Date: May 25, 2018

From: Krishnakumar Devadas, Chair of the Review Committee

BLA/ STN#: 125658.0

Applicant Name: National Genetics Institute

Date of Submission: May 08, 2017

MDUFA Goal Date: June 03, 2018

Proprietary Name: *UltraQual® Multiplex PCR Assay for HCV, HIV-1, HIV-2 and* HBV Hepatitis B Virus.

Established Name (common or usual name): NGI UltraQual® Multiplex PCR Assay for HCV, HIV-1, HIV-2 and HBV

Intended Use/Indications for Use:

The intended use of the National Genetics Institute's (NGI) UltraQual® Multiplex PCR Assay is to screen source plasma for HCV, HIV-1, HIV-2 and HBV. A maximum number of 512 aliquots from individual donations will be pooled using (b) (4) devices. The pools will then be tested using the multiplex assay to detect the presence of hepatitis C virus, human immunodeficiency virus types 1 and 2, and hepatitis B virus. Any pooled samples that test positive for any of these viruses will then be retested, per NGI's resolution algorithm, to identify the individual positive donation. The assay will discriminate between HCV, HIV-1, HIV-2 and HBV, but will not discriminate between HIV-1 Group M and Group O viruses.

Recommended Action: The Review Committee recommends approval of this product.

Review Office Signatory Authority: Nicole Verdun, M.D., Acting Director, OBRR/CBER

☐ **I concur with the summary review.**

☐ **I concur with the summary review and include a separate review to add further analysis.**

☐ **I do not concur with the summary review and include a separate review.**

The table below indicates the material reviewed when developing the SBRA.

Table 1: Reviews submitted

Document Title	Reviewer Name
Product Review(s)(DETTD) • *Clinical*	Julia Lathrop Krishnamurthy Konduru
• *Non-Clinical*	Andriyan Grinev Pawan Jain Jiangqin Zhao
• *Software / Instrumentation*	Yongqing Chen Lisa Simone
Statistical Review(s) • *Clinical*	Paul Hshieh
CMC Review • *CMC (DETTD)*	Cyrus Bett Guang Gao Luisa Gregory Swati Verma Xue Wang
• *Facilities Review (OCBQ/DMPQ)*	Sean Byrd Deborah Trout
• *Establishment Inspection Report(s) (OCBQ/DMPQ)*	Sean Byrd Deborah Trout
Labeling Review(s) • *APLB (OCBQ/APLB)*	Dana Jones
Lot Release Protocols/Testing Plans	Varsha Garnepudi Swati Verma Kori Francis
Bioresearch Monitoring Review	Anthony Hawkins

1. Introduction

National Genetics Institute (NGI), located in Los Angeles, CA, submitted an original Biologic License Application (BLA) for the licensure of the UltraQual® Multiplex PCR Assay for the detection of HCV RNA, HIV-1 RNA (HIV-1 Group O, HIV-1 Group M), HIV-2 RNA and HBV DNA in pooled and individual human source plasma using NGI's Pooling and Testing Algorithm. The UltraQual® Multiplex PCR Assay is an "in-house" test performed only by NGI and not distributed as a kit.
The application was submitted on May 8, 2017 and filed on July 7, 2017. The mid-cycle meeting was held on October 5, 2017. Information requests were sent to the sponsor on May 16, May 25, May 31, June 2, June 20, June 27, July 20, September 12, October 31, November 3, November 9, December 19, 2017 and January 08, 2018. Teleconferences were held on October 11 and November 13, 2017. A Complete Response (CR) letter was forwarded to the sponsor on January 25, 2018. Responses to the CR letter were submitted on February 08, 2018 and April 03, 2018 and classified as Class 1 resubmissions.

Table 2: Chronological Summary of Submission and FDA Correspondence

Date	Action	Amendment to BL125658
May 08, 2017	BLA CBER receipt	
May 15, 2017	Acknowledgement Letter	
May 16, 2017	Information Request- line listing data	/0/1
May 25, 2017	Information Request- date of NAT testing	/0/2
May 31, 2017	Information Request- Hyperlinks to TOC	/0/3
June 02, 2017	Information Request- Hyperlinks to TOC	/0/4/05
June 20, 2017	Information Request- Process Validation	/0/7
June 27, 2017	Information Request – Draft Lot Release protocol	/0/6/10
July 07, 2017	Filing Notification Letter	
July 20, 2017	Information Request – Software	/0/8
September 12, 2017	Information Request - Software	/0/9
October 23, 2017	Teleconference to discuss software issues	
October 31, 2017	Information Request - Software	/0/11/12
November 03, 2017	Information Request – Clinical and Pre-clinical test results interpretation	/0/14/15
November 09, 2017	Information Request –Precision study, revised lot release protocol	/0/13
November 16, 2017	Advice – Software and risk assessment	
November 20, 2017	Teleconference – Software and risk assessment	
December 19, 2017	Information Request - Software	/0/16
December 29, 2017	Information Request – Pre-clinical	/0/17
January 08, 2018	Information Request – Revised lot release protocol, Precision studies, Pre-	/0/18/19/20

Date	Action	Amendment to BL125658
	clinical studies	
January 25, 2018	Complete Response (CR)Letter	
February 08, 2018	Response to CR -Lot Release testing data	/0/21
April 03, 2018	Response to CR –Precision study data	/0/22
April 04, 2018	Information Request – Precision studies	/0/23

2. Background

All source plasma collections in the United States are tested for HIV-1, HIV-2, HCV and HBV in plasma derivatives. Testing Source Plasma for HCV RNA, HIV-1 RNA, HIV-2 RNA, and HBV DNA by nucleic acid testing (NAT) increases the margin of safety of plasma derivatives by detecting these pathogens in donations that test negative by a licensed test that uses antibody or antigen detection technology.
The UltraQual® Multiplex PCR Assay is intended to screen pooled source plasma in conjunction with NGI's pooling and testing algorithm. The assay simultaneously detects HCV RNA, HIV-1 RNA (including Group M and Group O), HIV-2 RNA, and HBV DNA. This assay discriminates between HCV, HIV-1, HIV-2 and HBV positive samples but not between HIV-1 Group M and Group O positive samples. NGI currently uses three separate FDA-licensed assays for HCV (STN: 103868), HBV (STN: 125193), and HIV-1 Group M (STN: 103902) in testing pooled source plasma. The UltraQual® Multiplex PCR Assay is intended to replace the three individual FDA-licensed assays currently in use.

a) Principles of the Assay

Pooling and Testing Algorithm

The UltraQual® Multiplex Assay uses NGI's pooling and testing algorithm (STN: 103868/5002). This algorithm is the same method used as previously submitted in NGI's licensed UltraQual® HCV, HIV-1 and HBV assays. Pooling is carried out in a two-step process. The first step consists of pooling sixty-four individual donations into "primary pools". In the second step, aliquots of the primary pools are combined to create "master pools". The master pool is made up of 512 individual plasma samples. Resolution testing is performed when a master pool is found to be positive for a target by testing the primary pools which leads to the identification of individual donation(s) that is/are virus positive.

