Summary Basis for Regulatory Action

Date:	
From:	Guang Gao, Ph.D., Scientific Lead, BLA Review Committee
BLA STN#:	125576.0
Applicant Name:	Roche Molecular Systems, Inc.
Date of Submission:	November 13, 2014
Complete Response Letter:	June 16, 2015
Response to CR letter:	September 22, 2016
MDUFA Goal Date:	November 22, 2016
Proprietary Name:	cobas® MPX Test, for use on the cobas® 6800/8800 Systems
Biological Name:	HIV-1 Group O and M, HIV-2, HCV and/or HBV (HIV-1/HIV-2/HCV/HBV/Multiplex Discriminatory NAT Pool Testing)
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 in human donor plasma or serum specimens
Recommended Action:	Approval

Signatory Authorities Action:

Offices Signatory Authorities: 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.

Offices Signatory Authorities: Mary A. Malarkey, Director, OCBQ/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	Pawan Jain, OBRR/DETTD/PRB Guang Gao, OBRR/DETTD/PRB
Non-clinical/Analytical Review	Viswanath Ragupathy, OBRR/DETTD/LMV Laura Ulitzky* Jianqin Zhao, OBRR/DETTD/LMV Susan Zullo, OBRR/DETTD/PRB Luisa Gregori, OBRR/DETTD, LBTSEA Erica Silberstein. OBRR/DETTD/LEP
CMC Review	Jianqin Zhao OBRR/DETTD/LMV Erica Silberstein OBRR/DETTD/LEP Viswanath Ragupathy OBRR/DETTD/LMV Susan Zullo, OBRR/DETTD/PRB Krishnamurthy Konduru, OBRR/DETTD/LEP Deborah Trout, OCBQ/DMPQ/MRB1
Statistical Review	Zhen Jiang, OBE/DB/TEB Tie-Hua Ng, OBE/DB/TEB
Regulatory Project Manager	Alisha Miller* IO/OBRR Cherry
Facility Review	Deborah Trout, OCBQ/DMPQ/MRB1
Software and Instrumentation Review	Lisa Simone OBRR/DETTD/PRB Babita Mahajan, OBRR/DETTD/PRB Yongqing Chen, OBRR/DETTD/PRB
Living Organ Donor Claim Review	Michelle McClure, OTAT/DHT/HTRB
Bioresearch Monitoring	Anthony Hawkins, OCBQ/DIS/BIMO
Lot Release Testing Plan / In-Support Testing	Steve Kerby** Leslyn Aaron, OCBQ/DBSQC/LACBRP Kori Francis, OCBQ/DBSQC/LACBRP Susan Zullo, OBRR/DETTD/PRB
Labeling	Guang Gao, OBRR/DETTD/PRB Pradip Akolkar, OBRR/DETTD/PRB David Leiby, OBRR/DETTD/PRB J. Peyton Hobson, OBRR/DETTD Dana Jones, OCBQ/DCM/APLB

* No longer with DETTD/OBRR/CBER

**No longer with Agency

I. INTRODUCTION

Roche Molecular Systems, Inc. (RMS), submitted an original Biologics License Application (BLA) for the licensure of the cobas® MPX Test, for use on the cobas® 6800 and cobas® 8800 Systems (cobas® MPX Test).

Intended Use

The cobas® MPX Test, for use on the cobas® 6800 and cobas® 8800 Systems, 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 and serum. The cobas® MPX Test simultaneously detects and discriminates for HIV, HCV, and HBV and does not discriminate between HIV-1 Group M, HIV-1 Group O, and HIV-2.

This test is intended for use to screen for HIV-1 Group M 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 specimens to screen individual organ and tissue donors when specimens are obtained while the donor's heart is still beating.

For donations of Whole Blood and blood components, plasma specimens may be tested individually or in pools comprised of equal aliquots of not more than six (6) 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 individually or in pools comprised of equal aliquots of not more than six (6) individual donor specimens. For donations of Source Plasma, the samples may be tested in pools comprised of equal aliquots of not more than 96 individual Source Plasma donor specimens. Additionally, this test is intended to be used in conjunction with licensed serology tests for HIV, HCV and HBV.

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

This test is not intended for use on samples of cord blood.

This test is not intended for testing cadaveric blood specimens.

II. BACKGROUND

Serological screening assays have greatly reduced, but not eliminated, the risk of transmitting HIV, HCV and HBV 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 donated 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 viral markers has been developed. In MPX PCR, target sequences for more than one viral agent are amplified and detected using multiple pairs of primers and probes in a single reaction tube. The cobas® MPX Test is a qualitative

multiplex test that enables the simultaneous detection and discrimination of HIV RNA, HCV RNA, and HBV DNA on cobas® 6800 and cobas® 8800 Systems in a single test. 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 these instrument systems. The test does not discriminate between HIV-1 Group M, HIV-1 Group O, and HIV-2. The test incorporates an Internal Control (IC) for monitoring each individual test. The test also includes the AmpErase enzyme to reduce potential contamination by previously amplified material (amplicon).

III. CHEMISTRY, MANUFACTURING, AND CONTROLS (CMC)

The manufacture of the cobas® MPX Test is performed in in an environmentally controlled facility. The application 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."

1. Overview of the Assay

The cobas® MPX Test, for use on the cobas® 6800 and cobas® 8800 Systems, is based on real time PCR technology. The cobas® MPX Test consists of test kit, control kits, and omni reagents. The cobas® omni reagent kits [Wash Reagent, Lysis Reagent, and Magnetic Glass Particles (MGP) Reagent] are common to all tests performed on the cobas® 6800 and cobas® 8800 Systems and are used for sample preparation.

2. Kit Components

The cobas® MPX Test for the detection of HIV-1 Group M RNA, HIV-1 Group O RNA, HIV-2 RNA, HCV RNA, and HBV DNA in human plasma and serum consists of: a) cobas® MPX Test kit, b) cobas® MPX Control Kit, c) cobas® NHP Negative Control Kit, and d) the individual cobas® omni reagents for sample preparation.

a) cobas® MPX Test Kit

The cobas® MPX test kit has two configurations, the first is the cobas® MPX-96 (96 test cassette) and the second is the cobas® MPX-480 (480 test cassette). Each test kit contains the following components for specimen preparation, amplification, and detection of HIV-1 Group M RNA, HIV-1 Group O RNA, HIV-2 RNA, HCV RNA, and HBV DNA.

- Proteinase Solution (PASE)
- Internal Control (IC)
- Elution Buffer (EB)
- Master Mix Reagent 1 (MMX-R1)
- MPX Master Mix Reagent 2 (MPX MMX-R2)

b) cobas® MPX Test Control Kit

The cobas® MPX Test Control Kit consists of six sets of control reagents. Each set contains the following single use vials:

- MPX Multi- Positive Control (MPX M (+) C)
- MPX HIV-1 O Positive Control (MPX O (+) C)
- MPX HIV-2 Positive Control (MPX 2 (+) C)

c) cobas® NHP Negative Control Kit (NHP-NC)

cobas® MPX Test negative control uses normal human plasma, non-reactive by licensed tests for antibody to HCV, antibody to HIV-1/2, HBsAg, antibody to HBc; HIV-1 RNA, HIV-2 RNA, HCV RNA, HBV DNA, HEV RNA, WNV RNA, and CMV DNA not detectable by PCR methods.

d) cobas® Omni Reagents for Sample Preparation

The following reagents are used for sample preparation and are universal reagents for MPX assays.

- cobas® omni MGP Reagent (MGP)
- cobas® omni Lysis Reagent (LYS)
- cobas® omni Wash Reagent (WASH)

3. Primers, Probes, and Assay Controls

The design of the virus-specific oligonucleotides for the cobas® MPX test, for use with the cobas® 6800 and the cobas® 8800 Systems, is derived from sequences in conserved regions of their respective target viral genomes. In the cobas® MPX Test oligonucleotides are introduced for all five target viruses that achieve the (desired) analytical sensitivity and inclusivity. Specifically, the primers and probes were as follows. The HIV-1 Group M component of the cobas® MPX Test targets (b) (4) . The HIV-1 Group O component of the cobas® MPX Test targets (b) (4) . The HIV-2 component of the cobas® MPX Test targets (b) (4) . For the detection of HBV (b) are used in the cobas® MPX Test. For the HCV component of the cobas® MPX Test, (b) (4) of the genome is targeted. Furthermore, (b) (4) are used to improve HCV sensitivity and inclusivity.

The Internal Control (IC) for the cobas® MPX Test is a unique sequence with no homology to the target viral sequences.

The cobas® MPX Control Kit is comprised of the three positive controls. Positive Controls for HIV-1, HCV and HBV are (b) (4) for the MPX Multi-Positive Control. The other two Positive Controls are for MPX HIV-1 group O and MPX HIV-2.

