SUMMARY OF SAFETY AND EFFECTIVENESS (SSE)

I. GENERAL INFORMATION

Device Generic Name:	Real-time HIV-1 PCR test
Device Trade Name:	cobas® HIV-1
Product Code:	MZF
Applicant's Name and Address:	Roche Molecular Systems, Inc. 4300 Hacienda Drive Pleasanton, CA 94588-2722
Manufacturer:	Roche Molecular Systems, Inc. 4300 Hacienda Drive Pleasanton, CA 94588-2722
Date of Panel Recommendation:	Not Applicable
Premarket Approval Application (PMA) Number:	BP150262

Office's Signatory Authority:

Jay S. Epstein, M.D.
Director, OBRR/CBER

X 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.

Date of Notice of Approval:	December 18, 2015.
Expedited:	Not Applicable

Material Reviewed/Consulted: The PMA, amendments to the PMA, and other specific documentation used in developing the Summary of Safety and Effectiveness (SSE)

Review memos from the following reviewers were used in developing the SSE:

Discipline Reviewed	Reviewer Names
Preclinical Studies and Clinical Studies	Viswanath Ragupathy Jianqgin Zhao Pawan Jain Mohan Haleyurgirisetty Laura Juompan
Product Design	Viswanath Ragupathy Jianqgin Zhao
Chemistry/Manufacturing/Controls (CMC)	Viswanath Ragupathy Mohan Haleyurgirisetty Subhash Dhawan Jianqgin Zhao Susan Yu
Instrumentation and Software	Babita Mahajan Lisa Simone
Statistician	Zhen Jiang Tie-Hua Ng
Bioresearch Monitoring Inspection (BIMO)	Colonius King
DMPQ/pre-approval inspection	Susan Yu
Labeling OCBQ/DCM/APLB	Dana Martin Viswanath Ragupathy
Policy	Sayah Nedjar David Leiby Pradip Akolkar Indira Hewlett Hira Nakhasi J. Peyton Hobson

II. INTENDED USE

The cobas® HIV-1 is an in vitro nucleic acid amplification test for the quantitation of human immunodeficiency virus type 1 (HIV-1) in EDTA plasma of HIV-1-infected individuals using the automated cobas 6800/8800 specimen processing, amplification and detection systems. The test can quantitate HIV-1 RNA over the range of 20-10,000,000 copies/mL (33 to 1.67 x 10^7 International Units/mL).

This test is intended for use in conjunction with clinical presentation and other laboratory markers for the clinical management of HIV-1infected patients. The test can be used to assess patient prognosis by measuring the baseline HIV-1 level or to monitor the effects of antiretroviral therapy by measuring changes in HIV-1 RNA levels during the course of antiretroviral treatment.

The cobas HIV-1 test is not intended for use as a screening test for the presence of HIV-1 in donated blood or plasma or as a diagnostic test to confirm the presence of HIV-1 infection.

CLIA COMPLEXITY: MODERATE

III. DEVICE DESCRIPTION

The cobas HIV-1 is a quantitative test performed on the cobas 6800 System and the cobas 8800 System. The cobas 6800 System is configured for medium throughput and the cobas 8800 System is for high throughput users. The cobas HIV-1 utilizes a fully automated sample preparation (nucleic acid extraction and purification) followed by PCR amplification and detection. The cobas 6800/8800 Systems consists of the sample supply module, the transfer module, the processing module, and the analytic module. Automated data management is performed by the cobas 6800/8800 systems assay specific software which assigns results to all specimens and controls tested. Results can be viewed directly on the system screen, exported, or printed as a report.

The cobas HIV-1 test system consists of

- cobas 6800/8800 Systems
- cobas HIV-1 assay specific analysis package (ASAP) software
- cobas HIV-1 reagents in cassettes
- cobas HBV/HCV/HIV-1 Control Kit (HPC and LPC) in cassettes
- cobas NHP Negative Control Kit in cassettes
- Specimen preparation reagents (cobas omni Reagents)