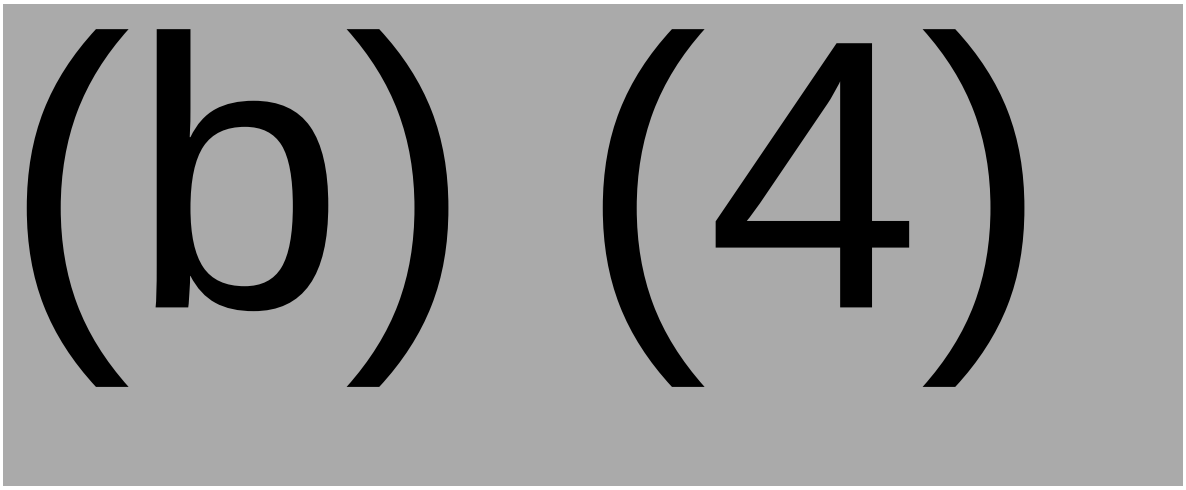
Description of the UltraQual® Multiplex PCR Assay

Nucleic acid extraction is carried out using a (b) (4) are used to perform the nucleic acid extractions. An internal control (b) (4) Each sample contains (b) (4) of the (b) (4) internal control. Master pool samples are processed with a starting volume of 4-mL of plasma. Primary pool samples are processed with a starting volume of 1- mL. In addition to the (b) (4)

1 Page determined to be not relesable: (b)(4)

(b) (4)

(b) (4)



3. Chemistry Manufacturing and Controls (CMC)

a) Manufacturing Summary

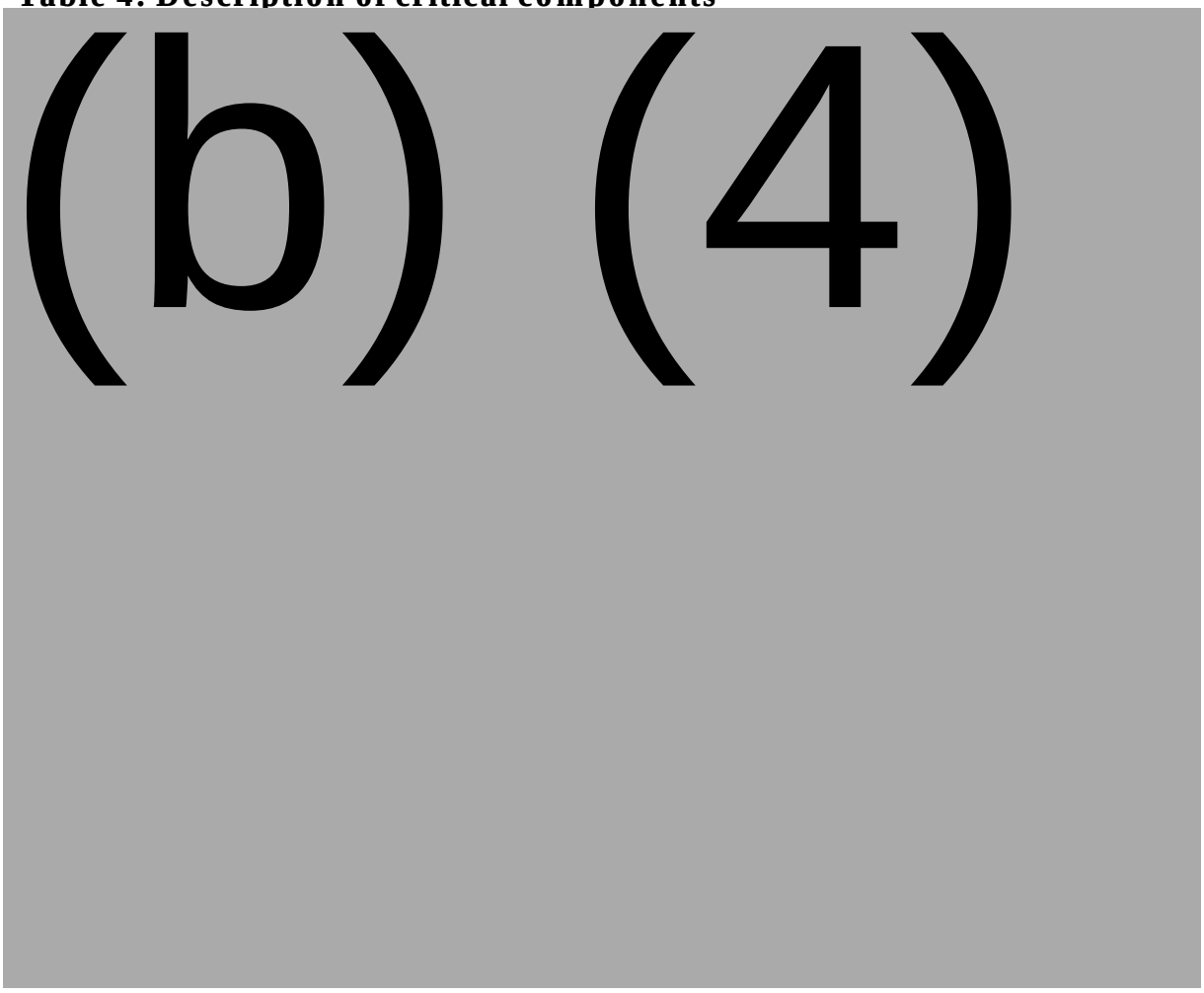
The UltraQual® Multiplex Assay consists of reagents used in nucleic acid extraction, reverse transcription, polymerase chain reaction (PCR) and detection of amplified targets. Nucleic acid extraction is carried out using a (b) (4) each run is conducted with positive, negative and blank controls to assure the validity of the assay. The UltraQual® Multiplex PCR Assay is an "in-house" test performed only by NGI and not distributed as a kit. NGI purchases raw materials to be used in the UltraQual® Multiplex Assay from authorized vendors. NGI has classified reagents as critical reagents and non-critical reagents. Critical reagents are vital for the performance of the assay and must meet predetermined acceptance criteria and FDA performance standards with FDA-CBER-provided blinded panels (lot release panels). NGI releases these reagents only after receipt of FDA CBER reports on acceptable reagent performance.

