Summary Basis for Regulatory Action

Date:	December 19, 2014
From:	Guang Gao, Ph.D., Scientific Lead, BLA Review Committee
BLA STN#:	125459.0
Applicant Name:	Roche Molecular Systems, Inc.
Date of Submission:	October 26, 2012
MDUFMA Goal Date:	January 7, 2015
Proprietary Name:	cobas® TaqScreen MPX Test, version 2.0 for use with the cobas s 201 system
Biological Name:	HIV-1 Group O and M, HIV-2, HCV and/or HBV (HIV-1/HIV-2/HCV/HBV/Multiplex Discriminatory NAT)
Indications:	Direct detection of Human Immunodeficiency Virus Type 1 (HIV-1) Group M RNA, HIV-1 Group O RNA, Human Immunodeficiency Virus Type 2 (HIV-2) RNA, Hepatitis C Virus (HCV) RNA, and Hepatitis B Virus (HBV) DNA in human plasma Simultaneous detection and discrimination of HIV RNA, HCV RNA, and HBV DNA
Recommended Action:	Approval

Signatory Authorities Action: Jay S. Epstein, M.D., 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.

Material Reviewed/Consulted:

Review memos from the following reviewers were used in developing this SBRA:

Discipline Reviewed	**Reviewer Names**
Clinical Review	Andy Dayton Krishnamurthy Konduru Luisa Gregori
Non-clinical/Analytical Review	Viswanath Ragupathy Laura Ulitzky
CMC Review	Jianqin Zhao Erica Silberstein
Statistical Review	Jean Wang Tie-Hua Ng
Facility Review	Debbie Trout
Software and Instrumentation Review	Diane Gubernot
Living Organ Donor Claim Review	Karoll Cortez Laura St. Martin
Bioresearch Monitoring	Dennis Cato
Lot Release Testing	Steve Kerby Kori Francis Susan Zullo
Labeling and Promotional Advertising	Dana Martin
Package Insert	Judy Ciaraldi Guang Gao Pradip Akolkar
Policy	Guang Gao Pradip Akolkar Indira Hewlett Sayah Nedjar Robin Biswas

I. INTRODUCTION

Roche Molecular Systems, located in Somerville, New Jersey, submitted a Biologics License Application (BLA) for the licensure of the cobas® TaqScreen MPX Test, version 2.0 for use with the cobas s 201 system (MPX v2).

Intended Use

The cobas® TaqScreen MPX Test, version 2.0 for use with the cobas s 201 system (MPX v2), is a qualitative *in vitro* test for the direct detection of Human Immunodeficiency Virus Type 1 (HIV-1) Group M RNA, HIV-1 Group O RNA, Human Immunodeficiency Virus Type 2 (HIV-2) RNA, Hepatitis C Virus (HCV) RNA, and Hepatitis B Virus (HBV) DNA in human plasma. This test is intended for use to screen for HIV RNA, HCV RNA, and HBV DNA in plasma specimens from human donors, including donors of Whole Blood, blood components, Source Plasma, and other living donors. This test is also intended for use in testing plasma to screen organ and tissue donors when specimens are obtained while the donor's heart is still beating. This test does not discriminate between HIV-1 Group M, HIV-1 Group O, and HIV-2 RNA.

For donations of Whole Blood and blood components, plasma specimens may be tested individually or in pools comprised of not more than six (6) equal aliquots of individual specimens. For donors of hematopoietic stem/progenitor cells (HPCs) sourced from bone marrow, peripheral blood, or cord blood, and for donors of donor lymphocytes for infusion (DLI), plasma may be tested in pools comprised of equal aliquots of not more than six (6) individual donor specimens. For donations of Source Plasma, the sample may be tested in pools comprised of equal aliquots of not more than 96 individual specimens.

Whereas this test can detect HIV-1 Group O RNA and HIV-2 RNA, detection of HIV-1 Group O RNA or HIV-2 RNA in donor specimens negative for anti-HIV-1 Group O antibodies or anti-HIV-2 antibodies, respectively, has not been demonstrated in clinical studies. This test is intended to be used in conjunction with licensed serology tests for HIV, HCV, and HBV. For an individual specimen, MPX v2 results are simultaneously detected and discriminated for HIV, HCV, and HBV.

This test is not intended for use as an aid in diagnosis of infection with HIV, HCV, or HBV.

II. BACKGROUND

Serological screening assays have greatly reduced, but not eliminated, the risk of transmission of viral infections by transfusion of blood and blood products. Studies have shown that testing for viral nucleic acids (HIV-1 RNA, HCV RNA, and HBV DNA) can further reduce the transmission risk of these agents in blood donations made during the seroconversion window period. To improve the efficiency of testing for multiple targets, a multiplex (MPX) polymerase chain reaction (PCR) for simultaneous detection of multiple viruses has been developed. In MPX PCR, more than one target sequence is amplified and detected by using multiple pairs of primers and probes in one reaction tube. The MPX v2 is a qualitative multiplex test that enables the simultaneous detection and discrimination of HIV RNA, HCV RNA, and HBV DNA in a single test. The MPX v2 uses a nucleic acid preparation technique on the cobas AmpliPrep Instrument. HIV RNA (HIV-1 Groups M and O RNA and HIV-2 RNA), HCV RNA, and HBV DNA are amplified, detected, and discriminated using automated, real time PCR on the cobas TaqMan Analyzer s 201 (the "cobas s 201 system"). The test does not discriminate among HIV-1 Group M, HIV-1 Group O, and HIV-2 RNA. The test incorporates an Internal Control (IC) for monitoring test performance in each individual test, as well as the AmpErase enzyme to reduce potential contamination by previously amplified material (amplicon). The Roche Molecular Systems (RMS) cobas TaqScreen MXP Test (MPX), the previous version of the test, was licensed by the Food and Drug Administration in December, 2008. This previous version also uses real-time PCR technology to simultaneously detect HIV-1 Group M RNA, HIV-1 Group O RNA, HIV-2 RNA, HCV RNA, and HBV DNA but does not discriminate among the analytes. The discriminatory testing of the MPX reactive specimens is conducted using a separately licensed cobas AmpliScreen HIV-1 Test v1.5, cobas AmpliScreen HCV Test v2.0, and cobas AmpliScreen HBV Test.

Principles of the Assay

The MPX v2 for use with the cobas s 201 system is based on four major processes:

1. **Automated Specimen Pooling and Pipetting using the Hamilton MICROLAB STAR/STARlet IVD Pipettor (optional)**

The Hamilton MICROLAB STAR/STARlet IVD Pipettor automates the pipetting of pools and individual donor specimens and Test Controls, and the transfer of aliquots to Deep Well Plates. Manually pipetted specimens may be loaded directly onto the COBAS AmpliPrep Instrument without prior use of the Hamilton MICROLAB STAR/STARlet IVD Pipettor.

2. **Automated Specimen Preparation using the COBAS AmpliPrep Instrument**

Nucleic acids from the targets and added Armored RNA Internal Control (IC) molecules are simultaneously processed. The MPX v2 contains reagents that accomplish five sequential steps on the COBAS AmpliPrep Instrument. The Proteinase Solution digests proteins to promote lysis of the viral particles, inactivates nucleases, and facilitates the release of RNA and DNA. Addition of the Lysis Reagent to the specimen results in viral lysis and nuclease inactivation by denaturation of proteins. RNA and DNA are released and simultaneously protected from nucleases. The released nucleic acids bind to the silica surface of the added Magnetic Glass Particles due to the net positive charge on the glass particle surface and net negative charge of the nucleic acids under the chaotropic salt concentration and ionic strength of the Lysis reaction. The Wash Reagent removes unbound substances and impurities and reduces the salt concentration. Purified nucleic acids are released from the Magnetic Glass Particles at an elevated temperature with Elution Buffer.