Assay Principle

The cobas HIV-1 enables the detection and quantitation of HIV-1 RNA in EDTA plasma from HIV-1 infected patients. This device detects and quantitates two genomic targets of HIV-1 (the LTR and gag) using two probes to detect and quantify, but not discriminate group M, N, and O subtypes of HIV-1. The viral load is quantified against a non-HIV-1 armored RNA quantitation standard (RNA-QS), which is introduced into each specimen at a known copy number and is

carried through the specimen preparation, reverse transcription, PCR amplification and detection steps along with the HIV-1 target. Using the RNA-QS (internal control) the assay system automatically detects and compensates for minor effects of inhibitors, thereby controlling for the preparation and amplification processes and allowing for more accurate quantitation of HIV-1 RNA in each specimen. The QS is a non-infectious armored RNA construct that contains a non HIV-1 sequence with no homology to the target viral sequences. The RNA-QS for the cobas HIV-1 test uses a unique primer/probe that allows the QS amplicon to be distinguished from the HIV-1 target amplicon. In addition, the test utilizes three external controls: a high titer positive, a low titer positive, and a negative validation control. The cobas HIV-1 Assay Specific Analysis Package (ASAP) software calculates the HIV-1 RNA (cp/mL) concentration in the test specimens by comparing the HIV-1 signal to the QS signal for each specimen and control using a pre-established linear relationship.

Assay Procedure

The cobas HIV-1 test is performed on the fully automated cobas 6800 or 8800 systems. The process involves sample preparation, amplification and detection.

Sample Preparation (Nucleic Acid Extraction and Purification): The purpose of sample preparation is to isolate and concentrate target RNA from samples and controls so that the target sequence is available for amplification, and to remove any potential inhibitors of amplification. Briefly, viral nucleic acid is released by addition of proteinase and lysis reagent to the sample and controls. The free nucleic acid binds to the silica surface of magnetic glass particles. Unbound material and impurities, including potential PCR inhibitors, are removed with subsequent wash steps with a wash reagent, and purified nucleic acid is eluted from the magnetic glass particles with elution buffer at elevated temperature.

Nucleic Acid Amplification: HIV-1 amplification is achieved with the use of primers and probes that are designed for the highly conserved regions of HIV-1. In order to compensate for primer /probe mismatch in certain HIV-1 variants, this assay is designed to detect two separate regions of the HIV-1 genome, the gag and the long terminal repeat (LTR) region. In addition, this assay contains unique HIV-1 group O LTR amplification primers. However it uses a shared common probe, and therefore does not discriminate between HIV-1 group M and O. The amplification of the RNA-QS is achieved by the use of sequence-specific forward and reverse primers which are selected to have no homology with the HIV-1 genome. The master mix contains thermostable DNA polymerase which is used for both reverse transcription and PCR amplification. Viral targets and RNA-QS are amplified simultaneously. The master mix also contains deoxyuridine triphosphate (dUTP), instead of deoxythymidine triphosphate (dTTP), which is incorporated into the newly synthesized DNA (amplicon). Contamination due to carryover of amplicons from any previous PCR is eliminated by AmpErase (Uracil N-Glycosylase) enzyme, which is included in the PCR master mix, during the first thermal cycling step. However, newly formed amplicons are not eliminated since the AmpErase enzyme is inactivated once exposed to temperatures above 55°C.

Nucleic Acid Detection: The cobas HIV-1 master mix contains two detection probe sequences specific for the HIV-1 target sequences and one for the RNA-QS. The probes are labeled with target specific fluorescent reporter dyes allowing simultaneous detection of HIV-1 target and

RNA-QS in two different target channels. When not bound to the target sequence, the fluorescent signal of the intact probes is suppressed by a quencher dye. During the PCR amplification step, hybridization of the probes to the specific single-stranded DNA template results in cleavage of the probe by the 5' to 3' exonuclease activity of the DNA polymerase resulting in separation of the reporter and quencher dyes and the generation of a fluorescent signal. With each PCR cycle, increasing amounts of cleaved probes are generated and the cumulative signal of the reporter dye increases concomitantly. Real-time detection and discrimination of PCR products is accomplished by measuring the fluorescence of the released reporter dyes for the viral targets and RNA-QS, respectively.

Components of the cobas HIV-1

The assay kit consists of seven components including: cobas HIV-1, HIV/HBV/HCV control kit, Negative Control (NC) control kit, Omni Magnetic Glass Particles (MGP) reagent, protease and lysis reagent, wash reagent and sample diluent. The cobas HIV-1 reagents and controls are described below in Table 1.