The critical reagents include:

- Deoxynucleotide triphosphate (dNTPs)
- Amplification primers
- Reverse Transcriptase (RT)
- Taq Polymerase

- Probe-(b) (4)

Table 4: Description of critical components



Non-critical reagents are common regents and buffers that are used in the UltraQual® Multiplex Assay. All incoming quality control specifications must be met for these reagents and generally the expiry date is specified by the manufacturer. NGI creates (b) (4) aliquots of non-critical reagents to minimize the effects of (b) (4) Working reagents for the NGI UltraQual® Multiplex Assay are made by the technical staff performing the assay and are generally used soon after they are prepared.

In Vitro Substances: Primers and Probe-(b) (4)

The Initial Performance Report described the design process for primers and probes used in the kit. The UltraQual® Multiplex Assay uses (b) (4) primers for the amplification of the conserved genomic regions of each target virus and the (b) (4) internal control. The UltraQual® Multiplex Assay uses (b) (4) different primers for viral and internal control detection. To facilitate the detection of all isolates and genotypes of each target virus more than one forward and /or

reverse primer sequences were designed for some targets. There is (b) (4) each for HBV, HCV, and (b) (4) internal control detection. There are (b) (4) each for HIV-1 and HIV-2 detection. Primers are purchased from qualified vendors and all incoming quality control specifications must be met.
The UltraQual® Multiplex Assay hybridization probes bind to conserved regions of each target virus genome. (b) (4) different oligonucleotide probes or probe mixtures are used for detecting the amplicons to facilitate detection of all isolates and genotypes of the target viruses. The UltraQual® Multiplex Assay hybridization probes are (b) (4) oligonucleotides. Each probe consists of a single-stranded sequence of DNA bases that is complementary to one of the strands of the intended target amplicon. Each of the (b) (4) probes or probe (b) (4)

The In Vitro Substance Report section contained reports of the manufacturing process, characterization of the materials and quality control of the components used in the UltraQual® Multiplex Assay. NGI has provided information regarding the storage, concentration, and expiry date of critical and non-critical reagents. Review of the reports indicates that overall manufacturing of the test was performed according to validated and approved procedures.

Stability Studies
NGI conducted stability studies for critical and non-critical reagents and assay controls. If NGI has not performed a stability study for a specific reagent, the expiration date specified by the manufacturer for each raw material is used.

Review Issues
The review identified some minor issues regarding storage temperature and shelf-life claim for primers, probes, reverse transcriptase and the internal control. NGI has addressed these issues satisfactorily.

b) CBER Lot Release
The lot release protocol template was submitted to CBER for review and found to be acceptable after revisions. A lot release testing plan was developed by CBER and will be used for routine lot release.

c) Facilities review/inspection
Facility information and data provided in the BLA were reviewed by CBER and found to be sufficient and acceptable. The manufacturer of the UltraQual® Multiplex PCR Assay is National Genetics Institute (NGI). NGI uses the UltraQual® Multiplex PCR Assay in-house to perform screening of pooled and individual source plasma for detection of HCV, HIV-1 (Group O and Group M), HIV-2 and HBV. The activities performed and inspectional history is noted in the table below and are further described in the paragraph that follows.

Table 5: Manufacturing Facility Information

Name/Address	FEI number	Inspection/ waiver	Justification/ Results
• *Manufacturer of the UltraQual® Multiplex PCR Assay* • *Performs plasma screening of HCV, HIV-1 and -2, and HBV* National Genetics Institute 2440 S. Sepulveda Blvd., Suite 235 Los Angeles, CA 90064	3003082450	Waived	Team Biologics inspection, January 10 – 19, 2017 VAI

Team Biologics performed a surveillance inspection of the National Genetics Institute manufacturing facility located at 2440 S. Sepulveda Blvd., Los Angeles, CA from January 10 – 19, 2017. Inspectional observations were noted on FDA Form 483 and the corrective actions were deemed satisfactory. The inspection was classified as voluntary action indicated (VAI).

d) Environmental Assessment (only when categorical exclusion is acceptable for this reporting)

The BLA included a request for categorical exclusion from an Environmental Assessment under 21 CFR 25.31(c). The FDA concluded that this request is justified as the manufacturing of this product will not alter significantly the concentration and distribution of naturally occurring substances and no extraordinary circumstances exist that would require an environmental assessment.

e) Container Closure

N/A

4. Software and Instrumentation

The UltraQual® Multiplex Assay System is a collection of separate instruments and software modules used in a very manual process to perform the multiplex assay. The following is a summary overview of software, instrumentation and risk management information provided to support a reasonable assurance that the device is safe and effective for its intended uses and conditions of use.

Versioning: This system includes several software components and instruments.

Custom software includes:

- (b) (4) for pool sample management (b) (4)
- (b) (4) for Plasma pooling liquid handlers (b) (4)
- (b) (4) master pool and primary pool (b) (4)

- Sample data verification worksheet (b) (4)
- Extraction batch setup worksheet, Runsheet (b) (4)
- (b) (4)
- Multiplex assay result analysis sheet (b) (4)
- (b) (4)

The instruments include:

- (b) (4)

Device Description:
The UltraQual® Multiplex Assay System is a collection of separate instruments and software modules used in a highly manual process to perform the assay. The custom software components include functionality to oversee pooling, verify the scanned sample tube barcode ID, organize sample orders, perform result analysis, and export data to an external intranet database. None of the UltraQual® Multiplex Assay instruments or software is functionally connected to each other or to internet for any aspect of data collection.

Risk Management:
The risk assessments have been performed using information and instructions given in ISO 14971:2012 "Medical devices – application of risk management to medical devices." The identified risks are associated with sample receipt, accessioning, pooling, performance of the assay itself, data analysis, and result reporting. There were (b) (4) risks in the pre-mitigation assessment and (b) (4) risks in post-mitigation. The major risks to harm are associated with releasing a false negative result, allowing a positive unit to be included in the manufacturing pool, and risk of operator infection. NGI performed a benefit-risk assessment, and when considering all treatments and procedures to reduce risk of harm outside of NGI (e.g., viral inactivation), in addition to mitigations in place at NGI, the applicant concluded that the benefits to patients

receiving potentially life-saving plasma products outweigh the remaining residual risk of infection.

Unresolved Anomalies: Two unresolved anomalies in (b) (4) were reported in the April 16, 2014 reply to the April 07, 2014 FDA email from the Regulatory Project Manager for submission BL103868/5105, BL103902/5098, and BL125193/12. In brief, one anomaly causes a sample to be incorrectly reported on a Not Tested Sample report, leading to a possible delay in reporting. The second is a misleading error message for a file import failure.The anomalies still hold true for (b) (4) Both anomalies are low risk with minor severity and low probability after mitigation. Correction of both is intended for a future (b) (4) version.