3. Automated Amplification of Nucleic Acid using the COBAS TaqMan Analyzer

After isolation of the purified nucleic acids from human plasma during automated specimen preparation, the MPX v2 Master Mix is used for the amplification, detection, and discrimination of HIV RNA (HIV-1 Group M RNA, HIV-1 Group O RNA, and HIV-2 RNA collectively, without discrimination), HCV RNA, HBV DNA, and IC RNA. Once activated by the addition of manganese acetate, the MPX v2 Master Mix permits reverse transcription (for RNA targets), followed by PCR amplification of highly conserved regions of HIV-1 Group M RNA, HIV-1 Group O RNA, HIV-2 RNA, HCV RNA, HBV DNA, and IC RNA using specific primers. Concurrent detection of the amplified nucleic acid is accomplished by the generation of fluorescent signals from 5'- nucleolytic degradation of the HIV (HIV-1 Groups M and O, and HIV-2), HCV, HBV, and IC probes that bind to the amplified target sequences.

Reverse transcription and amplification reactions are performed with a thermostable recombinant enzyme, Z05D DNA Polymerase. In the presence of manganese ($Mn2^{+}$), Z05D DNA Polymerase has reverse transcriptase, DNA polymerase and 5’ to 3’ nuclease activities. This allows reverse transcription, PCR amplification, and detection to occur in the same reaction tube. Compared to Z05 DNA polymerase used in MPX, the new version of polymerase, Z05 D DNA Polymerase, has improved inclusivity of the different enzymatic activities and improved efficiency of reverse transcription.

4. Real Time Automated Detection and Discrimination of PCR Products using the COBAS TaqMan Analyzer

During PCR amplification, the intermittent high temperature during the cycling denatures the Target and IC amplicon to form single stranded DNA. The specific detection oligonucleotide

probes hybridize to the single stranded form of the amplified DNA. Amplification, Hybridization, and Detection occur simultaneously. The MPX v2 Master Mix contains detection probes which are specific for HIV-1 (Groups M and O), HIV-2, HCV, HBV, or IC nucleic acid. Three unique reporter dyes are associated with the HIV (-(b)(4)-), HCV (-(b)(4)-), or HBV (-(b)(4)-) specific probes and are measured at defined wavelengths at -(b)(4)- for HIV, -(b)(4)- for HCV, and -(b)(4)- for HBV. A fourth reporter dye is associated with the IC specific probe (-(b)(4)-) and is measured at -(b)(4)- wavelength. A single type of quencher dye is used for all probes. This system permits simultaneous detection and discrimination of the amplified HIV, HCV, and HBV targets, and the IC. Before PCR amplification begins, the probes are intact and the reporter dye fluorescence is suppressed by the quencher dye due to Förster-type energy transfer. During PCR amplification, the probes hybridize to specific single stranded DNA sequences and are cleaved by the 5' to 3' nuclease activity of the Z05D DNA Polymerase at the same time that amplification is occurring. Once the reporter and quencher dyes are separated by this cleavage, the fluorescent activity of the reporter dye is unmasked. With each PCR cycle, increasing amounts of cleaved probes are generated and the cumulative signal of the reporter dye is concomitantly increased. Real time detection and discrimination of PCR products is accomplished by measuring the fluorescence of the released reporter dyes representing the viral targets and IC.

Automated Data Management using the Pooling and Data Management (PDM) Software

Roche PDM software allows the user to review and report results. The Roche PDM software assigns test results for all tests as non-reactive, reactive, or invalid. In addition to retrieving and examining PCR results, the Roche PDM software allows the operator to print reports, search for results, accept donor results, and optionally transmit results to a Laboratory Information System (LIS).

III. CHEMISTRY, MANUFACTURING, AND CONTROLS (CMC)

The manufacturing of the MPX v2 is performed in accordance with current Good Manufacturing Practices in an environmentally controlled facility. The application complies with 21 CFR 809, and follows the recommendations in “Guidance for Industry: Content and Format of Chemistry, Manufacturing, and Controls Information and Establishment Description Information for Biological *In Vitro* Diagnostic Products.”

Quality oversight is managed by complying with 21 CFR 820, Quality System Regulation.

a. Kit Components

Three kits are required for performing the MPX v2 assay:

1. cobas TaqScreen MPX Test, v2

The MPX v2 kit contains seven components for the specimen preparation, amplification, and detection of HIV-1 Group M RNA, HIV-1 Group O RNA, HIV-2 RNA, HCV RNA, and HBV DNA. These seven components are configured into four plastic cassette carriers. Each kit includes 2 sets of each of the following four cassettes and provides sufficient materials for processing a total of 96 samples in a single batch or 48 tests in each of two separate batches. Each cassette has one or more of the seven components:

- MPX2 CS1
  Magnetic Glass Particles (MPG)
- MPX2 CS2
  Lysis Reagent (LYS)
- MPX2 CS3
  - Proteinase Solution (Pase)
  - Elution Buffer (EB)
- MPX2 CS4
  - MPX v2 Master Mix Reagent 1 (MPX v2 MMX-R1)
  - MPX v2 Master Mix Reagent 2 (MPX v2 MMX-R2)
  - MPX v2 Internal Control (MPX v2 IC)

2. cobas MPX Control Kit, v2

The MPX Control Kit, v2 (MPX CTL v2) consists of six sets of control reagents. Each set consists of each of the following as a single use vial:

- MPX M(+)C, v2 [MPX Multi-Positive Control, v2] (HIV-1 M Positive Control, HBV Positive Control, and HCV Positive Control)
- MPX O(+)C, v2 [cobas TaqScreen MPX HIV-1 O Positive Control, v2]
- MPX 2(+)C, v2 [cobas TaqScreen MPX HIV-2 Positive Control, v2]
- MPX(-)C, v2 [cobas TaqScreen MPX Negative Control, v2]

Each kit contains reagents sufficient for processing six separate batches with one set required for each batch.

3. cobas TaqScreen Wash Reagent

The Wash Reagent (TS WR) consists of an aqueous solution of -(b)(4)- Sodium Citrate Dihydrate, -(b)(4)- with 0.1% (w/v) Methylparaben. This is the same solution that is used in the MPX test.

b. Primers, Probes, and Assay Controls

The MPX v2 includes (b)(4) primers and 5 dual-labeled fluorescent probes for the amplification and detection of HIV-1 Group M, HIV-1 Group O, HIV-2, HCV, HBV, and the MPX v2 Internal Control. The probes also allow for discrimination of HIV, HCV, HBV, and the Internal Control. The HIV-1 Group M, HIV-1 Group O, and HIV-2 target sequences are independently amplified and detected by specific primers and probes. However, the probes for these targets share the same fluorescent detection dye, and thus these targets are not discriminated.

1. HIV-1 Group M and HIV-1 Group O Primers and Probes

The primers for HIV-1 Group M, the reverse primer for HIV-1 Group O, and the DNA sequence of the HIV-1 probe used in the licensed MPX, are maintained in the MPX v2. Although limited sequence information for HIV-1 Group N exists, ----------(b)(4)----------.

The HIV-1 probe sequence is able to detect both HIV-1 Group M and HIV-1 Group O. This probe is labeled with the ----------(b)(4)----------, as compared to the licensed MPX test, for ease of ---(b)(4)---.

2. HIV-2 Primers and Probe

The HIV-2 primer and probe system is updated for the MPX v2. ----------(b)(4)----------

3. HCV Primers and Probe

----------(b)(4)---------- used in the MPX are maintained for the MPX v2. ----------(b)(4)----------. Furthermore,

----------(b)(4)---------- channel 3.

4. HBV Primers and Probe

Although the ----------(b)(4)---------- region remains the same as for the licensed MPX, the ----------(b)(4)---------- channel 2.

5. MPX v2 Internal Control Primers and Probe

The MPX v2 Internal Control (MPX v2 IC) is a single stranded armored RNA which is added to each specimen prior to specimen preparation to monitor the entire specimen preparation and amplification/detection process. This IC contains unique primer and probe binding regions which have ----------(b)(4)----------. The new MPX v2 IC contains ----------(b)(4)----------

The MPX v2 IC sequence was changed ----------(b)(4)----------. The MPX v2 IC probe is labeled with the reporter dye -(b)(4)- for reporting in --(b)(4)--.