Table 1: Components in the cobas HIV-1 Reagent Kit Cassettes

Component Name	Formulation
Elution Buffer	Tris buffer, 0.2% methyl-4 hydroxybenzoate
Master Mix Reagent 1	Manganese acetate, potassium hydroxide, < 0.1% sodium azide
Master Mix Reagent 2	Tricine buffer, potassium acetate, 18% dimethyl sulfoxide, glycerol, < 0.1% Tween 20, EDTA, < 0.12% dATP, dCTP, dGTP, dUTPs, < 0.01% upstream and downstream HIV primers, < 0.01% Quantitation Standard forward and reverse primers, < 0.01% fluorescent-labeled oligonucleotide probes specific for HIV and the Quantitation Standard,< 0.01% oligonucleotide aptamer, < 0.1% ZO5D DNA polymerase (microbial), < 0.10% AmpErase (uracil-N- glycosylase) enzyme (microbial), < 0.1% sodium azide
Proteinase Solution	Tris buffer, < 0.05% EDTA, calcium chloride, calcium acetate, 8% (w/v) proteinase
RNA Quantitation Standard	Tris buffer, < 0.05% EDTA, < 0.001% non-HIV related armored RNA construct containing primer and probe specific primer sequence regions (non-infectious RNA in MS2 bacteriophage), < 0.1% sodium azide

Positive and Negative Control Kit Components

The positive controls for use with the cobas HIV-1 assay (which are co-formulated for use with HBV, HCV, and HIV-1) and the negative control are described in Table 2 below.

Table 2: Positive and Negative Controls for the cobas HBV/HCV/HIV-1

Control	Description
HBV/HCV/HIV-1 Low Positive Control (L(+)C) Titer assignment for each analyte is lot specific with the following target concentrations: HBV Target: ~2.3 Log10 IU/mL HCV Target: ~2.3 Log10 IU/mL HIV-1 Target: ~2.6 Log10 cp/mL	< 0.001% armored HIV-1 Group M RNA (non-infectious RNA in MS2 bacteriophage), < 0.001% synthetic (plasmid) HBV DNA encapsulated in Lambda bacteriophage coat protein, < 0.001% synthetic (armored) HCV RNA encapsulated in MS2 bacteriophage coat protein, 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. 0.1% ProClin 300 preservative.
HBV/HCV/HIV-1 High Positive Control (H(+)C) Titer assignment for each analyte is lot specific with the following target concentrations: HBV Target: ~ 6.3 Log10 IU/mL HCV Target: ~6.3 Log10 IU/mL HIV-1 Target:~ 5.3 Log10 cp/mL	< 0.001% armored HIV-1 Group M RNA (non-infectious RNA in MS2 bacteriophage), < 0.001% synthetic (plasmid) HBV DNA encapsulated in Lambda bacteriophage coat protein, < 0.001% synthetic (armored) HCV RNA encapsulated in MS2 bacteriophage coat protein, 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. 0.1% ProClin 300 preservative.
Normal Human Plasma Negative Control (NHP-NC)	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. < 0.1% ProClin 300 preservative.

cobas omni Reagents

The cobas omni reagents are common sample preparation reagents that are used with all assays run on the cobas 6800/8800 Systems. The cobas omni reagents are described in Table 3 below.

Table 3: cobas omni Reagents

Component Name	Formulation
cobas omni MGP Reagent	Magnetic glass particles, Tris buffer, 0.1% methyl-4 hydroxybenzoate, < 0.1% sodium azide
cobas omni Lysis Reagent	42.56% (w/w) guanidine thiocyanate, 5% (w/v) polydocanol, 2% (w/v) dithiothreitol, di-hydro sodium citrate
cobas omni Specimen Diluent	Tris buffer, 0.1% methyl-4 hydroxybenzoate, < 0.1% sodium azide
cobas omni Wash Reagent	Sodium Citrate Dihydrate; 0.1% (w/v) Methyl 4-Hydroxybenzoate

