master pools (or diluted samples designed to simulate master pools) with calculated HCV RNA concentrations of $\geq$ 20 copies/mL were detected. These clinical sensitivity data obtained on diluted window-period samples were consistent with the results of analytic sensitivity studies that demonstrated an assay sensitivity of approximately 20 RNA copies/mL at 95% detectability.

All anomalies during the clinical trial period were documented, investigated and resolved. The investigations for all anomalies have been completed and the anomalies for all pools were resolved. In each case, there was a formal investigation and report, which attempted to identify the cause and to suggest corrective action. The corrective actions instituted have resulted in an improved system with greater reliability.

The data show that the NGI UltraQual™ HCV RT-PCR test on pools of not more than 512 samples is safe and effective when used in combination with FDA approved pooling algorithm and confirmation procedure.

VI. Benefit Analysis

The NGI UltraQual™ Hepatitis C Virus (HCV) Reverse Transcription (RT) Polymerase Chain Reaction (PCR) assay is an *in-vitro* nucleic acid amplification test (NAT) for the detection of HCV ribonucleic acid (RNA) in pooled human Source Plasma. The NGI UltraQual™ HCV 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 HCV RNA in pools of not more than 512 samples.

LICENSING REVIEW COMMITTEE

Signature Date

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