6. MPX v2 Positive Controls

The MPX v2 contains three positive control formulations: the co-formulated MPX Multi-Positive Control, v2 [MPX M(+)C, v2] containing three target Positive Control constructs (HIV-1 Group M, HBV, and HCV), the HIV-1 Group O Positive Control [MPX O(+)C, v2, and the HIV-2 Positive Control [MPX 2(+)C, v2]. This control configuration differs from the licensed MPX which contains five positive controls formulated individually. The HIV-1 Group M, HIV-1 Group O, HIV-2, and HCV Positive Controls are non-infectious particles containing the viral target sequences from each respective virus as single stranded armored RNA. The HBV Positive Control is double stranded HBV lambda DNA. All are encapsulated by bacteriophage coat proteins, which protect the control RNA or DNA from nucleases. The Positive Controls are formulated in Negative Human Plasma (NHP), and are used to monitor the full test process from specimen preparation through amplification and detection.

Four of the five Positive Control sequences (HIV-1 Group M, HIV-1 Group O, HIV-2, and HCV) are identical to the licensed MPX Positive Control sequences. These controls are formulated from the ---(b)(4)--- material for both tests. The sequence of the HBV Positive Control for the MPX v2 is very similar to the HBV Positive Control for the licensed MPX, except that the new control contains ----------(b)(4)----------.

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

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

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

7. MPX v2 Negative Control

The MPX Negative Control, v2 [MPX(-)C, v2] is formulated with NHP containing 0.1% ProClin 300. This is the same Negative Control that is used in the licensed MPX.

c. Manufacturing Quality Control

Various raw materials and components used in the production of the MPX v2 lots used in the Clinical and Non-Clinical Studies were manufactured at the Roche Molecular Systems, Inc. (RMS) Branchburg manufacturing facility located in Somerville, New Jersey, the Roche Diagnostics facility located in --------(b)(4)---------, the RMS facility located in -------(b)(4)----------, and the ----------(b)(4)---------- facility located in ----------(b)(4)----------.

Since the manufacture of the Clinical and Non-Clinical Study lots, the manufacture of the MPX v2 Positive Control Stocks and Positive Controls (PCs) has been moved from the RMS ----------(b)(4)----------, respectively, to the RMS Branchburg facility. The relocation of PC manufacturing from RMS ---------(b)(4)--------- to RMS Branchburg was executed to consolidate all control manufacturing activities in one RMS facility where Quantitation Standards and Internal Controls (QS/IC) for other assays are currently produced under the same process and environmental conditions, and by the same trained core personnel.

The manufacture of Magnetic Glass Particles (MGP) and Proteinase Solution (Pase) will be conducted at Roche Diagnostics GmbH --(b)(4)-- Facility, ----------(b)(4)----------,

--(b)(4)--, under FDA Registration Number --(b)(4)--. The rest of the kit components and the final kit assembly and testing will be performed at Roche Molecular Systems, Inc., Somerville, New Jersey 08876, under FDA Registration Number 2243471.

d. CBER Lot Release

Each new lot of a licensed retroviral test kit is subject to lot release, including testing at the Center for Biologics Evaluation and Research (CBER) using panels developed for that purpose.

Three conformance lots of MPX v2 were received and tested in the Division of Biological Standards and Quality Control (DBSQC). CBER Reference Panels (CBER HIV-1 Group M, HIV-1 Group O, HIV-2, HCV, and HBV) were used to evaluate kit function. All three lots demonstrated the expected reactivity on all of the CBER Reference Panels. The following is a list of the lots submitted in support of approval:

Lot Number	Expiration Date	Lot Release Determination
T0595	March, 2015	Pass
T09552	March, 2015	Pass
T09553	March, 2015	Pass

e. Facilities Review/Inspection

RMS will manufacture the MPX v2 in the currently licensed multi-product RMS Somerville facility.

The review of facility and equipment-related issues conducted by a Division of Manufacturing and Product Quality/Office of Compliance and Biologics Quality reviewer did not identify any major concerns. The major equipment/instrumentation used for the MPX v2 is identical to the licensed MPX.

A pre-licensure inspection of the RMS Somerville facility was not required because of the following:

- RMS Somerville is already approved to manufacture the licensed MPX assay and cobas AmpliScreen assays.
- The facility was inspected in December, 2012 by TeamBio of the Office of Regulatory Affairs at FDA. The inspection was classified as Voluntary Action Indicated (VAI). There are no outstanding cGMP issues from the previous inspections.

f. Environmental Assessment

The request for Categorical Exclusion from the Environmental Assessment for the manufacture of the MPX v2 was reviewed under 21 CFR 25.31(c) and is justified as the product is composed of naturally occurring substances and no extraordinary circumstances exist.

IV. NON-CLINICAL STUDIES

a. Determination of Limits of Detection (LODs)

Standards used for LOD Determination

The LODs of the MPX v2 were determined using the following standards:

- WHO Second International Standard for HIV-1 RNA (NIBSC code 97/650)
- WHO International Standard for Hepatitis B Virus DNA for Nucleic Acid Amplification Technology (NAT) Assays (NIBSC code 97/746)
- WHO Second International Standard for Hepatitis C Virus RNA for Genomic Amplification Technology Assays (NIBSC code 96/798)
- WHO International Standard for HIV-2 RNA (NIBSC code 08/150)
- Roche Primary Standards for HIV-1 Group O and HIV-2.

No International Standard is currently available for HIV-1 Group O RNA. The Roche Primary Standards for HIV-1 Group O RNA and HIV-2 RNA are commercially available cultured virus stocks. The Roche HIV-1 Group O and HIV-2 Standards are traceable to the CBER HIV-1 Subtype RNA Reference Panel #1 Lot 01 and to the CBER HIV-2 RNA Lot Release Panel ISD, respectively.

LOD Determination

For the WHO HIV-1, HCV, and HBV Standards and the Roche Primary Standards, three independent dilution series of each viral standard were prepared with pooled human plasma collected in EDTA anticoagulant. Each dilution series was tested using 3 different lots of MPX v2 kits with approximately 20 replicates per lot, for a total of approximately 180 replicates per concentration. For the WHO HIV-2 International Standard, 10 replicates per lot from 3 independent dilutions and 3 reagent lots were tested, for a total of 90 replicates per concentration.

PROBIT analysis was used to estimate average 95% LOD and 2-sided 95% fiducial confidence intervals for each virus (see Table 2).

The conversion factors for the MPX v2 are: 1 IU = 0.6 copy for HIV-1 Group M RNA, 1 IU = 2.7 copies for HCV RNA, and 1 IU = 5.0 copies for HBV DNA.

Table 2: Limits of Detection for MPX v2

Analyte	Standard	Units	Average 95% LOD	95% Confidence Interval
HIV-1 Group M	WHO International Standard	IU/mL	46.2 IU/mL	35.5 - 65.9
HIV-1 Group O	Roche Primary Standard	Copies/mL	18.3 cp/mL	13.1 - 30.9
HIV-2	Roche Primary Standard	Copies/mL	56.1 cp/mL	48.6 - 66.5
HIV-2	WHO International Standard	IU/mL	7.9 IU/mL	5.6 - 13.8
HCV	WHO International Standard	IU/mL	6.8 IU/mL	5.8 - 8.3
HBV	WHO International Standard	IU/mL	2.3 IU/mL	2.0 - 2.8

HBV Donor Reentry

Testing in singular, duplicate, and triplicate was performed to detect HBV WHO International Standard 97/746 at less than 2 IU/mL with greater than 95% probability (see Table 3). These data support testing in duplicate using the MPX v2 in reentry of donors deferred because of HBV test results.