4. **CBER Lot Release**

The lot release protocol templates were submitted to CBER for review and found to be acceptable after revisions. Data from three lots were submitted to CBER in support of the BLA. All results met the acceptance criteria. For routine lot release, CBER will review lot specific kit performance data reported on the approved Lot Release Protocol. A lot release testing plan was developed by CBER and will be used for routine lot release.

5. **Facilities Review/Inspection**

Facility information and data provided in the BLA were reviewed by CBER and found to be sufficient and acceptable. The facilities involved in the manufacture of cobas® MPX Test are listed in the table below. The activities performed and inspectional histories are noted in the table and are further described in the paragraphs that follow.

Table 1: Manufacturing Facilities for cobas® MPX Test

Name/address	FEI number	Inspection/ waiver	Results/ Justification
Final Device Manufacturer Roche Molecular System, Inc. (b) (4)	(b) (4)	Waived	CDRH Level 1 QSIT and Postmarket PMA inspection of Class II, III and licensed IVD medical devices (b) (4) NAI Team Biologics (b) (4) VAI

NAI – No Action Indicated

A Level 1 QSIT (Quality System Inspection Technique) and Postmarket PMA inspection of Class II, III and licensed IVD medical devices was conducted (b) (4). The inspection covered the cobas (b) (4). Limited coverage was given to the Design Control subsystem. No deficiencies were noted and no FDA Form 483 was issued.

The previous routine surveillance inspection was conducted (b) (4) by Team Biologics using CP 7342.008, Inspection of Licensed In-Vitro Diagnostic (IVD) Devices Regulated by CBER. The inspection was classified as Voluntary Action Indicated (VAI).

6. Stability Program

Real time stability studies completed to date support the following shelf life claims: cobas® MPX -96 and cobas® MPX -480 at 2-8ºC for 24 months, cobas® MPX Test Control Kit at 2-8ºC for 24 months, cobas® NHP Negative Control Kit at 2-8ºC for 24 months, cobas® omni MGP Reagent and cobas® omni Lysis Reagent at 2-8ºC for 24 months, and cobas® omni Wash Reagent at 15-30ºC for 24 months.

7. Environmental Assessment

The BLA included a request for a 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.

IV. NON-CLINICAL STUDIES

1. Determination of Limits of Detection (LODs)

The LODs of the cobas® MPX Test for HIV-1 Group M RNA, HIV-1 Group O RNA, HIV-2 RNA, HCV RNA, and HBV DNA were determined using the following standards:

- WHO 3rd International Standard for HIV-1 Group M RNA (NIBSC code 10/152)
- WHO International Standard for HIV-2 RNA (NIBSC code 08/150)
- Roche Primary Standards for HIV-1 Group O RNA
- WHO 2nd International Standard for HCV RNA (NIBSC code 96/798)
- WHO 3rd International Standard for HBV DNA (NIBSC 10/264)

No international standard is currently available for HIV-1 Group O RNA. The Roche Primary Standards for HIV-1 Group O RNA were derived from commercially available cultured virus stocks, P/N 2420 (Cat. No. 500493, SeraCare Life Sciences) and are traceable to the CBER HIV-1 Subtype RNA Reference Panel No. 1 Lot 01. For these studies of LOD determination, HIV-1 Group M, HCV, and HBV standards were co-formulated, while HIV-1 Group O and HIV-2 standards were individually formulated. For each formulated standard, three independent dilution series were prepared with normal, HIV, HBV and HCV negative human EDTA-plasma. Each dilution series was tested using three different lots of cobas® MPX Test kits with approximately 63 replicates per lot, for a total of approximately 189 replicates per concentration. For the HIV-2 Standard, 33 replicates per lot from three independent dilutions and three reagent lots were tested for a total of 99 replicates per concentration. For each virus, PROBIT analysis on the data combined across dilution series and reagent lots were used to estimate the LOD. Table 2 shows the LOD for each virus in EDTA plasma and serum along with the lower and upper limit of the 95% confidence interval. There was no significant difference in LOD for serum and plasma in this study.

Table 2: LOD in EDTA Plasma and Serum

Matrices	Analyte	Units	LOD	Lower 95% Confidence Limit	Upper 95% Confidence Limit
EDTA Plasma	HIV-1 M	IU/mL	25.7	21.1	32.8
	HIV-1 O	Cp/mL	8.2	7.0	10.0
	HIV-2	IU/mL	4.0	3.0	5.2
	HCV	IU/mL	7.0	5.9	8.6
	HBV	IU/mL	1.4	1.2	1.7
Serum	HIV-1 M	IU/mL	23.7	20.0	29.1
	HIV-1 O	Cp/mL	12.2	10.3	14.9
	HIV-2	IU/mL	4.4	3.5	5.8
	HCV	IU/mL	8.1	6.8	10.1
	HBV	IU/mL	1.3	1.1	1.5

2. Genotype Detection

The ability of the cobas® MPX Test to detect the subtypes of HIV-1 Group M, HIV-1 Group O, HIV-1 Group N and HIV-2, and the genotypes of HCV and HBV was determined using clinical samples and/or culture isolates.

a) HIV-1 Group M

A total of 115 unique HIV-1 Group M clinical samples with known HIV-1 subtype were quantified for HIV-1 concentrations using the COBAS® AmpliPrep/COBAS® TaqMan HIV-1 Test, v2.0. All 115 samples were tested with the cobas® MPX Test after dilution with normal, HIV, HCV, and HBV negative human EDTA-plasma to 5x LOD of cobas® MPX Test. Of 115 HIV-1 Group M subtype samples, 102 samples were also tested neat (undiluted). As demonstrated in Table 3, all clinical samples of HIV-1 group M subtypes were detected neat and/or at 5x LOD.

Table 3: HIV-1 Group M Clinical Samples

Genotype	% Reactive (Reactive/Samples Tested) Neat	% Reactive (Reactive/Samples Tested) Diluted to 5X LOD
A	100% (12/12)	100% (12/12)
CRF01_AE	100% (12/12)	100% (12/12)
CRF02_AG	100% (12/12)	100% (12/12)
B	100% (11/11)	100% (11/11)

C	100% (12/12)	100% (12/12)
D	100% (11/11)	100% (11/11)
F	100% (10/10)	100% (10/10)
G	100% (12/12)	100% (12/12)
H	100% (10/10)	100% (10/10)
BF	Not tested*	100% (3/3)
BG	Not tested*	100% (4/4)
J	Not tested*	100% (2/2)
K	Not tested*	100% (4/4)

*Insufficient volume to test neat

b) HIV-1 Group O and HIV-1 Group N

A total of seven (7) HIV-1 Group O and two (2) HIV-1 Group N culture isolates were tested after log dilutions were prepared in normal, HIV, HCV, and HBV negative human EDTA-plasma.

For HIV-1 Group O isolates, seven isolates (obtained from individual clinical specimens) were tested in 4 replicates at each dilution for a total of 28 replicates per dilution. HIV-1 Group O culture isolates were detected up to a dilution of 1:1.00E+07 (Table 4) with a reactive rate of 71.4% for all replicates tested. At these dilutions the viral concentrations of these isolates were below the estimated LOD of the assay (Table 2). For HIV-1 Group N isolates, a total of 4 replicates were tested for one cultured isolate from dilution 1:1.00E+02 to 1:1.00E+07 with 100% reactivity rate of 100% up to 1:1.00E+03. Another HIV-1 Group N clinical specimen was only tested at one dilution (1:1.00E+04) with one replicate and was reactive by the cobas® MPX Test. The reactive result at 1:1.00E+04 was not included in Table 4 (see footnote).

Table 4: HIV-1 Group O and HIV-1 Group N Culture Isolates

Sample Dilution	**% Reactive (Reactive/Valid Replicates Tested)**	
	HIV-1 Group O	**HIV-1 Group N**
1:1.00E+02	100% (28/28)	100% (4/4)
1:1.00E+03	100% (28/28)	100% (4/4)
1:1.00E+04	89.3% (25/28)	0.0% (0/4)*
1:1.00E+05	71.4% (20/28)	0.0% (0/4)
1:1.00E+06	71.4% (20/28)	0.0% (0/4)
1:1.00E+07	71.4% (20/28)	0.0% (0/4)

*One HIV-1 Group N clinical specimen was tested at only one dilution (1:1.00E+04), with only one replicate. The reactive result was not included in the calculation in this table.

c) HIV-2

Four culture isolates of HIV-2 subtype A and one culture isolate of HIV-2 subtype B were tested with the cobas® MPX Test after log dilutions were prepared in normal, HIV, HCV, and HBV virus negative human EDTA plasma.

For subtype A, four isolates were tested in replicates of 4 at each dilution for a total of 16 replicates across each dilution. For one isolate of subtype B, four total replicates were tested for each dilution. In addition, clinical specimens of HIV-2 subtype A (n=5) and subtype B (n=6) were also tested with the cobas® MPX Test in replicates of 4 at each dilution after log dilutions were prepared in normal, virus-negative human EDTA-plasma for a total of 20 replicates at each dilution for subtype A and a total of 24 replicates across six clinical samples for subtype B.