Testing: This submission included validation for all (b) (4) instruments. Validation testing of custom software was completed (Sample data verification Worksheet, Extraction batch setup Runsheet, Multiplex assay result analysis sheet, and Entry of NAT results script), along with assay system level validation testing, traceability analysis, integrity of data flow among the instruments, and cybersecurity analysis.

Development Management: The software development activities included establishing detailed software requirements, linking requirements with associate verification tests, verification and validation testing, defect tracking, configuration management and maintenance activities to ensure the software conforms to user needs and intended uses.

Review Issues: As the UltraQual® Multiplex Assay System is a collection of separate instruments and software modules used in a very manual process to perform the assay, the review focused on system component integrity, risk assessment methodology, and hazard analysis of the entire system. After two teleconferences (held on October 23, 2017 and November 09, 2017) and three rounds of interactive review, the applicant was able to provide a new system architecture chart, significantly-updated risk analysis methodology and resulting risk and hazard analysis documentation in amendments 11, 12, and 16 which are adequate.

The system architecture chart was expanded to include missing system components: 1) Pooling process steps with related hardware and software (b) (4) software, and 3) Customized scripts in data flow to better capture the scope of the system and allow all appropriate risks to be identified.

Risk assessment methodology was significantly updated to align with ISO 14971. The original processes were based on an arithmetic method of harms and mitigations that was not based on any industry standard or published methodology. Failure Mode Effects Analysis (FMEA) was utilized in different assay procedures to inform the risk analysis. NGI has removed claims that risk reduction activities outside their control (e.g., downstream viral inactivation) can be used to lower the probability of harm in their risk analyses, and developed more comprehensive pre-mitigation and

post-mitigation methodologies, including clear enumeration of hazards, potential causes and impacts, individual assessments of pre- and post-mitigation severity and probability, and a defined method to determine the adequacy of residual risks.

Revised hazard analysis included the risks associated with 1) NGI pooling procedures as well as software and instrumentation, 2) UltraQual® Multiplex Assay Procedure, 3) the equipment and software used during the UltraQual® Multiplex Assay procedure, and 4) NGI cybersecurity risks.

Software/Instrumentation Changes: No changes were required in the custom software or any other system component or labeling as a result of this review.

5. Analytical Studies

Analytical Sensitivity

Three lots of normal human plasma samples were spiked with different concentrations of HBV, HCV, HIV-1 (Group M Subtype B and Group O VQA Isolate (b) (4) and HIV-2 positive standards and tested to determine the analytical sensitivity for the UltraQual® Multiplex assay using four mL and one mL input volumes. The panels were tested by two different operators on three different days using three different lots of reagents. Data for each viral type panel member at a particular input volume was calculated using Probit statistics and the average 95% detection limits were determined. The results are provided in table 6.

Table 6: Analytical sensitivity of the UltraQual® Multiplex Assay

Virus Type	Average 95% Detection Limit 4-mL Assay (95% Confidence Intervals)	Average 95% Detection Limit 1-mL Assay (95% Confidence Intervals)
HBV	0.66 IU/mL (0.54 – 0.90)	2.45 IU/mL (2.02 – 3.25)
HCV	1.72 IU/mL (1.41 – 2.35)	6.69 IU/mL (5.50 – 9.01)
HIV-1 (Group M)	9.95 IU/mL (8.30 – 12.89)	30.76 IU/mL (25.28 – 41.53)
HIV-1 (Group O)	5.39 Copies/mL (4.53 – 6.90)	16.99 Copies/mL (14.16 – 22.10)
HIV-2	3.04 IU/mL (2.49 – 4.14)	9.11 IU/mL (7.40 – 12.34)

Testing of Known Positive Samples

Two thousand three hundred (2300) plasma samples positive for HCV, HIV-1 (Groups M and O), or HBV, were tested. Two hundred HIV-2 positive plasma or serum samples were tested. The viral loads of these samples were unknown prior to testing. The HCV, HIV-1 Group M (of unspecified subtypes) and HBV samples were previously determined to be positive using either FDA-licensed serology or NGI's FDA-licensed UltraQual® assays. HIV-2 samples were confirmed positive using NGI's in-house developed HIV-2 (b) (4) Assay validated to CLIA standards. Samples were initially tested at a 512-fold dilution. Samples that were not found to be positive were re-tested at lower dilutions.

Of the 525 HCV positive samples tested, 523 were found positive at a 512-fold dilution. The remaining two samples were found positive at a lower dilution (b) (4) fold and (b) (4) -fold). The results are summarized in table 7.

Table 7: Summary of Results for HCV Samples

Category	Number of Samples
Detected at a 512-fold dilution	523
Detected at a lower dilution	2
Total	525

Of the 1022 HIV-1 samples of unspecified subtypes that were tested, 1006 samples were positive and sixteen samples were negative at a 512-fold dilution. Of the sixteen samples, fifteen were detected at lower dilutions and one sample was found to be negative at any dilution tested. This sample also could not be confirmed as positive using NGI's licensed HIV-1 UltraQual® Assay. The results are summarized in table 8.

Table 8: Summary of Results for HIV-1 Group M (Unspecified Subtypes) Samples

Category	Number of Samples
Detected at a 512-fold dilution	1006
Detected at lower dilutions	15
Not confirmed positive with NGI's licensed HIV-1 UltraQual® Assay	1
Total	1022

Of the 242 HIV-1 subtype and CRF plasma samples diluted 512-fold and tested, 227 samples were found to be positive. Of the remaining fifteen samples, nine samples were positive at lower dilutions, five were co-infected with HBV and one HIV-1 subtype B sample was negative at any dilution tested. This HIV-1 subtype B sample was not detected with NGI's licensed HIV-1 UltraQual® Assay and was confirmed positive by (b) (4) sequencing. In addition, three HIV-1 Group O plasma samples and four HIV-1 Group O virus isolates were diluted 512-fold and tested. Test results indicated that all seven HIV-1 Group O samples diluted 512-fold were found to be positive. The results are summarized in tables 9 and 10.

Table 9: Summary of Results for HIV-1 Group M Subtypes and HIV-1 Circulating Recombinant Forms (CRFs)

Category	Number of Samples
Detected at a 512-fold dilution	227
Detected at lower dilutions	9
HBV co-infected samples (Detected when HBV primers were omitted from PCR Mix)	5
Not confirmed positive with NGI's licensed HIV-1 UltraQual® Assay	1
Total	242

Table 10: Summary of Results for HIV-1 Group O Samples

Category	Number of Samples
Detected at a 512-fold dilution	7
Total	7

Of the 515 HBV samples tested at a 512-fold dilution, 440 samples were found to be positive and 75 samples were found to be negative. Of the 75 samples, 67 samples were detected at lower dilutions, two samples were co-infected with HIV-1 or HCV and six samples were not detected by the multiplex assay. The results are summarized in table 11.