Table 3: Probability of Detecting HBV DNA at 2 IU/mL in Multiple Replicates

Replicates (N)	Probability of Detection at 2 IU/mL
1	87.3%
2	98.4%
3	99.8%

b. Analytical Sensitivity – CBER Panels for HIV-1 Group M, HCV, HBV, HIV-1 Group O, and CBER HIV-2 Panel Stock

The FDA CBER panels for HIV-1 Group M, HIV-1 Group O, HIV-2, HCV, and HBV were tested with the MPX v2. The MPX v2 detected all panel members with titers of 25 - 500 copies /mL for HIV-1 Group M, 25-1000 copies/mL for HIV-1 Group O, 5-100 copies/mL for HIV-2, 5-500 copies/mL for HCV, and 10-500 copies/mL for HBV.

c. Genotype/Subtype Sensitivity and Inclusivity

The performance of the MPX v2 for detection of the subtypes of HIV-1 Groups M, N, and O, and the genotypes of HCV and HBV was evaluated using specimens tested neat and diluted 1:6 to simulate testing in pools of whole blood donor samples.

HIV-1 Group M

A total of 77 HIV-1 Group M neat clinical specimens of different subtypes and 81 HIV-1 Group M clinical specimens of different subtypes at 1:6 dilution were tested with the MPX v2. For HIV-1 Group M specimens tested neat, the MPX v2 detected all 77 specimens (see Table 4). For 1:6 diluted specimens, all subtype specimens were detected by the MPX v2 except one Subtype B that was non-reactive with the MPX v2 (see Table 5). This diluted specimen had a low RNA titer (below the LOD for MPX v2).

Table 4. Test Results for Neat HIV-1 Group M Subtype Specimens

Genotype	Total Number of Specimens	Number of MPX v2 Reactive
A	7	7
AE	10	10
AG	10	10
B	10	10
C	10	10
D	3	3
E	8	8
F	7	7
G	10	10
G-BG	1	1
J	1	1

Table 5. Test Results for 1:6 Diluted HIV-1 Group M Subtype Specimens

Genotype	Total Number of Specimens	Number of MPX v2 Reactive
A	7	7
AE	10	10
AG	10	10
B	10 [a]	9
C	10	10
D	3	3
E	8	8
F	10	10
G	10	10
G-BG	1	1
J	1	1

[a]. One of the specimens was confirmed to have a low titer when tested diluted (below the LOD for MPX v2) with the COBAS® AmpliPrep /COBAS® TaqMan® HIV-1 Test, v2.0.

HIV-1 Group O and HIV-1 Group N

Culture supernatants from a total of nine HIV-1 Group O culture isolates and one HIV-1 Group N culture isolate were tested on the MPX v2. Six of the nine HIV-1 Group O isolates were tested in triplicate (18 replicates) at 10 particles/mL and 100 particles/mL. The remaining three HIV-1 Group O isolate supernatants were tested in triplicate as diluted specimens by preparing log dilutions in normal, virus-negative human plasma to 1 x 10^{-7} (see Table 6). The MPX v2 showed a high rate of detection of the virus at these low levels. At least two replicates were detected for each of the nine isolates.

Table 6. Test Results for HIV-1 Group O Isolates

Subtype	Culture Supernatants	Detected at 10 particles/mL	Detected at 100 particles/mL	Detected at 1x10^{-7} dilution
HIV-1 Group O	9	83% (15/18)[a]	89% (16/18)[a]	67% (6/9) [b]

[a] Three replicates for each of six isolates

[b] The culture supernatant was diluted as stated and three replicates for each of three isolates were tested

The HIV-1 Group N culture isolate was quantified with the COBAS AmpliPrep/COBAS TaqMan HIV-1 Test, v2.0. The culture supernatant was diluted with normal, virus-negative human plasma to 3X LOD and 1X LOD of the MPX v2 for HIV-1 Group M. Twenty-four HIV-1 Group N replicates were tested with the MPX v2. The isolate was detected at a 100% rate (24/24) at both of the levels tested.

HIV-2

One HIV-2 subtype A culture isolate and one HIV-2 subtype B culture isolate were tested. The HIV-2 Subtype A culture isolate was diluted in pooled virus-negative human plasma and was detected 100% of the time at 91.5 copies/mL (~12.9 IU/mL after conversion to WHO International Units) and was detected at a level as low as 6 copies/mL (~0.85 IU/mL after conversion to WHO International units). For HIV-2 Subtype B, log dilutions of the culture supernatant were prepared in normal, virus-negative human plasma and three of three replicates of each dilution were detected with the MPX v2 in all dilutions up to 1×10^{-5}. No clinical specimens were available for this study.

HCV

A total of 91 HCV clinical specimens of different genotypes were tested neat and diluted 1:6 with the MPX v2. For HCV specimens tested neat, the MPX v2 detected all HCV genotypes; one genotype 1b specimen was non-reactive (see Table 7). For all diluted specimens, the MPX v2 detected all HCV genotypes tested (see Table 8).

Table 7. Test Results for Neat HCV Genotype Specimens

Genotype	Total Number of Specimens	Number of MPX v2 Reactive
1a	10	10
1b	10	9
2	10	10
2a	5	5
2b	10	10
3a	10	10
4	10	10
4a	5	5
4acd	4	4
4d	1	1
5a	10	10
6	5	5
6ab	3	3
6c	1	1

Table 8. Test Results for 1:6 Diluted HCV Genotype Specimens

Genotype	Total Number of Specimens	Number of MPX v2 Reactive
1a	10	10
1b	10	9
2	10	10
2a	5	5
2b	8	8
3a	10	10
4	10	10
4a	5	5
4acd	4	4
4d	1	1
5a	10	10
6	5	5
6ab	2	2
6c	1	1

HBV

A total of 51 HBV clinical specimens of different genotypes were tested neat and diluted 1:6 with the MPX v2. The MPX v2 detected each genotype tested neat and diluted (see Tables 9 and 10).

Table 9. Test Results for Neat HBV Genotype Specimens

Genotype	Total Number of Specimens	Number of MPX v2 Reactive
A	10	10
B	8	8
C	10	10
D	10	10
E	3	3
F	4	4
Pre-core mutant	6	6

Table 10. Test Results for 1:6 Diluted HBV Genotype Specimens

Genotype	Total Number of Specimens	Number of MPX v2 Reactive
A	10	10
B	8	8
C	10	10
D	10	10
E	3	3
F	4	4
Pre-core mutant	6	6

d. Seroconversion Panels

The performance of the MPX v2 during seroconversion was determined for HIV-1 Group M, HCV, and HBV using commercially available seroconversion panels collected from plasmapheresis donors. The MPX v2 results for neat and 1:6 diluted specimens were compared with licensed serology tests that used neat specimens. Panels that were consistently reactive with the MPX v2 beginning on the first bleed were excluded from the summary calculations for the minimum, average, and maximum number of days earlier detection than by the serology tests.

HIV-1 Seroconversion Panels

Ten commercially available seroconversion panels collected from plasmapheresis donors that seroconverted for HIV antibody were tested with the MPX v2. Each sample was tested with the

MPX v2 neat and diluted 1:6 to simulate testing in pools of blood donor samples. The MPX v2 results for neat and 1:6 dilution samples were compared to the results obtained with serological assays, the Abbott PRISM HIV O Plus and the Abbott PRISM anti-HIV-1/2 (CE Marked) (see Table 11).

Table 11: Performance of MPX v2 on HIV-1 Seroconversion Panels

HIV-1 Seroconversion Panels	Days Earlier Detection by the MPX v2 than by Tests for HIV-1/2 Antibody			
	Abbott PRISM HIV O Plus Neat		Abbott PRISM anti-HIV-1/2 Neat	
	MPX v2 for Neat Samples	MPX v2 for 1:6 Dilution Samples	MPX v2 for Neat Samples	MPX v2 for 1:6 Dilution Samples
1	14	9	14	9
2	14	14	14	14
3	12	12	12	12
4	14	14	14	14
5	11	11	11	11
6	14	14	14	14
7	9	9	9	9
8	14	14	14	14
9	15	15	15	15
10	22	22	22	22
Minimum	9	9	9	9
Average	13.9	13.4	13.9	13.4
Maximum	22	22	22	22

Overall, the MPX v2 was able to detect HIV-1 RNA several bleeds ahead of the tests for HIV-1 antibody.