As demonstrated in Table 5, culture isolates of HIV-2 subtypes A and B were detected with 100% reactivity rate by the cobas® MPX Test at dilutions up to 1:1.00E+06. Beyond this dilution the viral load in the samples was below the LOD demonstrated in Table 2.

Also, the clinical samples tested were detected by the cobas® MPX Test only at dilutions of up to 1:1.00E+03 for both subtypes A and B of HIV-2 (Table 5). However, subtype A could be detected up to 1:1.00E+05 dilution. The lower dilution at which the HIV-2 clinical samples were detected by cobas® MPX Test compared to culture isolate is due to the fact that HIV-2 clinical specimens generally have lower viral load.

Table 5: HIV-2 Culture Isolates and Clinical Samples

Sample Dilution	**% Reactive (Reactive/Valid Replicates Tested)**			
	Culture Isolate		**Clinical Sample**	
	Subtype A	**Subtype B**	**Subtype A**	**Subtype B**
1:1.00E+02	100.0% (16/16)	100.0% (4/4)	100% (20/20)	100.0% (24/24)
1:1.00E+03	100.0% (16/16)	100.0% (4/4)	65.0% (13/20)	50.0 (12/24)
1:1.00E+04	100.0 (15/15)	100.0% (4/4)	25.0% (5/20)	0.0% (0/24)
1:1.00E+05	100.0% (16/16)	100.0% (4/4)	5.0% (1/20)	0.0% (0/24)
1:1.00E+06	100.0% (16/16)	100.0% (4/4)	0.0% (0/20)	0.0% (0/24)
1:1.00E+07	81.2% (13/16)	0.0% (0/4)	0.0 (0/20)	0.0% (0/24)

d) HCV

A total of 107 unique HCV clinical samples with known HCV genotypes were quantified for HCV concentrations using the COBAS® AmpliPrep/COBAS® TaqMan HCV Test, v2.0. All HCV clinical samples with known genotypes were tested after dilution with normal, HIV, HCV, and HBV negative human EDTA-plasma (106) or serum (1) to 5x LOD of the cobas® MPX Test. All samples were tested with a single replicate. Table 6 shows that all HCV positive clinical

samples were detected neat and/or diluted by the cobas® MPX Test in this study except for one which had insufficient volume to test neat.

Table 6: HCV Clinical Samples

Genotype	% Reactive (Reactive/Samples Tested) Neat	% Reactive (Reactive/Samples Tested) Diluted to 5X LOD
1a	100% (9/9)	100% (9/9)
1b	100% (12/12)	100% (12/12)
1	100% (12/12)	100% (12/12)
2b*	100% (11/11)	100% (12/12)
2	100% (13/13)	100% (13/13)
3a	100% (12/12)	100% (12/12)
3	100% (1/1)	100% (1/1)
4	100% (13/13)	100%13/13)
5a	100% (10/10)	100% (10/10)
5	100% (2/2)	100% (2/2)
6**	100% (10/10)	100% (11/11)

*One (1) HCV 2b neat testing was not complete due to invalid initial result and insufficient sample volume for repeat testing

**One (1) HCV 6 neat testing was not completed due to clot detection

e) HBV

A total of 94 unique HBV clinical samples with known HBV genotypes and pre-core mutants were quantified for HBV concentrations using the COBAS® AmpliPrep/COBAS® TaqMan HBV Test. All 94 HBV clinical samples with known genotypes were tested neat and/or diluted with normal, HIV, HCV and HBV negative EDTA-plasma to 5x LOD of the cobas® MPX Test.
All samples were tested with a single replicate. As shown in Table 7, all HBV-positive clinical samples were detected both neat and/or diluted in this study except for one which had insufficient volume to test neat.

Table 7: HBV Clinical Samples

Genotype	% Reactive (Reactive/Samples Tested) Neat	% Reactive (Reactive/Samples Tested) Diluted to 5 X LOD
A	100% (15/15)	100% (15/15)
B	100% (12/12)	100% (11/11)
C	100% (10/10)	100% (9/9)
D	100% (12/12)	100% (11/11)
E	100% (12/12)	100% (11/11)

F	100% (12/12)	100% (12/12)
G	Not tested*	100% (1/1)
H	100% (8/8)	100% (8/8)
Pre Core Mutant	100% (12/12)	100% (12/12)

*Insufficient volume to test neat

3. Seroconversion Panels

The cobas® MPX Test was evaluated using seroconversion panels for HIV-1 Group M, HCV, and HBV.

The results of the cobas® MPX Test were compared to results for the same panels tested using the FDA licensed cobas® TaqScreen MPX Test on the cobas® s 201 System. Each panel member is tested neat, at a 1:6 dilution, and at a 1:96 dilution with the cobas® MPX Test and the FDA licensed cobas® TaqScreen MPX Test on the cobas® s 201 System, to reflect the use of the test as individual donation, in pools of 6, or pools of 96. The results of the cobas® MPX at each dilution were compared to the results for the serology assays tested with neat sample (panel member) for each target.

a) HIV-1 Group M

Twenty commercially available seroconversion panels were used. Each panel member was tested neat, and at 1:6 and 1:96 dilutions, to simulate testing in pools with the cobas® MPX Test and the cobas® TaqScreen MPX Test. The cobas® MPX Test results were compared to the results obtained with a FDA licensed multiplex NAT. The cobas® MPX Test results obtained at each dilution were also compared to the results of two serology tests obtained by testing the neat panel members. The two serology tests used are a CE-marked HIV Ag/Ab Combo assay and an FDA approved diagnostic HIV Ag/Ab combo assay.

Table 8: Performance of the cobas® MPX Test on HIV-1 Seroconversion Panels

HIV-1 Sero-conversion Panels	Days Earlier Detection by cobas® MPX than by HIV-1/2 Ag/Ab or Licensed HIV-1 RNA NAT								
	FDA Approved HIV Ag/Ab Combo			CE Marked HIV Ag/Ab Combo			FDA Licensed Multiplex NAT		
	Neat	1:6	1:96	Neat	1:6	1:96	Neat	1:6	1:96
1	7	7	7	0	0	0	-4	0	-4
2	7	7	3	7	7	3	0	0	0
3	3	3	3	3	3	3	0	0	0
4	8	4	4	8	4	4	4	0	4
5	15	8	2	15	8	2	13	6	2
6	7	2	2	7	2	2	5	0	0

7	7	5	5	7	5	5	2	0	0
8	15	15	8	15	15	8	0	0	0
9	12	7	7	12	7	7	5	0	0
10	2	2	0	2	2	0	0	0	2
11	6	0	0	6	0	0	6	0	0
12	8	8	6	8	8	6	-5	2	0
13	7	7	7	7	7	7	0	0	2
14	10	3	3	10	3	3	2	0	0
15	12	7	7	12	7	7	0	-5	0
16	9	9	7	9	9	7	0	0	0
17	11	11	9	11	11	9	0	0	0
18	2	2	2	2	2	2	0	0	0
19	7	7	7	7	7	7	0	0	2
20	7	7	5	7	7	5	0	2	0
Min	2	0	0	0	0	0	-5	-5	-4
Ave	8.1	6.1	4.7	7.8	5.7	4.4	1.4	0.3	0.2
Max	15	15	9	15	15	9	13	6	4

As demonstrated in Table 8, the cobas® MPX Test was able to detect HIV-1 RNA several bleeds earlier than the CE-Marked IVD and FDA approved serology combo tests for HIV antigens/antibodies. There was no significant difference between the cobas® MPX Test and the FDA licensed multiplex NAT in testing seroconversion panels for HIV-1.

b) HCV

Twenty-five HCV seroconversion panels were tested using the cobas® MPX Test. Each panel member was tested neat and at 1:6 and 1:96 dilutions to simulate testing in pools with the cobas® MPX Test and the cobas® TaqScreen MPX Test. The cobas® MPX Test results obtained at each dilution were also compared to the results of two serology tests obtained by testing the neat panel members. The serology tests used are a CE-Marked IVD and a FDA licensed anti-HCV assay.