Table 11: Summary of Results for HBV Samples

Category	Number of Samples
Detected at a 512-fold dilution	440
Detected at lower dilutions	67
Not detected at lower dilutions (Confirmed positive by an FDA licensed HBV NAT assay)	6
HIV/HCV co-infected samples (Detected when HIV/HCV primers were omitted from PCR Mix)	2
Total	515

Of the 200 HIV-2 positive samples diluted 512-fold and tested, 105 samples were found to be positive. Of the 95 negative samples, 87 samples were detected at lower dilutions, six samples were detected when tested undiluted and two samples were found to be co-infected with HIV-1 or HCV. The results are summarized in table 12.

Table 12: Summary of Results for HIV-2 Samples

Category	Number of Samples
Detected at a 512-fold dilution	105
Detected at a lower dilution	93
HIV/HCV co-infected samples (Detected when HIV/HCV primers were omitted from PCR Mix)	2
Total	200

The multiplex assay was able to detect the majority of the samples at a 512-fold dilution. Samples not detected at 512-fold dilution were found positive at lower dilutions, with the exception of HBV samples and samples with co-infections.

Detection of Viral Genotypes

To demonstrate that the UltraQual® Multiplex assay can detect all viral genotypes, different samples of each genotype of HCV, HBV, HIV-1 Group M and HIV-2 were tested. In addition, seven HIV-1 Group O samples were also tested.

Table 13: Viral Genotypes or Subtypes Tested

Virus	Genotypes or Subtypes Tested	Total # Samples Tested
HCV	(b) (4)	30
HBV	(b) (4)	33
HIV-1 Group M	(b) (4)	57
HIV-1 Group O	Isolates	7
HIV-2	(b) (4) isolates of unknown genotype	10

All HCV, HBV, HIV-1 group M, and HIV-2 genotype samples were detected at a concentration of 10X the 95% detection limit of the multiplex assay. All samples (except for one HBV sample) were detected as positive at a viral load of 3X the 95% detection limit of the multiplex assay. The one HBV sample not detected at 3X was detected as positive at the 0.5X detection limit. Six HIV-1 Group O samples were detected at a concentration of 10X, 3X, and 1X the 95% detection limit of the multiplex assay. One HIV-1 Group O sample was not detected at any of the initial dilutions tested. It was, however, detected when a concentration greater than 10X the 95% detection limit was tested. The multiplex assay was able to detect all the viral genotypes and subtypes tested.

HIV-1 and HIV-2 CBER/WHO Panel Testing

To demonstrate that the UltraQual® Multiplex Assay could detect a variety of genotypes and isolates at varying concentrations the WHO 12/24 HIV-1 genotype panel, the CBER Reference Panel #1 HIV-1 genotype panel, the WHO 13/24 HIV-1 circulating recombinant forms (CRF) panel, and one CBER lot release panel each for HIV-1 and HIV-2 were tested. The panel members were initially tested at a 512-fold dilution and if not found positive, they were tested in a less dilute form. The results indicate that the UltraQual® Multiplex Assay could detect HIV-1 and HIV-2 members in WHO and CBER panels. These results demonstrate that the UltraQual® Multiplex Assay could detect a variety of HIV-1 genotypes, HIV-1 isolates and HIV-2.

Testing of Seroconversion Panels

Seroconversion panels were tested to demonstrate that the NGI UltraQual® Multiplex Assay can detect HCV, HBV and HIV-1 in window-period samples. Ten different seroconversion panels for each of these three viruses were purchased from (b) (4). Each panel member was initially tested at a 512-fold dilution to simulate its inclusion in a master pool. Any sample testing negative at a 512-fold dilution was quantitated using the (b) (4) HBV (b) (4) Assay, (b) (4) HCV (b) (4) Assay, or the (b) (4)
The samples found negative at a 512-fold dilution were retested at a (b) (4) dilution. The results are summarized in tables 14 and 15. The results indicate that, at least one 512-fold diluted member of each seroconversion panel was detected as positive. All

samples found positive at this dilution using the multiplex assay were also found positive by the panel supplier. The NGI UltraQual® Multiplex Assay can detect HCV, HBV and HIV-1 in seroconversion panels. In all instances where a sample was not detected at a 512-fold dilution, the diluted concentration was near or below the detection limit of the assay.

Table 14: Seroconversion Panel Results after 512-fold Dilution

Panel Members Positive / Total Panel Members						
Panel #	**HCV**		**HIV-1**		**HBV**	
	HCV MPX* Result	**Anti-HCV Assay Result**	**HIV-1 MPX* Result**	**Anti-HIV-1 Assay Result**	**HBV MPX* Result**	**HBsAg Assay Result**
1	7 / 7	2 / 7	3 / 7	2 / 7	2 /3	2 /3
2	4 / 7	3 / 7	3 / 6	1 / 6	4 / 5	4 / 5
3	6 / 6	5 / 6	4 / 10	3 / 10	7 / 8	6 / 8
4	6 / 6	2 / 6	4 / 6	3 / 6	7 / 9	5 / 9
5	6 / 6	3 / 6	6 / 10	4 / 10	5 / 5	5 / 5
6	5 / 5	1 / 5	5 / 10	4 / 10	8 / 8	8 / 8
7	5 / 5	1 / 5	4 / 4	4 / 4	5 / 5	3 / 5
8	4 / 5	0 / 5	4 / 6	2 / 6	4 / 5	4 / 5
9	7 / 9	0 / 9	4 / 4	2 / 4	1 / 8	1 / 8
10	3 / 6	0 / 6	3 / 7	1 / 7	7 / 9	7 / 9
Total	53 / 62	17 / 62	40 / 70	26 / 70	50 / 65	45 / 65

* MPX --- NGI UltraQual® Multiplex Assay

Table 15: Seroconversion Panel Results after (b) (4) Dilution

Panel #	**Panel Members Positive at a (b) (4) Dilution / Total Panel Members Negative at 512-fold Dilution**		
	HCV	**HIV-1**	**HBV**
1	N/A*	0 / 4	1 / 1
2	0 / 3	0 / 3	0 / 1
3	N/A*	1 / 6	1 / 1
4	N/A*	0 / 2	2 / 2
5	N/A*	0 / 4	N/A*
6	N/A*	2 / 5	N/A*
7	N/A*	N/A*	N/A*
8	0 / 1	2 / 2	1 / 1
9	0 / 2	N/A*	6 / 7
10	0 / 3	0 / 4	2 / 2

* All panel members were previously found positive at a 512-fold dilution

Analytical Specificity

The sponsor tested replicates of citrate-plasma containing substances that may be present in donor samples, plasma collected with anticoagulants other than sodium citrate, and serum to demonstrate the analytical specificity of the UltraQual® Multiplex Assay. Three replicates of HBV, HCV, HIV-1 Group M, HIV-2 and Parvovirus B19 were separately diluted to (b) (4) and tested. Three replicates of HAV, HIV-1 Group O, HTLVI, HTLVII and WNV were separately diluted to (b) (4) copies/mL and tested. Three replicates of bacteremia plasma, lipemic plasma, hemolyzed plasma, icteric plasma, EDTA plasma and serum were also tested. Samples and test results are summarized in table 16 below.