HCV Seroconversion Panels

Twelve commercially available seroconversion panels collected from plasmapheresis donors that seroconverted for HCV antibody were tested with the MPX v2. Each sample was tested with the MPX v2 neat and diluted 1:6 to simulate testing in pools of blood donor samples. The MPX v2 results for neat and 1:6 dilution samples were compared to the results obtained with serological assays, the Abbott PRISM HCV and the ORTHO HCV Version 3.0 ELISA Test System (see Table 12).

Table 12: Performance of MPX v2 on HCV Seroconversion Panels

HCV Seroconversion Panels	Days Earlier Detection by the MPX v2 than by Tests for HCV Antibody			
	Abbott PRISM HCV Neat		Ortho HCV v3 ELISA Neat	
	MPX v2 for Neat Samples	MPX v2 for 1:6 Dilution Samples	MPX v2 for Neat Samples	MPX v2 for 1:6 Dilution Samples
1[a]	12	12	23	23
2[a]	30	30	32	32
3	23	23	23	23
4[a]	25	25	25	25
5[a]	28	28	28	28
6[a]	4	4	4	4
7[a]	11	11	11	11
8[a]	24	24	24	24
9[a]	7	7	18	18
10	33	33	33	33
11	32	32	32	32
12	30	30	30	30
Minimum	23	23	23	23
Average	29.5	29.5	29.5	29.5
Maximum	33	33	33	33

[a] Panels that were consistently reactive with the MPX v2 beginning on the first bleed were excluded from the summary calculations for the minimum, average, and maximum number of days earlier detection than HCV antibody.

Overall, the MPX v2 was able to detect HCV RNA several bleeds ahead of the tests for HCV antibody.

HBV Seroconversion Panels

Ten commercially available seroconversion panels collected from plasmapheresis donors that seroconverted for HBsAg were tested with the MPX v2. Each sample was tested with the MPX v2 neat and diluted 1:6 to simulate testing in pools of blood donor samples. The MPX v2 results for neat and 1:6 dilution samples were compared to the results obtained with serological assays, the Abbott PRISM HBsAg and the ORTHO HBsAg ELISA Test System 3 (see Table 13).

Table 13: Performance of MPX v2 on HBV Seroconversion Panels

HBV Seroconversion Panels	Days Earlier Detection by the MPX v2 than by Tests for HBsAg			
	Abbott PRISM HBsAg Neat		Ortho HBsAg ELISA 3 Neat	
	MPX v2 for Neat Samples	MPX v2 for 1:6 Dilution Samples	MPX v2 for Neat Samples	MPX v2 for 1:6 Dilution Samples
1[a]	3	0	25	22
2	12	12	19	19
3	8	0	19	11
4	-14	-14	14	14
5	22	13	22	13
6[a,b]	7	7	23	23
7	20	8	20	8
8	11	11	11	11
9	28	14	30	16
10	33	24	40	31
Minimum	-14	-14	11	8
Average	15	7.6	21.9	16.1
Maximum	33	24	40	31

[a] Panels that were consistently reactive with the MPX v2 for neat samples beginning on the first bleed were excluded from the summary calculations for the minimum, average, and maximum number of days earlier detection than HBsAg.

[b] Panels that were consistently reactive with the MPX v2 for 1:6 dilution samples beginning on the first bleed were excluded from the summary calculations for the minimum, average, and maximum number of days earlier detection than HBsAg.

Overall, the MPX v2 was able to detect HBV DNA several bleeds ahead of HBsAg (except in one panel where HBsAg was detected at an earlier bleed (panel 4) than HBV DNA by the MPX v2).

e. Analytical Specificity – Potentially Cross-Reactive and Interfering Microorganisms

The analytical specificity of the MPX v2 was evaluated by testing a panel of 20 microorganisms (see Table 14) including 13 viral isolates, 6 bacterial strains, and 1 yeast isolate. The microorganisms were added to normal, virus-negative, human plasma and tested with and without HIV-1 Group M, HIV-1 Group O, HIV-2, HCV, or HBV added to a concentration of 3X LOD of the MPX v2 for each virus.

Non-reactive results were obtained with the MPX v2 for all of the microorganism samples without added HIV-1 Group M, HIV-1 Group O, HIV-2, HCV, or HBV. The tested microorganisms did not cross-react with the MPX v2.

Reactive results were obtained for all of the microorganism samples with added HIV-1 Group M, HIV-1 Group O, HIV-2, HCV, or HBV. The tested microorganisms did not interfere with the MPX v2 under the test conditions.

Table 14: Analytical Specificity – Microorganisms Tested

Adenovirus 5	Human Herpes Virus 6A	*Candida albicans*
Cytomegalovirus	Human T-Lymphotropic Virus, I	*Propionibacterium acnes*
Epstein Barr Virus	Influenza Virus A	*Staphylococcus epidermidis*
Varicella-Zoster Virus, 1	West Nile Virus	*Staphylococcus haemolyticus*
Herpes Simplex Virus, 1	Dengue-1, Strain Hawaii	*Escherichia coli*
Herpes Simplex Virus, 2	Chikungunya Virus	*Streptococcus viridans*
Hepatitis G Virus	*Staphylococcus aureus*	

f. **Analytical Specificity — Disease States**

Plasma specimens from infected individuals in each of the following disease categories: Human Cytomegalovirus, Epstein-Barr Virus, Herpes Simplex Virus Type I, Herpes Simplex Virus Type 2, Human T-Lymphotropic Virus Type I, Human T-Lymphotropic Virus Type II, Human T-Lymphotropic Virus Type I/II, Hepatitis A Virus, West Nile Virus, and Parvovirus B19, were tested with and without HIV-1 Group M, HIV-1 Group O, HIV-2, HCV, or HBV added to a concentration of 3X LOD of the MPX v2 for each virus.

The MPX v2 yielded non-reactive results for all of the disease state specimens without added HIV-1 Group M, HIV-1 Group O, HIV-2, HCV, or HBV.

The MPX v2 yielded reactive results for all of the disease state specimens with added HIV-1 Group M, HIV-1 Group O, HIV-2, HCV, or HBV, except in one of six HTLV-I/II samples for which the test for added HCV was invalid due to operational error. These disease states did not interfere with the sensitivity or specificity of the MPX v2 under the test conditions.

g. **Potentially Interfering Substances**

Endogenous Interfering Substances

Plasma specimens with abnormally high levels of triglycerides (up to 3300 mg/dL), hemoglobin (up to 500 mg/dL), unconjugated bilirubin (50 mg/dL), albumin (up to 9.6 g/dL), or human DNA (up to 0.4 mg/dL) were tested with and without HIV-1 Group M, HIV-1 Group O, HIV-2, HCV,

or HBV added to a concentration of 3X LOD of the MPX v2 for each virus. The listed substances did not interfere with the sensitivity or specificity of the MPX v2 under the test conditions.

Plasma specimens with red blood cells added to abnormally high levels (up to 10% v/v) were tested with and without HIV-1 Group M, HIV-1 Group O, HIV-2, HCV, or HBV added to a concentration of 3X the LOD of the MPX v2 for each virus. Plasma with red blood cells added to 2.5% (v/v) did not interfere with the sensitivity or specificity of the MPX v2. Plasma with red blood cells added to 5.0% (v/v) reduced the sensitivity of the MPX v2 for detection of HCV. Plasma with red blood cells added to 7.5% (v/v) interfered with sensitivity of the MPX v2 for detection of HBV and HCV. Detection of HIV viral targets was 100% in plasma with red blood cells added to 10% (v/v). When viral targets were present at 3X LOD, the sensitivity of the MPX v2 for detection of the IC was not reduced in plasma with red blood cells added to 1.0% (v/v). When no viral targets were present, the sensitivity of the MPX v2 for detection of the IC was not reduced in plasma with red blood cells added to 2.5% (v/v). The IC monitors all steps in the testing process (sample preparation, amplification, and detection) and did fail under conditions that might affect the performance of the assay.