Table 9: Performance of the cobas® MPX Test on HCV Seroconversion Panels

HCV Sero-conversion Panels	**Days Earlier Detection by cobas® MPX than by Antibody or Licensed HCV RNA NAT**								
	FDA Licensed anti-HCV			**CE-Marked anti-HCV**			**FDA Licensed Multiplex NAT**		
	Neat	**1:6**	**1:96**	**Neat**	**1:6**	**1:96**	**Neat**	**1:6**	**1:96**
1	13	13	13	7	7	7	0	0	0
2*	20	20	20	20	20	20	0	0	0

3	23	23	23	23	23	23	0	0	0
4*	23	23	23	19	19	19	0	0	0
5	33	33	33	33	33	33	-6	0	0
6*	39	39	39	37	37	37	0	0	0
7	32	32	32	32	32	32	0	0	0
8	38	38	38	38	38	38	-24**	0	0
9*	34	34	32	34	34	32	0	0	0
10*	32	32	29	32	32	29	0	3	0
11	34	34	34	34	34	34	0	0	0
12*	11	11	11	11	11	11	0	0	0
13*	10	10	10	10	10	10	0	0	0
14*[a]	12	12	-2	12	12	-2	0	0	1
15	65	65	65	65	65	65	0	0	0
16	3	3	3	3	3	3	0	0	0
17*	13	13	13	16	16	16	0	0	0
18*	21	21	21	21	21	21	0	0	0
19	34	4	34	34	4	34	0	0	0
20	75	75	75	75	75	75	0	0	0
21	46	42	42	49	45	45	4	0	7
22	35	35	35	35	35	35	0	0	0
23	38	38	25	38	38	25	0	6	0
24	39	39	35	39	39	35	0	7	3
25	2	2	2	0	0	0	0	0	0
Min	2	2	-2	0	0	-2	-24	0	0
Ave*	34.0	31.7	30.4	33.7	31.4	30.1	-1.7	0.9	2.7
Max	75	75	75	75	75	75	4	7	30

* Panels were reactive on the first draw when tested with the cobas® MPX Test or did not show seroconversion. These panels were excluded from the summary calculations for the minimum, average and maximum number of days earlier detection than HCV antibody or RNA for each dilution.

** 24 day interval between adjacent draws

[a] The results for panel 14 at a l:96 dilution versus serology were used for the calculations. The first draw for this panel tested as neat and 1:6 dilution were reactive, thus these results were excluded from the calculations.

As shown in Table 9, the cobas® MPX Test was able to detect HCV RNA several bleeds earlier than the CE-IVD and FDA licensed tests for HCV antibodies. There was no significant difference between the cobas® MPX Test and the FDA licensed multiplex NAT assay in testing seroconversion panels for HCV.

c) HBV

Twenty-one commercially available HBV seroconversion panels were tested using the cobas® MPX Test. Each panel member was tested neat and at 1:6 and 1:96 dilutions to simulate testing in pools with the cobas® MPX Test and the cobas® TaqScreen MPX Test. The cobas® MPX Test results were compared to the results obtained with FDA licensed multiplex NAT. The cobas® MPX Test results observed at each dilution were also compared to the results for neat panel members tested by two HBsAg serology tests: a CE-Marked IVD and a FDA licensed HBsAg.

Table 10: Performance of the cobas® MPX Test on HBV Seroconversion Panels

HBV Sero-conversion Panels	Days Earlier Detection by the cobas MPX Test than by Licensed HBsAg Assay or by Licensed HBV DNA NAT								
	FDA Licensed HBsAg			CE-Marked HBsAg			FDA Licensed Multiplex NAT		
	Neat	1:6	1:96	Neat	1:6	1:96	Neat	1:6	1:96
1	29	12	0	29	12	0	17	0	0
2	19	11	7	15	7	3	0	-3	0
3**	9	9	2	-14	-14	-21	0	0	0
4	38	27	19	38	27	19	4	0	2
5	22	0	0	22	0	0	0	-13	0
6	24	24	0	24	24	0	-7	7	0
7	21	18	7	21	18	7	3	4	0
8	21	14	11	21	14	11	3	0	7
9	19	12	5	19	12	5	0	5	5
10*	12	12	7	19	19	14	0	0	0
11**	17	17	0	0	0	-17	0	0	0
12	28	28	7	28	28	7	0	0	7
13	18	18	7	18	18	7	-8	4	10
14	18	15	7	11	8	0	9	0	5
15**	30	28	14	0	-2	-16	2	12	0
16	17	17	6	17	17	6	0	2	6
17	29	33	18	29	33	18	-4	15	3
18	22	10	0	22	10	0	12	0	0
19	18	14	3	18	14	3	4	0	0
20	28	28	0	28	28	0	-5	14	0
21	22	20	5	17	15	0	2	7	0
Min	9	0	0	-14	-14	-21	-8	-13	0
Ave	22.5	17.8	6.0	18.2	13.5	2.2	1.6	2.7	2.1

Max	38	33	19	38	33	19	17	15	10

* Panel was consistently reactive with the cobas® MPX Test, beginning on the first bleed and was excluded from the neat and 1:6 summary calculations for the minimum, average and maximum number of days earlier detection than HBV antibody.

** Low concentrations of HBV DNA were present in diluted panel members which were detected later by the cobas® MPX Test than by serology; 1.7 IU/mL in Panel 3 at 1:96, 2.0 IU/mL in Panel 11 at 1:96, and 0.5 IU/mL in Panel 15 at 1:96.

As shown in Table 10, the cobas® MPX Test generally detected HBV DNA earlier than the FDA licensed serology assays for HBV surface antigen (HBsAg). However, panels with low viral titers (i.e., panels #3, 11 and 15) were not detected earlier than CE-Marked HBsAg assay. Although there was no significant difference between the cobas® MPX Test and FDA licensed multiplex NAT in testing seroconversion panels for HBV, there were variations among individual panels in detection of HBV by the cobas® MPX Test and the FDA licensed multiplex NAT that could be due to the difference in the intervals between bleeds for each panel.

4. **Analytical Specificity- Cross Reactivity**

The analytical specificity of cobas® MPX Test was evaluated for cross-reactivity using 25 microorganisms, which included 18 viral isolates, six bacterial strains and one yeast isolate (Table 11). The microorganisms were added to normal, HIV, HCV, and HBV negative human EDTA-plasma at 1.00E+06 particles, copies, or PFU/mL and tested unspiked or spiked with HIV-1 Group M, HCV, HBV (co-formulated), HIV-1 Group O and HIV-2 virus added to a concentration of approximately 3x LOD of the cobas® MPX Test for each virus. The tested microorganisms did not cross-react or interfere with the cobas® MPX Test.

Table 11: Microorganisms Tested for Analytical Specificity

Category	**Microorganism**
Virus	Adenovirus 5, Cytomegalovirus, Epstein-Barr virus, Herpes Simplex virus Type 1, Herpes Simplex virus Type 2, Hepatitis A virus, Hepatitis E virus, Hepatitis G virus, HTLV-I, HTLV-II, HHV-6, Influenza virus A, Parvovirus B19, Chikungunya virus , Varicella Zoster virus , West Nile virus, Dengue virus type 1, Usutu virus
Bacteria	*Escherichia coli, Propionibacterium acnes, Staphylococcus aureus, Staphyl epidermidis, Staphylococcus haemolyticus*
Yeast	*Candida albicans*

In addition, plasma samples from individuals infected with the agents listed in Table 12 were tested unspiked or spiked with HIV-1 Group M, HCV, HBV (co-formulated), HIV-1 Group O, and HIV-2 to a concentration of approximately 3x LOD of the cobas® MPX Test for each virus.

Plasma sample from individuals infected with these agents do not cross-react or interfere with the cobas® MPX Test.

Table 12: Analytical Specificity Determined Using Plasma Samples from Individuals Infected with the Following Agents

Etiologic Agents of Disease		
Adenovirus Type 5	Herpes Simplex virus 1	Human HTLV-I
Cytomegalovirus	Herpes Simplex virus 2	Human HTLV-II
Dengue virus	HAV	Parvovirus B19
EB virus	HEV	WNV

5. Analytical Specificity – Interfering Substances

a) Endogenous Interfering Substances

Plasma samples with abnormally high levels of triglycerides (up to 33.2 g/L), hemoglobin (up to 2 g/L), unconjugated bilirubin (up to 0.236 g/L), albumin (up to 60 g/L), and human DNA (up to 0.002 g/L) were tested with and without HIV-1 Group M, HCV, HBV (co-formulated), HIV-1 Group O and HIV-2 virus added to a concentration of 3x LOD of cobas® MPX Test. Samples containing these endogenous substances did not interfere with the sensitivity or specificity of the cobas® MPX Test.

b) Exogenous Interfering Substances Commonly Found in Blood Donations

Several common drugs were added to normal, HIV, HCV, and HBV negative human EDTA-plasma samples as stated in Table 13 and tested unspiked or spiked with HIV-1 Group M, HCV, HBV (co-formulated), HIV-1 Group O and HIV-2 to a concentration of 3x LOD of cobas® MPX Test for each virus. The exogenous substances tested did not interfere with the sensitivity or specificity of the cobas® MPX Test.

Table 13: Concentrations of the Drugs Added into EDTA-Plasma

Name of Drug Tested	Concentration
Acetaminophen	1324 µmol/L
Acetylsalicylic Acid	3620 µmol/L
Ascorbic Acid	342 µmol/L
Atorvastatin	600 µmol/L
Fluixetine	11.2 µmol/L
Ibuprofen	2425 µmol/mL
Loratadine	0.78 µmol/L

Nadolol	3.88 µmol/L
Naproxen	2170 µmol/L
Paroxetine	3.04 µmol/L
Phenylephrine HCl	491 µmol/L
Sertraline	1.96 µmol/L

6. Whole System Failure

The Whole System Failure rate for the cobas® MPX Test was determined by testing 100 replicates of EDTA plasma spiked either with HIV-1 Group M, HCV, HBV (co-formulated), HIV-1 Group O, or HIV-2, for a total of 300 replicates. These samples were tested at a target concentration of approximately 3x LOD and were run neat. The study was performed using the cobas® 8800 System with cobas® p 680 instrument (pipetting and pooling).