Additional Materials Required but Sold Separately

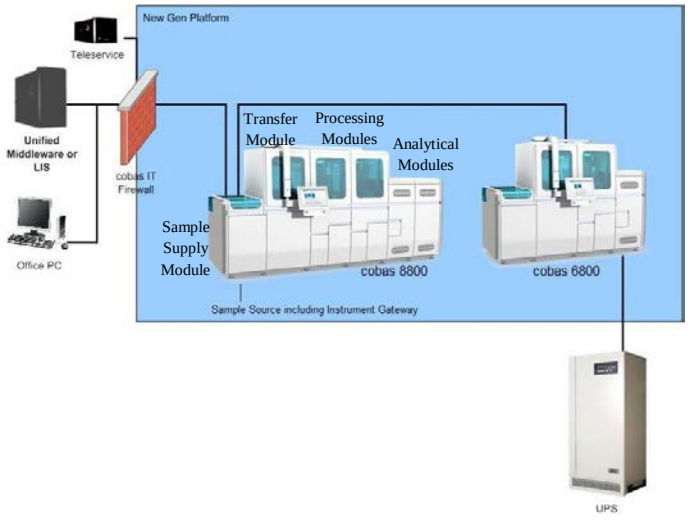
- cobas omni Processing Plate
- cobas omni Amplification Plate
- cobas omni Pipette Tips
- cobas omni Liquid Waste Container
- Solid Waste Bag
- Solid Waste Container

Instrumentation and Software

cobas 6800\8800 Platform Overview

There are two instrument versions of the cobas system: the cobas 6800 System, and the cobas 8800 System. The functionality of the cobas 6800 and 8800 instruments is identical except that sample throughput of cobas 8800 is higher compared to cobas 6800. Each system is comprised of the cobas 6800 or 8800 instrument, system software, Assay Specific Analysis Package (ASAP) and a sample source unit, which can be connected to a conveyor system for automated transport of samples to and from the system (Figure 1). The test kits consist of assay-specific reagents and omni reagents, which can be used with any of the cobas 6800/8800 assays, and on either the cobas 6800 or the cobas 8800 instrument.
The omni reagents and consumables, such as the P-plates, racks, AD-plates, waste bags, pipette tips, and secondary tubes, can be used by any of the cobas 6800/8800 System assays, and on either the cobas 6800 or the cobas 8800 instrument. Either system can be interfaced to an uninterruptible power supply, a customer's Laboratory Information System (LIS), or middleware, and office PCs for some remote viewing and messaging functionalities.

Figure 1: cobas 6800/8800 Platform



IV. TEST PROCEDURE

Specimen Collection, Preparation and Storage

- The cobas HIV-1 test can be performed on plasma samples collected in EDTA anticoagulant containing Lavender Top tubes (LT) and BD Vacutainer® plasma preparation (PPT) tubes.

- Whole blood collected in EDTA tubes may be stored and/or transported for up to 24 hours at 2°C to 25°C prior to plasma separation.
- Upon separation EDTA plasma samples may be stored for up to 6 days at 2°C to 8°C or up to 12 weeks at ≤ -18°C. For long-term storage up to 6 months, temperatures at ≤ -60°C are recommended.
- Plasma samples are stable for up to four freeze/thaw cycles when frozen at ≤ -18°C.
- For HIV-1 viral load quantitation plasma specimens may either be fresh or frozen but frozen samples should be completely thawed to room temperature (15-30°C) before analysis.

Procedural notes

- Do not use cobas HIV-1 reagents, cobas HBV/HCV/HIV-1 Control Kit, cobas NHP Negative Control Kit, or cobas omni reagents after their expiry dates.
- Do not reuse consumables. They are for one-time use only.
- Refer to the cobas 6800/8800 Systems Operator's Manual for proper maintenance of instruments.

Running the cobas HIV-1

The cobas HIV-1 test is run using a sample volume of 650 µL. The test procedure is described in detail in the cobas 6800/8800 Systems Operator's Manual. Procedural steps are summarized below;

Step 1
- Load reagents and consumables as prompted by the system
- Load wash reagent, lysis reagent and diluent
- Load processing plate and amplification plates
- Load Magnetic Glass Particles
- Load control cassettes
- Load tip racks
- Replace rack for clotted tips

Step 2
- Load racks with samples and controls and start the process.