Exogenous Interfering Substances

Normal, human plasma specimens containing abnormally high concentrations of acetaminophen (1324 µmol/L), acetylsalicylic acid (3.62 mmol/L), ascorbic acid (342 µmol/L), atorvastatin (600 µg Eq/L), fluoxetine (11.2 µmol/L), ibuprofen (2425 µmol/L), loratadine (0.78 µmol/L), nadolol (3.88 µmol/L), naproxen (2170 µmol/L), paroxetine (3.04 µmol/L), phenylephrine HCl (491 µmol/L), and sertraline (1.96 µmol/L), with and without HIV-1 Group M, HIV-1 Group O, HIV-2, HCV, or HBV added to a concentration of 3X LOD of the MPX v2 were tested for each virus. These exogenous substances did not interfere with the sensitivity or specificity of the MPX v2 under the test conditions.

V. CLINICAL STUDIES

a. Reproducibility

The reproducibility of the MPX v2 was established by testing a 32-member panel composed of two negative plasma samples, and two positive plasma samples each for HIV-1 Group M, HIV-1 Group O, HIV-2, HCV, and HBV at concentrations of approximately 0.5X, 1.0X, and 3.0X the LOD of the MPX v2 for each virus. Operators at each of three sites with one cobas s 201 system

per site performed five days of testing with each of three lots of reagents and two valid panel runs per day (two tests per run) to yield up to 180 tests per panel member virus type (see Table 15).

Table 15: MPX v2 –Reproducibility Study

Analyte	Concentration	% Agreement with expected results[a]	95% Confidence Interval
Negative	0	99.4% (167/168)	96.7% - 100.0%
HIV-1 Group M	0.5X LOD	82.7% (143/173)	76.2% - 88.0%
	1.0X LOD	94.2% (162/172)	89.6% - 97.2%
	3X LOD	98.9% (174/176)	96.0% - 99,9%
HIV-1 Group O	0.4X LOD	69.2% (119/172)	61.7% - 76.0%
	0.7X LOD	86.3% (145/168)	80.2% - 91.1%
	1.7X LOD	99.4% (170/171)	96.8% - 100.0%
HIV-2	0.5X LOD	83.7% (149/178)	77.4% - 99.8%
	1X LOD	98.3% (170/173)	95.0% - 99.6%
	3X LOD	100.0% (178/178)	97.9% - 100.0%
HCV	0.5X LOD	80.7% (142/176)	74.1% - 86.2%
	1X LOD	94.2% (161/171)	89.5% - 97.2%
	3X LOD[b]	100.0% (175/175)	97.9% - 100.0%
HBV	0.5X LOD	73.6% (131/178)	66.5% - 79.9%
	1X LOD	94.9% (168/177)	90.6% - 97.6%
	3X LOD	98.9% (172/174)	95.9% - 99.9%

[a] A denominator of less than 180 reflects the number of invalid results which were excluded from the analysis

[b] One test was also reactive for HBV.

This study demonstrated that the MPX v2 is highly reproducible across lots, across sites and on different days at concentrations of 3X LOD, with lesser reproducibility around or below the LOD, as expected.

b. Clinical Specificity

1. Specificity in a Whole Blood Donor Population

Specimens were collected from blood donors recruited at five test sites. Testing with the MPX v2 was performed according to two testing algorithms: one for individual blood donations (requiring a single level of testing), and one for pools of up to six donations (requiring a single level of testing for primary pools that were non-reactive and two levels of testing for primary pools that were reactive at primary pool testing and at individual donor resolution testing).

Specificity in Individual Donation Testing (Whole Blood)

For the individual donation testing, a total of 13,306 Whole Blood donations were tested. Of these, 29 specimens were excluded from further calculations because they were from donors who were presumed to be infection status positive due to repeatedly reactive serology test results (13,306 - 29 = 13,277). Of the remaining 13,277 donations, 17 were reactive on the MPX v2 (13,277 - 17 = 13,260). Three of these 17 donations were from donors subsequently shown to be infection status positive, and these three specimens were excluded from the calculation of specificity (13,277 - 3 = 13,274) (see Table 16). **The clinical specificity for individual donation testing in this study was 99.895% (13,260/13,274; 95% CI: 99.823% to 99.937%).**

Table 16: Individual Donation Reactivity in Whole Blood Donors

Category	# of Specimens	Percentage of Specimens Tested
Individual Donations Tested	13,277	100.00
Non-Reactive Donations	13,260	99.87
Reactive Donations	17	0.13
Reactive Donations for Donor Status Positives (True Positive)	3	0.02
Reactive Donations for Donor Status Negatives (False Positive)	14	0.11

Specificity in Pool Testing (Whole Blood)

The Whole Blood donations were also tested in pools of equal aliquots of not more than six individual donations. Of the 10,500 pools tested by MPX v2, 10,471 pools were negative. Of the 29 reactive pools (10,500 - 10,471 = 29), 15 pools contained specimens from donors who were shown to be infection status positive (i.e., true positive donor specimens in the pool), and those pools were excluded from the calculation of specificity. The remaining 14 reactive pools contained individual donation specimens that were all found non-reactive in pool resolution testing; therefore, the test result on the 14 pools was false positive (10,471 + 14 = 10,485) (see Table 17). Based on these data, **the specificity in pool testing was 99.866% (10,471/10,485, 95% CI: 99.78% to 99.92%).**

Table 17: Pool Reactivity in Whole Blood Donors

Category	# of Pools	Percentage of Pools Tested
Pools Tested	10,500	100.00
Non-Reactive Pools	10,471	99.72
Reactive Pools	29	0.28
Reactive Pools with Donor Status Positive Specimens (True Positive)	15	0.14
Reactive Pools with Donor Status Negative Specimens (False Positive)	14	0.13

Specificity in Individual Donation Testing in Pool Resolution Testing (Whole Blood)

A total of 64,030 individual donations were tested in 10,500 pools of up to six donations in this study. The individual donations that caused the reactive pools were identified by individual donation testing during the resolution testing of the reactive pools. Of the total donor specimens tested in this study, 372 specimens were excluded from further calculations due to infection-status-positive results on discriminatory testing or follow-up testing of the donors or resolved MPX Test results. Of the remaining 63,658 donations (64,030 - 372 = 63,658), six donations were false positive on MPX v2 after resolution by individual donation testing of the reactive pools (63,658 - 6 = 63,652). **The clinical specificity for individual donations in pools of not more than 6 in this study was 99.991% (63,652/63,658; 95% CI: 99.979x% to 99.996%).**

The invalid batch rate for the MPX v2 from initial testing of donations in pools of up to six donor specimens and of individual donations was 4.8% and 5.3%, respectively.

2. <u>Specificity in Source Plasma Donor Samples</u>

Specificity in Pool Testing (Source Plasma)

A total of 1,100 pools comprised of equal aliquots of up to 96 individual specimens were tested with the MPX v2, of which 1,049 (95.4%) were non-reactive and 51 (4.6%) were reactive. Of

the 1,049 non-reactive pools, 1,048 pools contained all donor infection-status-negative donations and two pools contained at least one donor infection-status-positive donation; these two pools were excluded from the calculation of specificity. Twenty-one of the 51 reactive pools of 96 were determined to be false positive on MPX v2, as these were resolved to contain all MPX v2 non-reactive donations, following resolution testing using the Pooled Testing Algorithm or follow-up of the donors (i.e., false positive pools) (1,048 + 21 = 1,069). The remaining 30 reactive pools that contained at least one donation from a donor with positive infection status confirmed by follow-up testing of the donors were excluded from the calculation of specificity (see Table 18). **The clinical specificity (at the pool level) of the MPX v2 for testing Source Plasma pools of up to 96 individual specimens was 1,0478/1,069 = 98.04% (95% CI: 97.02% to 98.71%) in this study.**