The whole system failure rate was obtained by dividing the number of replicates with valid results that were non-reactive by the total number of replicates with valid results. It was calculated separately for each of the five targets tested. The results of this study demonstrated that all replicates were reactive for each target, resulting in a Whole System Failure rate of 0%, with the two-sided 95% exact confidence interval of 0% to 1.22 %.

7. Cross Contamination

The Cross Contamination rate for the cobas® MPX Test was determined by testing 240 replicates of a normal, HIV, HCV, and HBV negative human EDTA-plasma sample and 220 replicates of a high titer HBV sample at 1.00E+08 IU/mL. The study was performed using the cobas® 8800 System. In total, 5 runs were performed with positive and negative samples in a checkerboard configuration.

All 240 replicates of the negative sample were non-reactive, resulting in a Cross Contamination rate of 0%, with the two-sided 95% exact confidence interval of 0% to 1.53%.

V. CLINICAL STUDIES

1. Reproducibility

The reproducibility of the cobas® MPX Test for use on the cobas® 6800/8800 Systems was established by testing a 32-member panel composed of two negative plasma samples and two plasma samples positive for each of the following viral targets: HIV-1 Group M and Group O, HIV-2, HCV, and HBV at three different concentrations approximately 0.5 x, 1.0 x, and 3.0 x the LOD of the cobas® MPX Test.

Operators at each of the three cobas® MPX Test sites performed five days of testing, using three lots of the cobas® MPX Test reagents to obtain two valid batches per day. Two replicates per concentration were tested to yield up to 180 tests per viral target at each of the three viral concentrations.

All valid batches and test results were analyzed by calculating the percentage of reactive test results for each panel member and the percentage of non-reactive results for the negative control panel member. This study demonstrated that the cobas® MPX Test for use on the cobas® 6800/8800 Systems shows reproducible performance across all variables assessed (lot, site/instrument, day, batch, and within batch) and for all five viral targets (Table 14).

Table 14: Reproducibility Study

		Site/Instrument		**Lot**		**Day**		**Batch**	
Viral Target	**Viral Concen-tration**	**ID**	**% Positive Results**	**ID**	**% Positive Results**	**ID**	**% Positive Results**	**ID**	**% Positive Results**
HIV-1 Group M	~0.5x LOD	1	81.7% (49/60)	1	81.7% (49/60)	1	91.7% (33/36)	1	84.3% (75/89)
		2	84.7% (50/59)	2	88.3% (53/60)	2	77.1% (27/35)	2	81.1% (73/90)
		3	81.7% (49/60)	3	78.0% (46/59)	3	83.3% (30/36)		
						4	83.3% (30/36)		
						5	77.8% (28/36)		
	~1x LOD	1	100.0% (60/60)	1	100.0% (60/60)	1	97.2% (35/36)	1	100.0% (90/90)
		2	100.0% (60/60)	2	100.0% (60/60)	2	97.2% (35/36)	2	97.8% (88/90)
		3	96.7% (58/60)	3	96.7% (58/60)	3	100.0% (36/36)		
						4	100.0% (36/36)		
						5	100.0% (36/36)		
	~3x LOD	1	100.0% (60/60)	1	100.0% (60/60)	1	100.0% (36/36)	1	100.0% (90/90)
		2	100.0% (60/60)	2	100.0% (60/60)	2	100.0% (36/36)	2	100.0% (90/90)
		3	100.0% (60/60)	3	100.0% (60/60)	3	100.0% (36/36)		
						4	100.0%		

							(36/36)		
						5	100.0% (36/36)		
		Site/Instrument		**Lot**		**Day**		**Batch**	
Viral Target	**Viral Concen-tration**	**ID**	**% Positive Results**	**ID**	**% Positive Results**	**ID**	**% Positive Results**	**ID**	**% Positive Results**
HIV-1 Group O	~0.5x LOD	1	78.3% (47/60)	1	83.3% (50/60)	1	72.2% (26/36)	1	73.3% (66/90)
		2	76.7% (46/60)	2	78.3% (47/60)	2	77.8% (28/36)	2	86.7% (78/90)
		3	85.0% (51/60)	3	78.3% (47/60)	3	77.8% (28/36)		
						4	86.1% (31/36)		
						5	86.1% (31/36)		
	~1x LOD	1	98.3% (59/60)	1	98.3% (59/60)	1	94.4% (34/36)	1	95.6% (86/90)
		2	100.0% (60/60)	2	96.7% (58/60)	2	100.0% (36/36)	2	98.9% (89/90)
		3	93.3% (56/60)	3	96.7% (58/60)	3	97.2% (35/36)		
						4	100.0% (36/36)		
						5	94.4% (34/36)		
	~3x LOD	1	100.0% (60/60)	1	100.0% (60/60)	1	100.0% (36/36)	1	100.0% (90/90)
		2	100.0% (60/60)	2	100.0% (60/60)	2	100.0% (36/36)	2	100.0% (90/90)
		3	100.0% (60/60)	3	100.0% (60/60)	3	100.0% (36/36)		
						4	100.0% (36/36)		
						5	100.0% (36/36)		

HIV-2	~0.5x LOD	1	74.1% (43/58)	1	73.3% (44/60)	1	77.8% (28/36)	1	69.7% (62/89)
		2	76.7% (46/60)	2	79.7% (47/59)	2	69.4% (25/36)	2	79.8% (71/89)
		3	73.3% (44/60)	3	71.2% (42/59)	3	75.0% (27/36)		
						4	71.4% (25/35)		
						5	80.0% (28/35)		
	~1x LOD	1	96.7% (58/60)	1	100.0% (60/60)	1	97.2% (35/36)	1	100.0% (90/90)
		2	98.3% (59/60)	2	96.7% (58/60)	2	100.0% (36/36)	2	96.7% (87/90)
		3	100.0% (60/60)	3	98.3% (59/60)	3	97.2% (35/36)		
						4	100.0% (36/36)		
						5	97.2% (35/36)		
	~3x LOD	1	100.0% (60/60)	1	100.0% (60/60)	1	100.0% (36/36)	1	100.0% (90/90)
		2	100.0% (60/60)	2	100.0% (60/60)	2	100.0% (36/36)	2	100.0% (90/90)
		3	100.0% (60/60)	3	100.0% (60/60)	3	100.0% (36/36)		
						4	100.0% (36/36)		
						5	100.0% (36/36)		
		Site/Instrument		**Lot**		**Day**		**Batch**	
Viral Target	**Viral Concen-tration**	**ID**	**% Positive Results**	**ID**	**% Positive Results**	**ID**	**% Positive Results**	**ID**	**% Positive Results**
HCV	~0.5x LOD	1	75.0% (45/60)	1	80.0% (48/60)	1	66.7% (24/36)	1	79.8% (71/89)
		2	70.7% (41/58)	2	76.7% (46/60)	2	77.8% (28/36)	2	74.2% (66/89)

		3	85.0% (51/60)	3	74.1% (43/58)	3	69.4% (25/36)		
						4	91.2% (31/34)		
						5	80.6% (29/36)		
	~1× LOD	1	100.0% (60/60)	1	98.3% (59/60)	1	97.2% (35/36)	1	100.0% (90/90)
		2	96.7% (58/60)	2	98.3% (59/60)	2	100.0% (36/36)	2	97.8% (88/90)
		3	100.0% (60/60)	3	100.0% (60/60)	3	97.2% (35/36)		
						4	100.0% (36/36)		
						5	100.0% (36/36)		
	~3× LOD	1	100.0% (60/60)	1	100.0% (60/60)	1	100.0% (36/36)	1	100.0% (90/90)
		2	100.0% (59/59)	2	100.0% (60/60)	2	100.0% (36/36)	2	100.0% (89/89)
		3	100.0% (60/60)	3	100.0% (59/59)	3	100.0% (36/36)		
						4	100.0% (35/35)		
						5	100.0% (36/36)		
HBV	~0.5× LOD	1	80.0% (48/60)	1	80.0% (48/60)	1	80.6% (29/36)	1	72.2% (65/90)
		2	78.3% (47/60)	2	73.3% (44/60)	2	80.6% (29/36)	2	82.2% (74/90)
		3	73.3% (44/60)	3	78.3% (47/60)	3	75.0% (27/36)		
						4	77.8% (28/36)		
						5	72.2% (26/36)		
	~1×	1	100.0% (60/60)	1	100.0% (60/60)	1	100.0% (36/36)	1	100.0% (90/90)

	LOD	2	100.0% (60/60)	2	100.0% (60/60)	2	100.0% (36/36)	2	100.0% (90/90)
		3	100.0% (60/60)	3	100.0% (60/60)	3	100.0% (36/36)		
						4	100.0% (36/36)		
						5	100.0% (36/36)		
	~3× LOD	1	100.0% (60/60)	1	100.0% (60/60)	1	100.0% (36/36)	1	100.0% (90/90)
		2	100.0% (60/60)	2	100.0% (60/60)	2	100.0% (36/36)	2	100.0% (90/90)
		3	100.0% (60/60)	3	100.0% (60/60)	3	100.0% (36/36)		
						4	100.0% (36/36)		
						5	100.0% (36/36)		

2. Clinical Specificity

a) Blood Donor Population

Samples were collected from consented blood donors recruited from designated blood donor centers and tested at four sites. Testing with the cobas® MPX Test was done according to two testing algorithms: one for individual donation testing, which required only a single level of testing; and one for pools of six testing, which required only a single level of testing for non-reactive pools, but two levels of testing for reactive pools (primary pool testing followed by individual donation resolution testing to identify the positive donation).