Table16: Analytical Specificity Samples Tested

Substance Present	Concentration	Result			
		HBV	HCV	HIV-1	HIV-2
HIV-1 Group M	(b) (4)	Neg	Neg	**POS**	Neg
HIV-1 Group O	(b) (4)	Neg	Neg	**POS**	Neg
HIV-2	(b) (4)	Neg	Neg	Neg	**POS**
HBV	(b) (4)	**POS**	Neg	Neg	Neg
HCV	(b) (4)	Neg	**POS**	Neg	Neg
HAV	(b) (4)	Neg	Neg	Neg	Neg
WNV	(b) (4)	Neg	Neg	Neg	Neg
Parvovirus	(b) (4)	Neg	Neg	Neg	Neg
HTLV I	(b) (4)	Neg	Neg	Neg	Neg
HTLV II	(b) (4)	Neg	Neg	Neg	Neg
Bacteremic Plasma	E. coli (b) (4) S. epi (b) (4)	Neg	Neg	Neg	Neg
Lipemic Plasma	(b) (4) Triglycerides	Neg	Neg	Neg	Neg
Hemolyzed Plasma	RBC (b) (4) Hemoglobin (b) (4)	Neg	Neg	Neg	Neg
Icteric Plasma	Bilirubin (b) (4)	Neg	Neg	Neg	Neg
EDTA Plasma	EDTA (b) (4)	Neg	Neg	Neg	Neg
Serum	Not Applicable	Neg	Neg	Neg	Neg
B-lymphocytes in plasma	(b) (4)	Neg	Neg	Neg	Neg

Substance Present	Concentration	Result			
		HBV	HCV	HIV-1	HIV-2
T-lymphocytes in plasma	(b) (4)	Neg	Neg	Neg	Neg
Epithelial cells in plasma	(b) (4)	Neg	Neg	Neg	Neg

The results indicate that, samples containing HIV-1, HCV, HBV or HIV-2 returned positive results for those specific targets. All other results were negative demonstrating the specificity of the assay.

Effect of Potentially Interfering Substances

The sponsor tested samples containing non-target viruses, plasmas containing potentially interfering substances, and twenty different master pool samples separately spiked with each of the assay target viruses to demonstrate that the presence of potentially interfering plasma components and non-target viruses do not interfere with the assay performance. HIV-1 Group M, HIV-1 Group O, HIV-2, HCV and HBV target viruses were added at 3X the 95% detection limit of the assay. In addition to demonstrate the ability of the assay to detect co-infected samples, each of the assay-specific target virus was separately diluted to 3X, 5X, 10X and 20X the 95% detection limit (DL) of the multiplex assay and then spiked with a relatively high concentration (b) (4) or (b) (4) copies/mL) of a second multiplex assay target virus and tested.

The UltraQual® Multiplex Assay yielded a positive result only for the specific target virus spiked into the different plasmas containing interfering substances and non-target viruses. All viruses were consistently detected at 20X the 95% detection limits of the assay in the presence of another target virus at (b) (4) IU/mL or (b) (4) copies/mL. The sensitivity of the assay was diminished for a low titer target virus in the presence of a high titer virus due to PCR competition.

Robustness

To demonstrate that minor variations in assay parameters do not adversely affect the UltraQual® Multiplex Assay, reagent volumes, incubation times and incubation temperatures used in extraction, reverse transcription, and PCR procedures were slightly varied compared to the standard protocol and tested. Results demonstrated that changes in these parameters did not adversely affect the performance of the assay.

Resolution of Matrices Containing HIV-1 Group O and HIV-2 Samples

To demonstrate the ability of the UltraQual® Multiplex Assay to correctly resolve pooled plasma matrices containing HIV-1 Group O or HIV-2 samples, 10 plasma matrices each containing one HIV-1 Group O sample and ten plasma matrices, each containing one HIV-2 sample, were manually contrived and tested. A matrix is

composed of 512 individual donations pooled (b) (4) . The results demonstrated that ten different pooled plasma matrices containing HIV-1 Group O were correctly resolved, with the individual positive samples identified. The ten different pooled plasma matrices containing HIV-2 samples were also correctly resolved, with the individual positive samples identified. Specifically, each master pool was determined to be positive. Upon primary pool testing, the appropriate row, layer, and column primary pools were identified, which led to the identification of each individual positive sample.

Resolution of Matrices Containing Two Positive Samples

To demonstrate that the UltraQual® Multiplex Assay can resolve pooled plasma matrices containing two positive samples, twenty-five different pooled plasma matrices were contrived, each containing two positive samples and tested. The HBV, HCV, and HIV-1 Group M positive samples used in the matrices were randomly selected from a list of samples previously identified as positive during performance of the multiplex assay clinical study. The matrices included a variety of different sample virus concentrations and represented all combinations of the different viruses that the assay detects. Results indicate that the UltraQual® Multiplex Assay accurately resolved the two positive samples in all matrices tested.

Resolution of Primary Pools Containing Two Infected Samples in Overlapping Primary Pools

When two different viruses are present in a single sample, the UltraQual® Multiplex Assay sensitivity may be diminished for the virus present at a lower concentration. The sensitivity for the lower concentration virus is reduced due to competition by the higher concentration virus during PCR. To demonstrate that the UltraQual® Multiplex Assay can accurately detect a low-concentration positive donation in the presence of a high-concentration positive donation, different pooled plasma matrices were contrived with different combinations of viruses (HCV/HBV, HCV/HIV-1 Group M, and HBV/HIV-1 Group M) and tested. Samples were added to each matrix such that one virus was at a relatively high concentration and the other was at a relatively low concentration, or both viruses were at relatively low concentrations. The test results demonstrate that in matrices containing a high-titer positive donation and a separate low-titer positive donation, both positive samples can be detected. In instances, where the low-concentration sample is not detected in the master pool or some of the primary pools due to PCR competition, it can still be detected and removed from the donor population when tested at the individual donation level. In the case of co-infected individuals, at least one of the viral types will be detected for that donation, causing that donation to be excluded from use.

Precision

Assay precision was evaluated at 4-mL and 1-mL input volume by testing panels of samples separately spiked with varying concentrations of HBV, HCV, HIV-1 Group M, HIV-1 Group O, and HIV-2 standards. Three panels of samples were prepared for each type of virus and tested by two different operators on three different days using different lots of reagents. Data for each viral type panel member at a particular input

volume was calculated using Probit statistics and the average 95% detection limits, the upper and lower bound confidence intervals were determined. To demonstrate acceptable assay precision, the 95% confidence intervals should overlap for the three panels for each target virus tested with the multiplex PCR assay. The results indicate that the 95% confidence intervals overlapped for the three panels of each target virus demonstrating acceptable precision for the multiplex assay. The precision study results for 4-mL and 1-mL plasma input volumes are summarized in table 17 and table 18 below.