Table 18: Reactivity in Pools of Up to 96 Source Plasma Donations

Category	# of Pools	Percentage of Pools Tested
Total Pools of up to 96 [a] Tested	1,100	100.0
Non-Reactive Pools [b]	1,049	95.4
Non-Reactive Pools with All Donations from Donors with Negative Infection Status	1,047	95.2
Non-Reactive Pools with At Least One Donation from a Donor with Positive Infection Status	2	0.2
Reactive Pools [b]	51	4.6
Reactive Pools with At Least One Donation from a Donor with Positive Infection Status	30	2.7
Reactive Pools with All Donations from Donors with Negative Infection Status (False Positive Pools)	21	1.9

[a] *Out of 1100 pools, 299 had fewer than 96 donations. Ninety-seven percent (1071) of these pools had 90 or more donations.*

[b] *Negative pool status was assigned based on initial MPX and CAS test results.*

Specificity in Individual Donation Testing in Pool Resolution Testing (Source Plasma)

Of the 103,981 donations tested, 103,950 were assigned a donation status of negative, of which 103,931 were MPX v2 non-reactive, for **a clinical specificity (at the donation level) of 103,931/103,950 = 99.982% (95% CI: 99.971% to 99.988%) in this study.**

3. Detection of Window Period Specimens in Source Plasma Testing

A total of 103,981 Source Plasma donations which tested negative individually for anti-HCV, anti-HIV-1, and HBsAg, from 14,776 unique donors were tested in pools of 96 with both the MPX v2 and the MPX, and individually with the COBAS AmpliScreen assay (CAS test) for HBV, HCV, and HIV. Initial donation status was assigned on the basis of the results of the MPX, the CAS tests, and a negative serology status.

The MPX v2 detected 12 window period cases: one HIV-1 window period infection, ten HCV window period infections, and one possible HBV occult infection were identified in the 14,776 unique donors tested. The reactive-NAT-only yield for this study was 1:14,776 for HIV-1, 1:1,478 for HCV, and 1:14,776 for HBV in Source Plasma donations.

c. Clinical Sensitivity

1. Studies in NAT Positive or Seropositive Specimens

A total of 2,949 HIV, HCV, and HBV NAT positive specimens (known to be NAT positive by viral load assays) were tested, neat and at 1:6 dilution, at four test sites with the MPX v2 (4 lots of reagents) and the MPX.

HIV NAT Positive Specimens

There were 1,106 and 1,123 samples with test results, respectively, for neat and 1:6 diluted HIV samples. The MPX v2 was reactive for 1,098 (99.3%) neat samples and 1,086 (96.7%) diluted samples (see Table 19). The MPX was reactive for 1,095 (99.0%) neat samples and 1,078 (96.0%) diluted samples. There were 37 specimens with non-reactive results at 1:6 dilution that had viral loads that were below the LOD of MPX v2 (or MPX) at this dilution. The performance of the MPX v2 was comparable to the MPX in detecting known HIV-1 RNA positive specimens in this study.

HCV NAT Positive Specimens

There were 1,137 and 1,122 samples with test results, respectively, for neat and 1:6 diluted HCV samples. The MPX v2 was reactive for 1,117 (98.2%) neat samples and 1,106 (98.6%) diluted samples (see Table 19). The MPX was reactive for 1,118 (98.3%) neat samples and 1,106 (98.6%) diluted samples. The performance of the MPX v2 was comparable to the MPX in detecting known HCV RNA positive specimens in this study.

HBV NAT Positive Specimens

There were 491 and 498 samples with test results, respectively, for neat and diluted HBV samples. The MPX v2 was reactive for 491 (100.0%) neat samples and 493 (99.0%) diluted samples (see Table 19). The MPX was reactive for 491 (100.0%) neat samples and 489 (98.2%) diluted samples. The performance of the MPX v2 was comparable to the MPX in detecting known HBV DNA positive specimens in this study.

Table 19: Test Results for Known NAT Positive Specimens

		Total Tested	**MPX v2 Positive**	**MPX Positive**
HIV-1 Group M	Neat	1,106	1,098	1,095
	1:6 diluted	1,123	1,086	1,078
HCV	Neat	1,137	1,117	1,118
	1:6 diluted	1,122	1,106	1,106
HBV	Neat	491	491	491
	1:6 diluted	498	493	489
HIV-1 Group O	Seropositive, diluted	11	8	8
	Cultured, diluted	9	9	9
HIV-2	Seropositive, Neat	312	181	172
	Seropositive, Diluted	318	137	137

HIV-1 Group O Seropositive Specimens

A total of 11 HIV-1 Group O seropositive repository specimens were tested after 1:6 dilution using the MPX v2. Eight of the 11 specimens were reactive. The three non-reactive specimens

had viral loads below the Limit of Detection of the Abbott Real Time HIV-1 Test (< 60 Copies/mL), and none of the three were reactive using the MPX v2 or the MPX (see Tables 19 and 20). In addition, culture supernatants from a total of nine different HIV-1 Group O cultured specimens were diluted and tested with the MPX v2 and the MPX. All diluted cultured specimens were reactive using both tests (see Tables 19 and 21).

Table 20: Test Results for HIV-1 Group O Seropositive Specimens

Specimen ID_Dilution	MPX v2	MPX
BSE191_1:6	R	R
HJ1230_1:6	R	R
JI1357_1:6	NR	NR
HJ162_1:6	R	R
HJ1977_1:6	R	R
HJ367_1:6	NR	NR
HJ736_1:6	NR	NR
HJ2044_1:6	R	R
K1043_1:6	R	R
HJ100_1:6	R	R
HJ1322_1:6	R	R

R = Reactive; NR = Non-Reactive

Table 21: Test results for HIV-1 Group O Culture Isolates

Culture ID_Dilution	MPX v2	MPX
60736_1:1000	R	R
BCF02_1:1000	R	R
MVP5180_1:1000	R	R
BV5003_1:2000	R	R
BV5051_1:1000	R	R
BV5024_1:1000	R	R
BCF11_1:2000	R	R
BCF101_1:2000	R	R
BCF06_1:2000	R	R

R = Reactive; NR = Non-Reactive

HIV-2 Seropositive Specimens

A total of 312 HIV-2 seropositive specimens were tested neat using the MPX v2 and the MPX. A total of 181 specimens of the 312 were reactive using the MPX v2 compared to 172 for the MPX. Of the 131 non-reactive specimens on the MPX v2, none were reactive using an alternate quantitative NAT method, suggesting that no detectable RNA was present in these specimens and that there were no false negative results using MPX v2.

2. Pool Deconstruction Studies for Source Plasma

Ten pools of 96 plasma specimens were prepared that contained a varying number of specimens positive for HIV-1 RNA, HCV RNA, and HBV DNA, and were tested using MPX v2. The testing process using MPX v2 correctly identified the infected members of the pools.

3. Studies in High Risk Populations

High-risk factors for HIV, HCV, and HBV included, but were not limited to, tattoo or body piercing, injection drug use, multiple sex partners, needle stick accidents, blood or blood product transfusion, history of a sexually transmitted disease, and dialysis. A total of 570 specimens from a high risk population were distributed evenly across four sites for testing with the MPX v2 and the MPX.

Samples were tested neat and diluted (1:6) with the MPX v2 and the MPX. Target resolution testing of specimens that tested reactive with the MPX was conducted using the cobas AmpliScreen HIV-1, HCV, and HBV tests using neat specimens only.

There were 567 neat samples with results from the MPX v2 and the MPX (see Table 22). The MPX v2 identified a total of 99 reactive specimens (8 HIV, 87 HCV, and 4 HBV) compared to 87 identified by the MPX (5 HIV, 71 HCV, 0 HBV and 11 were unresolved). In this population the MPX v2 identified more specimens than the MPX, which also had 11 unresolved results, demonstrating a greater ability of the MPX v2 to correctly identify specimens from infected individuals.