Specificity in Individual Donation Testing for Whole Blood

For individual donation testing, a total of 11,198 specimens from Whole Blood donations were tested. About half of these donations were plasma specimens (n=5528) and the others were serum specimens (n=5670). Donor specimens that are non-reactive on both cobas® MPX Test and comparator test were determined to be negative and no further testing was required. Samples reactive by either test were further tested by alternate NAT and along with serology test results the true status of the sample was determined. Of the samples tested as individual donation testing, 6 specimens were false reactive (five plasma specimens and one serum specimen) on the cobas® MPX Test (Table 15). As shown in Table 16, the overall clinical specificity for individual donation testing in this study was 99.95% (95% CI: 99.88% to 99.98%). The specificity of

individual donation testing for plasma specimens was 99.91% (95% CI: 99.79 to 99.96%) and for serum specimens it was 99.98% (95% CI: 99.90 to 100%). There was no significant difference in the specificity for serum and plasma.

Table 15: Individual Donation Reactivity in Whole Blood Donations

Category		Number of Donations	Percentage of Specimens Tested
Plasma	Individual Donations Tested	5528	100
	Nonreactive Donations	5523	99.91
	Reactive Donations	5	0.09
	Reactive Donations for Donor Status Negative (False Positive)	1 (HBV) 4 (HIV)	0.09
Serum	Individual Donations Tested	5670	100
	Nonreactive Donations	5669	99.98
	Reactive Donations	1	0.02
	Reactive Donations for Donor Status Negative (False Positive)	1 (HIV)	0.02

Table 16: Clinical Specificity – Individual Donations

Specimens	Frequency (n/N)*	Estimated in Percent (95% Score Confidence Interval)
Individual Plasma	5,523/5,528	99.91% (99.79 to 99.96%)
Individual Serum	5,669/5,670	99.98% (99.90 to 100 %)
Individual (Plasma/Serum)	11,192/11,198	99.95% (99.88 to 99.98%)

*n= number tested negative/N= total number tested

Specificity in Pool Testing for Whole Blood

A total of 63,012 evaluable donations were tested in primary pools consisting of equal aliquots from 6 different donations. Of these, 62,982 donations were status negative and yielded negative results on the cobas® MPX Test. One donation gave a false negative result and 29 were identified as true positive (Table 17).

Table 17: Donation Reactivity for cobas® MPX Test in Whole Blood Tested in Pools of Six

Category	Number of Donations	Percentage of Specimens Tested
Donations in the Pool Tested	63,012	100
Status Negative Donations (True negative)	62, 982	99.95

Negative Results for Donor Status Negative (True Negative) Donation	62,982	99.95
Negative Results for Donor Status Positive (False Negative) Donation	1	0.00
Reactive Donations	29	0.05
Reactive Results for Donor Status Positive (True Positive) Donations	29*	0.05
Reactive Donations for Donor Status Negative (False Positive)	0	0

*Two HCV window cases were identified in this category.

For donations tested in pools of 6, 10,524 of 10,563 (99.63%) qualified pools, were non-reactive, while 39 (0.37%) were reactive on the cobas® MPX Test. Among 39 reactive pools, twenty-nine (29) had at least one status positive donation, while ten (10) pools consisted of only status negative donations (i.e., false positive, with respect to donation status) (Table 18).

Table 18: Pool Testing Reactivity for cobas® MPX Test in Whole Blood

Category	Number of Pools	Percentage of Specimens Tested
Pools Tested	10,563	100
Nonreactive Pools	10,524	99.63
Reactive Pools	39	0.37
Reactive Pools with Donor Status Positive Donations (True Positive Pools)	29*	0.27
Reactive Pools with Donor Status Negative Donations (False Positive Pools)	10 (1 HIV, 4 HCV, 5 HBV)	0.10

*Two HCV window cases were identified in this category.

As shown in Table 19, the clinical specificity at donation level in pool testing of the cobas® MPX Test was 100. % (95% CI: 99.99% to 100%) and specificity at pool level was 99.91% (95% CI; 99.83%-99.95%) in this study.

Table 19: Clinical Specificity – Pool Testing

Specificity	Frequency (n/N)	Estimated in Percent (95% Score Confidence Interval)
At Donation Level	62, 982/62, 982	100% (99.99 to 100%)
At Pool Level (Pools of 6 Plasma)	10,524/10,534	99.91% (99.83 to 99.95%)

The invalid batch rate for the cobas® MPX Test was 6.8% for individual donation testing and 3.5% for initial testing donations in pools of six. The observed invalid rates for individual donation testing and pool testing are comparable with those for the FDA licensed MPX NAT test.

HCV Window Period Cases

Two plasma specimens from Whole Blood donations tested in pools were HCV positive on the cobas® MPX Test and on the cobas® TaqScreen MPX Test plus cobas® AmpliScreen HCV, but serologically negative. One donor seroconverted on the first follow-up test. This follow up specimen was also positive on both the cobas® MPX Test and on the cobas® TaqScreen MPX Test plus cobas® AmpliScreen HCV. The second donor showed the same pattern of results as its index test (i.e., HCV-positive on both the cobas® MPX Test and on the cobas® TaqScreen MPX Test plus cobas® AmpliScreen HCV) on several subsequent follow up specimens up to 8 weeks, without evidence of seroconversion. Thus, both the cobas® MPX Test and the cobas® TaqScreen MPX Test plus cobas® AmpliScreen HCV identified 2 HCV-infected donors prior to seroconversion (window period HCV cases).

b) Reactivity in Source Plasma Donor Population

A total of 108,306 evaluable donations, from 24,514 unique donors, that tested serologically negative for anti-HIV, anti-HCV, and HBsAg, were tested in pools of 96 with both the cobas® MPX Test and an FDA licensed multiplex NAT. Donation status was assigned based on the concordance of two virus-specific tests (e.g., two NAT results or NAT and serology) on the index donation or the results of follow-up testing. A total of 1,106 evaluable pools were tested with the cobas® MPX Test, of which 1,092 (98.7%) were non-reactive and 14 (1.3%) were reactive. Of the 1,092 nonreactive pools, 1,090 pools contained all status-negative donations, and two pools contained at least 1 donation status-positive donation (false negative pools) (Table 20).

Table 20: Pool Reactivity in Source Plasma Donations

Category	Number of Pools	Percentage of Pools Tested
Total Pools of 96[a] Tested	1,106	100
Negative Pools[b]	1,092	98.7
Negative Pools with All Donations Status Negative	1,090	98.6

(True Negative)		
Negative Pools with One Positive Donation (True Positive)	2[c]	0.2
Positive Pools	14	1.3
Positive Pools with At Least One Status Positive Donation (True Positive)	7	0.6
Positive Pools with Status Negative (False Positive)	7	0.6

[a] 479/1,106 pools had <96 donations

[b] Donation status was assigned based on the concordance of two virus-specific tests on the index donation, or the results of follow-up testing

[c] These two nonreactive pools contained donations from an HBV positive donor. The donor's index donation was HBV-positive on the cobas® MPX Test but negative on cobas® TaqScreen MPX Test and was confirmed HBV-positive by alternative high sensitive NAT. This donor made three subsequent donations that were nonreactive on both NAT screening assays. One of these donations was contained within an HCV-positive pool.

The clinical specificity of the cobas® MPX Test for source plasma pools was determined by the analysis of 108,306 evaluable donations from 24,514 unique donors. Evaluable donations had valid cobas® MPX Test, cobas® TaqScreen MPX Test and CAS results from testing pools, and valid serology results (across analytes) from testing of individual donations. As shown in Table 20, 14 pools of 1,106 pool s of Source Plasma tested gave reactive results. Of the 14 reactive pools, 7 pools had at least one true positive donation and 7 were false reactive. Thus the specificity of cobas® MPX Test at pool level was 99.36% (1,092/1,099; 95% CI 98.69% to 99.74%). Of these 108,306 evaluable donations, 108,297 were assigned a donation status of negative, of which 108,291 were cobas® MPX Test non-reactive, for a clinical specificity of 99.99% (95% Confidence Interval: 99.99% to 100 %). Seven cobas® MPX Test false reactive pools of 96 resolved to contain all status-negative donations. Of the 24,514 unique donors tested, 24,509 contributed only status-negative donations, of which 24,503 were non-reactive on the cobas® MPX Test and six were false-reactive, resulting in specificity (at the donor level) of 99.98% (95% Confidence Interval: 99.95% to 99.99 %). Data are shown in Table 21.