Table 17: Precision Study Results for 4-mL input plasma volume

Virus Type	Units	Day	Average 95% detection limit	Lower 95% confidence interval	Upper 95% confidence interval	95% confidence intervals overlapped
HBV	IU/mL	Day 1	0.42	0.30	0.89	Yes
		Day 2	0.68	0.51	1.15	
		Day 3	0.81	0.56	1.85	
HCV	IU/mL	Day 1	1.86	1.34	3.96	Yes
		Day 2	1.49	1.08	3.13	
		Day 3	1.70	1.30	3.15	
HIV-1 Group M	IU/mL	Day 1	6.41	4.80	12.17	Yes
		Day 2	9.65	7.31	16.21	
		Day 3	12.44	9.33	21.69	
HIV-1 Group O	Copies/mL	Day 1	5.26	3.99	8.81	Yes
		Day 2	3.21	2.40	6.08	
		Day 3	6.19	4.93	10.01	
HIV-2	IU/mL	Day1	2.85	2.02	6.39	Yes
		Day2	2.66	1.95	5.13	
		Day3	3.35	2.53	6.28	

Table 18: Precision Study Results for 1-mL input plasma volume

Virus Type	Units	Day	Average 95% detection limit	Lower 95% confidence interval	Upper 95% confidence interval	95% confidence intervals overlapped
HBV	IU/mL	Day 1	1.64	1.23	3.53	Yes
		Day 2	2.71	2.08	4.69	
		Day 3	2.67	1.89	5.89	
HCV	IU/mL	Day 1	8.24	5.94	16.72	Yes
		Day 2	4.97	3.78	8.58	
		Day 3	6.71	5.07	12.57	
HIV-1 Group M	IU/mL	Day 1	28.95	20.91	60.23	Yes
		Day 2	33.71	24.85	64.79	
		Day 3	28.47	21.55	55.28	
HIV-1 Group O	Copies/mL	Day 1	13.81	10.85	27.64	Yes
		Day 2	12.46	9.29	23.64	

Virus Type	Units	Day	Average 95% detection limit	Lower 95% confidence interval	Upper 95% confidence interval	95% confidence intervals overlapped
		Day 3	21.08	15.79	36.48	
HIV-2	IU/mL	Day1	6.34	4.36	16.09	Yes
		Day2	9.81	7.21	17.83	
		Day3	9.90	7.35	17.45	

Review Issues

The following were the major issues identified by the committee during review of the pre-clinical / analytical studies and their resolution:

1. HBV genotype testing: Data presented by NGI indicates that the Multiplex assay can detect all HBV genotype samples at 3X LOD with one exception. One HBV genotype E sample out of five HBV genotype E samples tested was detected as positive at 10X and 0.5X LOD but negative at 3X and 1X LOD. To demonstrate that the multiplex assay can detect HBV genotype E samples, an information request was forwarded to NGI recommending testing ten additional HBV genotype E samples with the UltraQual® Multiplex Assay. Data provided in amendment 14, received on December 11, 2017, indicates that NGI tested fourteen additional HBV genotype E samples. Thirteen of the fourteen HBV genotype E samples were positive at 1x the 95% detection limit and one sample was positive at 10x the 95% detection limit. The results indicate that the UltraQual® Multiplex Assay can detect HBV genotype E samples. NGI has resolved this issue satisfactorily.

2. Co-infections: Data provided by NGI indicates that high concentrations of one target analyte can interfere with the detection of low concentrations of other target analytes leading to false negative results. In an information request forwarded on November 03, 2017, NGI was advised to incorporate into their results report a warning that when a donor is positive for a virus, there is the possibility that the testing missed other viruses and that this should be considered when treating physicians provide follow up care or qualify patients for reentry. In amendment 14, received on December 11, 2017, NGI has agreed to include a warning in the information sheet for the multiplex assay which will be provided to clients when the assay is licensed. This issue was resolved satisfactorily.

3. (b) (4) interference: The Agency recently has become aware of potential (b) (4) interference with several in vitro diagnostic devices that use (b) (4) interactions as part of the device technology. Since, NGI did not submit data for (b) (4) interference in their original submission; an information request was forwarded to NGI to evaluate the effect of (b) (4) interference with the UltraQual® Multiplex Assay. In amendment 14, received on December 11, 2017, NGI provided test data after evaluating the effect of (b) (4) on the UltraQual® Multiplex Assay. The results demonstrate that (b) (4) does not

interfere with the detection of the multiplex assay target viruses. This issue was resolved satisfactorily.

4. Precision: Precision studies were performed over three days using eight replicates of each panel, for 24 replicates per concentration, with two operators and several lots. Upon review, per (b) (4) the data from the precision study provided by NGI was not adequate. An information request was forwarded on November 03, 2017, requesting NGI to perform precision testing with three lots using the same reproducibility panel (each virus as a panel) on five non-consecutive days with two runs (AM, PM) per day, by two different operators and at least two replicates in each run. The sponsor responded in amendment 14, received on December 11, 2017 that critical components of the assay were back-ordered and NGI would only be able to complete testing by middle of January 2018. A Complete Response letter was forwarded to the sponsor on January 25, 2018, indicating that the precision study should be completed and data submitted for review. NGI has provided the results from the precision study in amendment 23, received on April 10, 2018 and the issue was resolved.

6. Clinical Studies

a) Clinical Program

NGI submitted IND 15851 for the UltraQual® Multiplex PCR Assay to FDA on December 2, 2013. NGI was notified on January 30, 2014 that the proposed clinical trials may proceed. On June 4, 2015, in an e-mail, NGI informed FDA that they had redesigned the PCR primers and modified the assay design to (b) (4) PCR method. A teleconference was held on June 24, 2015. During the teleconference FDA informed NGI that an IND amendment with the proposed changes in the assay design and PCR protocol should be submitted and all the studies in support of the future BLA must be performed using the modified device. NGI submitted an amendment (IND 1581/02) with the proposed changes in the assay design and PCR protocol to FDA on July 20, 2015.

Between August 3, 2015 and January 7, 2016, a total of 1,534,648 individual donations from 54 donor centers were tested in 3,000 master pools containing 512 donations each using the investigational UltraQual® Multiplex PCR Assay in addition to using NGI's FDA-licensed, individual assays for HCV, HIV-1, and HBV. Sample collection and pooling are conducted according to an FDA approved algorithm (BL 103868/5002). NGI prepares pools of not more than 512 samples using (b) (4)

Results were analyzed using approved protocols. When the result is concordant between the investigational UltraQual® Multiplex PCR Assay and NGI's FDA-licensed, individual assays for HCV, HIV-1, and HBV, it was considered confirmed. In instances when discordant results were obtained with the multiplex assay and the FDA-licensed assays, less dilute primary pools or individual donations were tested using both the investigational and licensed assays. All discordant samples according to the study protocol required testing with an additional FDA-licensed NAT assay. During the course of this study, all positive donations were confirmed and the use of

additional FDA-licensed NAT assay was not required. There were 1,534,341 donations that confirmed negative and 307 donations that confirmed positive for at least one target virus.

Clinical Specificity

The clinical specificities for each of the target viruses for both the investigational UltraQual® Multiplex assay and the individual licensed assays were 100% (table 19).