There were 570 diluted (1:6) specimens with results from the MPX v2 and the MPX. The MPX v2 identified a total of 80 reactive specimens (4 HIV, 74 HCV, and 2 HBV) compared to 78 identified by the MPX (4 HIV, 69 HCV, 0 HBV, and 5 were unresolved). The specimens that could not be detected at 1:6 dilution had viral loads below the LOD of MPX v2 at this dilution. There were no observed true reactive specimens from HBV infected individuals from this study for both neat and diluted samples. In this study the MPX v2 demonstrated a greater ability to correctly identify specimens from infected individuals compared to the MPX. Overall, in the high risk populations study the performance of MPX v2 was comparable to MPX in detecting the viral targets.

Table 22: MPX v2 Testing with Specimens from High Risk Populations

		MPX v2 Reactive	**MPX Reactive**
Neat	Nonreactive	468	480
	Total Reactive	99*	87*
	HIV	8	5
	HCV	87	71
	HBV	4[1]	0
	unresolved	0	11
	Total Tested	567	
1:6 Diluted	Nonreactive	490	492
	Total Reactive	80**	78**
	HIV	4	4
	HCV	74	69
	HBV	2[2]	0
	unresolved	0	5
	Total Tested	570	

*82 were reactive with both MPX v2 and MPX.

**73 were reactive with both MPX v2 and MPX.

[1] One MPX v2+/MPX+/CAS-/SuperQual- and 3 MPX v2+/MPX-/SuperQual-

[2] Two MPX v2+/MPX-/SuperQual-

VI. BIORESEARCH MONITORING (BIMO) INSPECTIONS

Inspections of the following clinical sites were conducted by BIMO inspectors in accordance with FDA's Compliance Program Guidance Manual 7348.811:

Site Number	Study Site	Location	Form FDA 483 Issued	Final Classification
Site 01/A01	Gulf Coast Regional Blood Center	Houston, Texas	None	NAI
Site 02/TAL	Talecris Biotherapeutics, Inc.	Raleigh, North Carolina	None	NAI
Site 03/R01	Mississippi Valley Regional Blood Center	Davenport, Iowa	None	NAI

NAI = No Action Indicated

REGULATORY REVIEW

The following are some of the issues that were identified during the review of the MPX v2. These review issues were discussed during Review Committee meetings, meetings with senior managers of DETTD and the Office of Blood, and RMS representatives.

1. <u>False Negative Results for Four HCV High Positive Specimens and the SP2 Software Patch</u>

In the study in which known HCV RNA positive specimens were tested (see section V.c. above), the MPX v2 failed to detect four HCV high viral load specimens. RMS attributed this to signal spiking during the real time PCR assay. During the meeting with FDA on June 13, 2014 and in their subsequent response to the FDA's Complete Response (CR) letter dated April 25, 2014, RMS explained the basis for this phenomenon of signal spiking in specimens with high viral load. They also explained that they had developed a software patch (SP2) to update the assay software. The SP2 will mitigate the problem by checking the raw data interpretation of non-reactive results and reporting this type of test result as invalid rather than non-reactive to avoid reporting false negative results. These invalid test results will require additional testing of the donation and follow-up testing of the donor.

RMS has committed to monitoring and reporting instances of HCV true positive samples that are reported as invalid due to the presence of signal spiking effects on the MPX v2. This will be accomplished through the RMS Complaint Handling Program. Moreover, RMS agreed to a Post Market Commitment to address this issue.

In the meantime, RMS has agreed to add the following warning statement to the package insert to mitigate the issue:

Warning: Some true positive HCV specimens may be reported as "invalid" due to signal spiking. These specimens will be flagged and required to be retested.

The user should report such results to RMS for further investigation.

2. HIV-1 Recombinants/Variants

The MPX v2 failed to detect two HIV-1 high viral load specimens due to mutations in the LTR region of HIV-1 RNA. RMS will reassess whether MPX v2 assay design or manufacturing changes are warranted based on feedback from post market surveillance and global surveillance programs. RMS agreed to a Post Market Commitment to monitor and address this issue.

RMS has agreed to add the following warning statement to the package insert to mitigate the issue:

Warning: This assay may fail to detect some HIV positive specimens due to LTR mutations in the primer/probe binding regions of HIV-1 genome. It is estimated that such LTR mutations occur in approximately 1.7% of HIV-1 NAT(+)/Antibody(-) donations.

3. Docked versus Undocked Configurations

In the docked versus undocked equivalency study, two discordant results were identified in the testing of neat samples and six discordant results were identified in the testing of diluted (1:6) samples. RMS has determined that most of the discordant results are likely the result of low viral titer samples. One discordant result for both neat and dilute testing occurred in an HIV-1 sample that was previously determined to have an LTR sequence variation that the MPX v2 did not detect.

4. "Non-target" Detection

There were 13 HIV reactive, 6 HCV reactive, and 1 HIV/HCV reactive donations out of a total of 77,176 donations tested in the study. These MPX v2 reactive results were concluded to be false reactive results relative to the true donor status.

Of these 20 total donations that were MPX v2 false reactive, only 5 of the donors completed follow-up, while 2 declined follow-up, and the remaining 13 donors were lost to follow-up.

VII. BENEFIT/RISK ANALYSIS

The MPX v2 assay has very high sensitivity and specificity for the detection of HIV-1 Group M RNA, HIV-1 Group O RNA, HIV-2 RNA, HCV RNA, and HBV DNA in plasma specimens. The Limits of Detection for these different analytes for the MPX v2 are equivalent to or better than those for the currently licensed MPX test. In addition, the MPX v2 can detect and simultaneously discriminate among HIV RNA (HIV-1 Group M or HIV-1 Group O or HIV-2), HCV RNA, and HBV DNA. This fully automated assay thus obviates the need for additional discriminatory testing, reducing the time needed for blood establishments to provide counseling to donors with reactive results.

Some specimens with very high HCV viral load could give invalid results due to a signal spiking effect, with a theoretical negative impact of about a 24-hour delay for donor notification and lookback. However, the estimated frequency of this signal spiking effect is 1 in approximately 180 million blood donations in the U.S. The MPX v2 assay may fail to detect some HIV-1 positive specimens due to mutations in the primer/probe binding sequences of the Long Terminal Repeat (LTR) region of the HIV-1 genome. It is estimated that such LTR mutations occur in approximately 1.7% of HIV-1 NAT-positive/antibody-negative donations; the estimated frequency of such a mutation is 1 in approximately 121 million blood donations in the U.S. The risk of invalid results due to signal spiking effect, and false negative results associated with HIV-1 mutations, is remote and does not outweigh the benefit of the MPX v2 assay.

VIII. FINAL REVIEW AND RECOMMENDATIONS

The Review Committee has reviewed the original submission and all of the material RMS provided during the interactive review, and all review issues are resolved or addressed in labeling. The Committee therefore recommends approval of the MPX v2 BLA with the following Post Market Commitments (PMCs).

IX. POST MARKET COMMITMENTS

Roche has committed to conduct the following activities after the MPX v2 is approved, as stated in the letter dated October 6, 2014 to FDA:

1. RMS commits to monitor for instances of HCV true positive samples that are reported as invalid due to the presence of signal spiking effects in the MPX v2 through RMS' Complaint Handling Program. The unnormalized raw data associated with complaints of this nature will be analyzed to verify the presence of signal spiking effects and samples will be requested for retesting. The complaint investigations will be stored in the RMS case handling system, will be periodically reported to FDA as a PMC Submission-Status Update, and will be included in the Annual Reports to FDA through the life cycle of the product.

2. RMS commits to monitor for the occurrence of mutations that could result in failure to detect some HIV-1 positive specimens through its Complaint Handling Program. Customer complaints that meet the criteria for a potentially critically complaint, (e.g., failure to detect HIV-1 mutations) are escalated for handling and investigation to the Complaint Investigation Resolution (CIR) group. Requests for samples that result in a failure to detect HIV-1 positive specimens are made for additional investigation activities, which may include sequence analysis or testing with an alternative platform that detects the LTR region of the HIV-1 genome. Specific information pertaining to HIV-1 mutations complaint investigations will be stored in the RMS case-handling system, will be periodically reported to FDA as a PMC Submission-Status Update, and will be included in the Annual Reports to FDA through the life cycle of the product.