Table 21: Observed Testing Reactivity Patterns from Initial Testing on Donation Level

cobas® MPX Test Result	**Donation Status**	**Number of Donations**
HCV+	Positive	5
HCV+	Negative	1
HBV+	Positive	4*
HBV+	Negative	2
HIV+	Negative	3
Nonreactive	Negative	108,291
	Total	108,306

* These donations are all from the same donor whose index donation was HBV+ and whose three subsequent donations were classified as status positive even though the cobas® MPX Test was nonreactive for HBV.

Eleven unique donors contributed 12 reactive donations (six HCV, three HIV, and three HBV). Seven donors completed follow-up testing: three of these donors did not show evidence of infection; four donors were confirmed to have infection, of these two seroconverted (HCV). One of the three HBV donors was determined to be a NAT HBV yield case.

Yield Cases in the Source Plasma Specificity Study

One HBV and 4 HCV yield cases were identified in the Source Plasma Specificity study.

The first donation from one Source Plasma donor was positive for HBV on the cobas® MPX Test and negative on the cobas® TaqScreen MPX Test. This donor made 3 subsequent donations (over a period of 8 weeks) that were non-reactive on both the cobas® MPX Test and the cobas® TaqScreen MPX Test. An alternative NAT performed on the HBV reactive donation from this donor was positive. Serology tests on all donations from this donor were negative. The reactive donation from this donor and the 3 subsequent nonreactive donations were classified as status positive and the donor is likely a yield case.

Among 11 donors with cobas® MPX Test reactive results, four donors were confirmed to have HCV infection, of these two seroconverted (HCV). All of these 4 donors are considered as yield cases.

3. Studies in High Risk Populations

Samples collected by third-party vendors from individuals at high risk for infection with HIV, HCV, or HBV were used to evaluate the performance of the cobas® MPX Test. Factors associated with high-risk included, but were not limited to, a history of incarceration; history of a diagnosis of a sexually-transmitted disease; history of multiple sex partners; and use of injection drugs. Some sample contributors indicated more than one risk factor. A total of 511 samples from high risk populations were distributed across four test sites and tested with the cobas® MPX Test and the cobas® TaqScreen MPX Test incorporating CAS (for target resolution).

All samples were prepared as panels. At the testing sites, samples were tested neat with both the cobas® MPX Test and the cobas® TaqScreen MPX Test incorporating CAS (for target resolution), as per the Standard Specimen Processing Procedure recommended in the cobas® TaqScreen MPX Test Package Insert. Diluted samples were also tested to simulate pools of six with both the cobas® MPX Test and the cobas® TaqScreen MPX Test. The diluted samples were manually diluted with pooled human plasma confirmed to be negative for HIV-1/2, HCV, and HBV. CAS was not performed on diluted samples.

When the 511 neat samples were tested, two samples had invalid cobas® MPX results and were not included in analyses of neat specimens. Overall, reactivity was similar between the cobas® MPX Test (71/509; 13.9%) and cobas® TaqScreen MPX Test (70/509; 13.8%) for neat specimens, as well as in diluted specimens, with 12.9% (66/511) for cobas® MPX Test and 11.9% (61/511) for cobas® TaqScreen MPX Test. Table 22 presents the overall performance of

the cobas® MPX Test in identifying the type of virus in 509 neat samples with valid results for both the cobas® MPX Test and the cobas® TaqScreen MPX Test. As shown in the Table 22, cobas® MPX Test correctly identified 97.8% and 97.5% viral targets when tested neat or at a 1:6 dilution, respectively.

Table 22: Overall Results for High Risk Study – Correct Versus Incorrect Identification of Virus

	cobas® MPX Test*	**Number**	**%**	**Total**
Neat	**True Positive**	**62**	**97.8**	**498**
	HIV	8		
	HCV	45		
	HBV	6		
	HIV/HCV	2		
	HCV/HBV	1		
	True Negative	**436**		
	False Positive	9[a]	1.8	11
	False Negative	2 (HBV)	0.4	
	Total	509	100	509
1:6 Diluted	**True Positive**	**59**	**97.5**	**498**
	HIV	7		
	HCV	46		
	HBV	3		
	HIV/HCV	2		
	HCV/HBV	1		
	True Negative	**439**		
	False Positive	7[b]	1.37	13
	False Negative	6 (1 HIV, 5 HBV)	1.17	
	Total	511	100.0	511

* Final status (as compared with CAS or alternative NAT [NGI testing] results)

Note: Correct identification = True positive and true negative results (shown in bold type)

Note: Two samples with invalid cobas® MPX Test results were not included in the analyses presented in this table

[a] A total of 9 false positive results were obtained when tested neat, 1 HIV, 2 HCV, 5 HBV, and 1 HIV/HCV

[b] A total of 7 false positive results were obtained when tested 1:6 dilution, 1 HIV/HCV, 5 HB, and 1 HCV

4. Studies in NAT Positive Populations

A total of 2,569 HIV, HCV, and HBV NAT-positive samples were tested across four test sites with the cobas® MPX Test and the cobas® TaqScreen MPX Test incorporating CAS. Four lots of the cobas® MPX Test reagents were used. The 2,569 samples known to be NAT-positive consisted of 1,015 HIV-positive samples, 1,016 HCV-positive samples, and 538 HBV-positive

samples. Out of 1015 initial HIV-positive specimens, 9 were excluded from the sensitivity calculation; 6 out of 9 were due to the viral loads below the inclusion criteria and 3 had invalid results. Out of 1016 initial HCV-positive specimens, one sample was excluded from the sensitivity calculation due to the invalid result when tested neat. Out of 528 initial HBV positive samples, 10 specimens were excluded from the sensitivity calculation due to invalid results. Each of these samples was tested neat and diluted (1:6) with the cobas® MPX Test and the cobas® TaqScreen MPX Test. Only neat, not diluted samples, were tested with the licensed CAS Tests per the Standard Specimen Processing Procedure recommended in the cobas® TaqScreen MPX Test Package Insert.

As shown in Table 23, the overall clinical sensitivity of the cobas® MPX Test for the evaluable results, was 100.00% (2,549/2,549) for neat, known positive samples and 100.00% (2,555/2,555) for diluted (1:6), known positive samples. The overall clinical sensitivity of the cobas® TaqScreen MPX Test was 99.96% (2,523/2,524) for neat, known positive samples and 99.84% (2,559/2,563) for diluted (1:6), known positive samples.

Table 23: Sensitivity Comparison for Known Positive Samples

		Sensitivity in Known Positive Samples[a]	
Dilution	**Target Virus**	**cobas® MPX Test**	**FDA Licensed Multiplex NAT**
Neat	Overall	100.00 % (2549/2549)	99.96% (2523/2524)
	HIV	100.00% (1006/1006)	99.90% (1007/1008)
	HCV	100.00% (1015/1015)	100.00% (1014/1014)
	HBV	100.00% (528/528)	100.00% (502/502)
1:6	Overall	100.00% (2555/2555)	99.84% (2559/2563)
	HIV	100.00% (1006/1006)	99.60% (1005/1009)
	HCV	100.00% (1016/1016)	100.00% (1016/1016)
	HBV	100.00% (533/533)	100.00% (538/538)

[a]Only known positive samples with valid test results for both the cobas® MPX Test and the cobas® TaqScreen MPX Test were included in the sensitivity analysis.

Clinical Sensitivity for HIV-1 Group O and HIV-2 Seropositive Population

a) HIV-1 Group O Seropositive Population

A total of 12 HIV-1 Group O seropositive samples were tested at a 1:6 dilution using the cobas® MPX Test and the cobas® TaqScreen MPX Test. All of the HIV-1 Group O samples were positive for HIV when tested with cobas® MPX Test at a 1:6 dilution, Table 24).

Table 24: Comparison of HIV-1 Group O Seropositive Sample Reactivity (1:6 Dilution)

FDA Licensed Multiplex NAT (1:6 Dilution)	cobas® MPX Test (1:6 Dilution)		Total
	Positive	Negative	
Positive	11	0	11
Negative	1	0	1
Total	12	0	12

b) HIV-2 Seropositive Population

A total of 319 HIV-2 seropositive samples were tested using the cobas® MPX Test and the cobas® TaqScreen MPX Test. Out of the 319 seropositive samples, 184 were tested neat and at a 1:6 dilution with the cobas® MPX Test and the cobas® TaqScreen MPX Test whereas the remaining 135 were only tested after a 1:6 dilution due to limited sample volume.