Clinical Sensitivity

Clinical sensitivities for HCV and HIV-1 were determined to be 100% for the UltraQual® Multiplex assay. The clinical sensitivity for HBV was determined to be 71.4% and was lower when compared to the FDA-licensed assay (76.2%). When the results for each assay were compared using McNemar's Test for matched pairs, the UltraQual® Multiplex assay and the licensed assays were similar (with p values > 0.05) in detecting HIV-1 and HBV. For HCV detection, the two assay results were statistically different (with p value = 0.0002), indicating that the UltraQual® Multiplex assay is better than the FDA-licensed assay at detecting HCV RNA (table 20).

Table19: UltraQual® Multiplex assay Clinical Specificity

		Investigational Assay		Licensed Assays	
Virus	**Confirmed Neg or NI Donations**	**Determined Neg or NI Donations***	**Clinical Specificity****	**Determined Neg or NI Donations***	**Clinical Specificity****
HCV	1,534,394	1,534,394	100.00%	1,534,394	100.00%
HIV-1	1,534,616	1,534,616	100.00%	1534,616	100.00%
HIV-2	1,534,648	1,534,648	100.00%	N/A	N/A
HBV	1,534,625	1,534,625	100.00%	1,534,625	100.00%
Total ***	1,534,341	1,534,341	100.00%	1,534,341	100.00%

*Identified as negative or not implicated (NI) following NGI's pooling and testing algorithm
**Lower bound 95% confidence intervals are 99.995% $\leq$ x <100%
*** Negative or NI for all four targets, HCV, HIV-1, HIV-2 and HBV

Table20: UltraQual® Multiplex Assay Clinical Sensitivity

		Investigational Assay		Licensed Assays	
Virus	**Confirmed Positive Donations**	**Determined Positive***	**Clinical Sensitivity (95% Confidence Intervals)**	**Determined Positive***	**Clinical Sensitivity (95% Confidence Intervals)**
HCV	254	254	100.00% (98.56%-100.00%)	238	93.70% (89.97% - 96.36%)

Virus	Confirmed Positive Donations	Investigational Assay		Licensed Assays	
		Determined Positive*	Clinical Sensitivity (95% Confidence Intervals)	Determined Positive*	Clinical Sensitivity (95% Confidence Intervals)
HIV-1	30	30	100.00% (88.43% - 100.00%)	29	96.67% (82.78% - 99.92%)
HIV-2	0	0	N/A	N/A	N/A
HBV	21	15	71.43%	16	76.19%
HBV and HIV-1	2	2	100.0%***	2	100.0%***
Total**	307				

* Identified positive following NGI's pooling and testing algorithm
** Positive for at least one of the four targets, HCV, HIV-1, HIV-2 and HBV
*** Confidence intervals not calculated given the small number of donations

Review Issues

The following were the major issues identified by the committee during review of the clinical studies and their resolution:

1. Clinical sensitivity performance for HBV positive samples: Clinical sensitivity performance data initially presented for HBV indicated that sensitivity was lower for the UltraQual® Multiplex Assay compared to other licensed assays. In addition, a high degree of discordance between the investigational and the licensed assays was noted. The investigational and the licensed assays did not detect HBV DNA in the same samples. Furthermore, the number of yield cases for HBV that were reported as positive in the investigational test was low. Thus, to evaluate sensitivity, an information request was forwarded to NGI on November 03, 2017 recommending that NGI test 250 additional known HBV positive samples. In amendments 17 and 18 NGI provided data after testing additional 251 HBV-positive samples using the investigational and licensed assays. Of the 251 samples tested 44 were yield samples. The results show that the UltraQual® Multiplex Assay sensitivity was slightly higher than that for the licensed assay (92.4% vs. 90.0%) when the 251 HBV-positive samples were tested at a 512-fold dilution. The calculated multiplex assay sensitivity when testing the 44 yield samples was slightly higher than that for the licensed assay (75.0% vs. 72.7%). The UltraQual® Multiplex Assay detected 33 out of the 44 yield samples. The 11 yield samples not detected by the UltraQual® Multiplex Assay had HBV viral loads below the limit of detection. Thus, the additional testing data provided by NGI indicates that the UltraQual® Multiplex Assay could detect yield samples

that would otherwise go undetected using current screening tests. NGI has addressed this issue satisfactorily.

Label considerations
There are no labeling restrictions other than those noted in the intended use statement.

Bioresearch Monitoring (BIMO) –
Clinical/Statistical/Pharmacovigilance; a) Clinical Program A Bioresearch Monitoring (BIMO) inspection was issued for the one domestic clinical study site that participated in the conduct of Clinical Study Protocol NGI 2013-01. The inspection did not reveal any issues that impact the data submitted in this original Biologics License Application (BLA).

b) Pediatrics
N/A

c) Other Special Populations
N/A

7. Advisory Committee Meeting
An advisory committee meeting was not needed.

8. Other Relevant Regulatory Issues
None

Post marketing commitments
No Post Marketing Requirements or Post Marketing Commitments have been requested for this application.

9. Labeling
The Advertising and Promotional Labeling Branch (APLB) found the proposed Standard Operating Procedures (SOPs) to be acceptable from a promotional and comprehension perspective.

10. Recommendations and Risk/ Benefit Assessment

a) Recommended Regulatory Action
The Review Committee reviewed the original submission and related amendments submitted by NGI. All review issues have been resolved; therefore, the Review Committee recommends licensure of the UltraQual® Multiplex Assay.

b) Risk/ Benefit Assessment
The UltraQual® Multiplex Assay is intended to screen pooled source plasma to simultaneously detect HCV RNA, HIV-1 Group M RNA, HIV-1 Group O RNA, HIV-2 RNA and HBV DNA. This assay has demonstrated an estimated 95% LoD

of (b) (4) of HCV RNA, (b) (4) of HIV-1 Group M RNA, (b) (4) of HIV-1 Group O RNA, (b) (4) of HIV-2 RNA and (b) (4) of HBV DNA. This assay has demonstrated 100% specificity in the clinical studies supporting this submission. NGI currently uses three separate FDA-licensed assays (for HCV, HBV, and HIV-1 Group M) in testing pooled source plasma. The UltraQual® Multiplex PCR Assay is intended to replace the three individual FDA-licensed assays currently in use. The NGI UltraQual® Multiplex PCR Assay has several benefits in comparison to the FDA-licensed HCV, HIV-1 and HBV tests currently used at NGI. The UltraQual® Multiplex PCR Assay has demonstrated greater sensitivity in detecting HCV compared to the licensed individual HCV assay. UltraQual® Multiplex PCR Assay has statistically similar clinical and analytical sensitivities for HBV and HIV-1 in comparison to the licensed individual HIV-1 and HBV assays. In addition, the UltraQual® Multiplex PCR Assay detects HIV-1 Group O and HIV-2 RNA. The combination of excellent analytical sensitivity (95% LOD), and data from the clinical trial showing strong evidence that the UltraQual® Multiplex PCR Assay detects donations that would otherwise be undetected and used for further manufacture of plasma-derived products, offer a significant public health benefit for the viral safety of Source Plasma donations.

c) Recommendation for Postmarketing Activities

No postmarketing activities have been proposed for this application.