A total of 137 of the 184 neat tested samples were positive, for a clinical sensitivity of 74.5% relative to serology using the cobas® MPX Test when tested neat. The sensitivity of cobas® MPX Test was significantly different (better) compared to FDA licensed comparator test (Table 25). Comparable sensitivity of the cobas® MPX Test towards HIV-2 was also demonstrated when samples were diluted 1:6 prior to testing with both methods. A total of 198 of the 318 were positive with the cobas® MPX Test when tested 1:6 dilution. The difference in the performance of the cobas MPX and the FDA licensed NAT assay for 1:6 diluted samples was not statistically significant (Table 26).

Table 25: Comparison of HIV-2 Seropositive Sample Reactivity (Neat)

FDA Licensed Multiplex NAT (Neat)	cobas® MPX Test (Neat)		Total
	Positive	Negative	
Positive	118	7	125
Negative	19	40	59
Total	137	47	184

p-value= 0.0310, chi-square value=4.654

Table 26: Comparison of HIV-2 Seropositive Sample Reactivity (1:6 Dilution)

FDA Licensed Multiplex NAT (1:6 Dilution)	cobas® MPX Test (1:6 Dilution)		Total
	Positive	Negative	
Positive	173	33	206
Negative	25	88	113
Total	198	121	319

p-value=0.2893, chi-square= 1.123

VI. BIORESEARCH MONITORING (BIMO) INSPECTIONS

The Bioresearch Monitoring (BIMO) inspections were conducted in accordance with FDA's Compliance Program Guidance Manual 7348.811. The clinical sites that were inspected and the outcome of the inspections are described in table 27.

Table 27: BIMO Inspections

Study Site	Location	Form FDA 483 Issued	Final Inspection Classification
Midwest Regional Blood Testing Services	Davenport, Iowa	No	NAI*
CSL Plasma, Inc.	Knoxville, Tennessee	No	NAI
Memorial Blood Center	St. Paul, Minnesota	No	NAI

*****NAI = No Action Indicated

VII. REGULATORY REVIEW

During the review of this BLA, the review committee identified deficiencies in the original submission. These issues were discussed during review committee meetings, meetings with senior management of the Division and the Office, and subsequently conveyed to the sponsor in the Complete Response (CR) letter dated June 16, 2015. The review committee conducted an interactive review with RMS to address these deficiencies and resolve all issues.

The following major review issues were identified by the committee and resolved:

1. Purchased/repository materials used in Reproducibility/System Equivalency studies were not well characterized before the initiation of studies. Instead, post-hoc investigations were conducted whenever the assay failed to generate correct results.

In response to the FDA CR letter, RMS repeated the Reproducibility study and part of the System Equivalency study. The Reproducibility study was conducted with 3 separate panels, one for each analyte. The panels for this study were characterized using 200-400 replicates in order to have at least 95% confidence that a minimum reactive rate of 95% would be observed. As a result, panel concentrations of ~1x LOD were used for each analyte. The replicate per lot and per system of this reproducibility study is consistent with that tested in Reproducibility study included in the original BLA submission. Both studies tested 180 replicates, using three lots and three systems, across five days.

RMS also completed a supplemental study to demonstrate system equivalency. The study was conducted with approximately 100 samples per target, with at least one-third of the specimens below 3x LOD, inclusive of some specimens around 2x LOD. The cobas® 6800 and the cobas® 8800 analytics systems were used to compare reactivity of living donor HIV-1, HBV and HCV

clinical specimens with the cobas® MPX Test. Specimens were characterized, with titers determined before the start of the study.

2. In several non-clinical studies of known positive specimens cobas® MPX Test, in addition to the analyte for which the specimens were positive, also detected additional analyte (e.g., in HIV-1 positive specimens HCV and/or HBV was detected). FDA asked RMS to demonstrate with independently performed PCR that the detection of additional analyte was not due to amplicon contamination or signal bleeding from target channel to non-target channel(s), RMS performed a study at an external laboratory to demonstrate that the signal in the non-target channel observed during non-clinical studies was not due to contamination during the testing. For this study, remaining clinical specimens from the non-clinical studies in which HBV was detected in the non-target channel (in addition to the target virus), were re-tested with an independently performed PCR. Testing was performed at National Genetics Institute (NGI) using the (b) (4) test that uses PCR regions, which do not correspond to the HBV sequence detected by the cobas® MPX Test. None of the aliquots sent to NGI for testing were opened or manipulated in the RMS laboratories. Of the 205 specimens tested, 172 had valid results and of those, 143 were confirmed positive for HBV. Twenty-nine specimens tested negative by NGI. Overall, the (b) (4) test at NGI confirmed the viral target in an alternate target region in 83.1% (143 out of 172) of the specimens indicating that the results using the cobas® MPX Test were not generated due to amplicon or viral target contamination during the testing, but due to the presence of the additional target in the specimens tested and that the cobas® MPX Test is a highly sensitive assay for detection of HBV target. RMS evaluated alternate nucleic acid testing methods that use HIV and HCV target regions different from that of the cobas® MPX Test at NGI using (b) (4) and (b) (4). The sensitivity of the (b) (4) and (b) (4) tests are comparable to the sensitivity of the cobas® MPX Test and provided independent confirmation of results at an external laboratory. The oligonucleotide sequence used in the NGI tests are within the same genetic regions as those detected by cobas® MPX Test, but may not completely overlap, providing for detection of different sequences. None of the aliquots sent to NGI for testing were opened or manipulated in the RMS laboratories, ensuring that any HIV or HCV viral target in these aliquots could not have originated from within the RMS facilities. Four specimens from the previously conducted non-clinical studies, which detected HIV in the non-target channel, were sent to NGI for external evaluation. Of the 4 HIV specimens tested, 3 had valid results of which 1 was confirmed positive for HIV-1. Of the 11 HCV specimens tested with (b) (4), all 11 had valid results and of those 8 were confirmed positive for HCV. Overall, the (b) (4) and (b) (4) tests confirmed the respective viral target in 64.2% (9 out of 14) of the specimens indicating that cobas® MPX Test is a highly sensitive assay for detection of HIV and HCV targets.

Based on the additional data, we agree with RMS conclusion that the additional reactivity has been confirmed in a large proportion of the evaluated samples and does not represent a safety risk. The performance capabilities, including the limit of detection for each analyte are included in the Package Insert and the technical performance verification (TPV) study results support the safety and efficacy of the cobas® MPX Test.

3. In the study of individuals at high risk for acquiring HIV, HCV, and/or HBV infection, 165 specimens from individuals who were either diagnosed with or treated for HIV and HCV infection were included. These subjects are not appropriate for inclusion in the High Risk study as per the objective of the study.

RMS has excluded these 165 specimens from the High Risk study. RMS has conducted a study testing 166 additional specimens that meet the revised inclusion criteria in order to satisfy the minimum requirement of 500 high risk specimens.

4. There were several issues related to full description of requirements, specifications, risk management and documentation for the assay specific analysis package functionality software used with the cobas® 6800 and 8800 systems to perform and interpret the test results. During the review, it was identified that several versions of software were used for different preclinical and clinical testing protocols. To resolve this, impact analyses were performed, the software version was updated from Version 1.0 (v1.00.12) to Version 1.1 (v1.01.09) to add new features and to fix anomalies, and corresponding updated design documentation was provided.

To resolve these all other issues identified in the CR letter FDA was engaged through interactive review with RMS. All the issues identified in the CR Letter have been addressed by RMS in the amendment responding to the CR letter and are determined by the committee as resolved.

VIII. LABELING

The Advertising and Promotional Labeling Branch (APLB) reviewed the Instructions for Use (IFU) and carton/container labels from a promotional and comprehension perspective and found them acceptable.

IX. BENEFIT/RISK ANALYSIS

The cobas® MPX Test 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/serum specimens. The Limits of Detection for these different analytes for the cobas® MPX Test are equivalent to or better than those for the currently licensed cobas® TaqScreen MPX Test, v 2.0. This fully automated with high throughput assay thus obviates the need for additional discriminatory testing, reducing the time needed for blood establishments to provide counseling to donors with reactive results.

In addition, the cobas® MPX Test utilizes (b) (4) of HIV-1 primers to target (b) (4) to increase the detection rate for HIV-1 positive specimens

(b) (4) . It is estimated that such (b) (4) occur in approximately 1.7% of HIV-1 NAT-positive/antibody-negative donations; the estimated frequency of such a (b) (4) is approximately 1 in 121 million blood donations in the U.S.

The benefit/risk analysis has demonstrated that the benefits of the cobas® MPX Test outweighs any risk to the blood donor and the safety and availability of nation's blood supply.

X. FINAL REVIEW AND RECOMMENDATIONS

The Review Committee has reviewed the original submission and all of the materials RMS provided as the amendments to the BLA. All the review issues have been resolved. The Committee therefore recommends licensure of the cobas® MPX Test.