Step 3
- Review and export results

Step 4
- Remove amplification plates from analytic module
- Unload empty control cassettes
- Empty solid waste
- Empty liquid waste

V. RESULTS

The cobas 6800/8800 Systems automatically determines the HIV-1 RNA concentration for the samples and controls. The HIV-1 RNA concentration is expressed in copies per milliliter (cp/mL) or International Units per milliliter (IU/mL). The conversion factor for the cobas HIV-1

is 0.6 cp/IU based on the 2nd WHO International Standard for HIV-1 RNA for Nucleic Acid Amplification Technology Assays group M subtype B obtained from the National Institute for Biological Standards and Control (NIBSC).

Quality Control and Validity of Results

- One negative control (–) C and two positive controls, a low positive control HIV-1 L (+) C and a high positive control HIV-1 H (+) C, are processed with each batch.
- In the cobas 6800/8800 software generated report, user checks for batch validity.
- The batch is valid if no flags appear for all three controls, which includes one negative control and two positive controls: HIV-1 L (+) C, HIV-1 H (+) C. The negative control result is displayed as (–) C and the low and high positive controls are displayed as HxV L (+) C and HxV H (+) C.

Control flags

Validation of results is performed automatically by the cobas 6800/8800 software based on negative and positive control failures (see Table 4).

Table 4: Control Flags for Negative and Positive Controls

Negative Control	Flag	Result	Interpretation
(-) C	Q02 (Control batch failed)	Invalid	An invalid result or the calculated titer result for the negative control is not negative
Positive Control	**Flag**	**Result**	**Interpretation**
HxV L (+) C	Q02 (Control batch failed)	Invalid	An invalid result or the calculated titer result for the low positive control is not within the assigned range.
HxV H (+) C	Q02 (Control batch failed)	Invalid	An invalid result or the calculated titer result for the high positive control is not within the assigned range.

- If the batch is invalid, repeat testing of the entire batch including samples and controls.
- HxV L (+) C stands for cobas HBV/HCV/HIV-1 low positive control and HxV H (+) C stands for cobas HBV/HCV/HIV-1 high positive control in the cobas 6800/8800 software.

VI. INTERPRETATION OF RESULTS

For a valid batch, check each individual sample for flags in the cobas 6800/8800 software and/or report. The result interpretation should be as follows:

- A valid batch may include both valid and invalid sample results.

Table 5: Target Results for Individual Target Result Interpretation

Results	Interpretation
Target Not Detected	HIV-1 RNA not detected Report results as "HIV-1 not detected"
< Titer Min	Calculated titer is below the Lower Limit of Quantitation (LLoQ) of the assay. Report results as "HIV-1 detected less than (Titer Min)." Titer min = 20 cp/mL and 33 IU/mL.
Titer	Calculated titer is within the Linear Range of the assay – greater than or equal to Titer Min and less than or equal to Titer Max Report results as "(Titer) of HIV-1 detected"
> Titer Max[a]	Calculated titer is above the Upper Limit of Quantitation (ULoQ) of the assay. Report results as "HIV-1 detected, greater than (Titer Max)" Titer max = 1.00E+07 cp/mL and 1.67E+07 IU/mL.

[a]Sample result > Titer Max refers to HIV-1 positive samples detected with titers above the upper limit of quantitation (ULoQ). If a quantitative result is desired, the original sample should be diluted with HIV-1 negative EDTA plasma, depending on the type of the original sample, and the test should be repeated. Multiply the reported result by the dilution factor.

VII. WARNINGS AND PRECAUTIONS

As with any test procedure, good laboratory practice is essential for the proper performance of this assay. Due to the high sensitivity of this test, care should be taken to keep reagents and amplification mixtures free of contamination (Refer to CLSI guideline, MM19-A).

- The cobas HIV-1 is only intended for quantitation of HIV-1 viral load and is not intended for initial clinical diagnosis of HIV-1 infection.
- The cobas HIV-1 test is not intended for use as a screening test for the presence of HIV-1 in donated blood or plasma or as a diagnostic test to confirm the presence of HIV-1 infection.
- All patient samples should be handled as if infectious, using good laboratory procedures as outlined in Biosafety in Microbiological and Biomedical Laboratories and in the CLSI Document M29-A4. Only personnel proficient in handling infectious materials and the use of cobas HIV-1 and cobas 6800/8800 Systems should perform this procedure.
- All human-sourced materials should be considered potentially infectious and should be handled with universal precautions. If spillage occurs, immediately disinfect with a freshly prepared solution of 0.5% sodium hypochlorite in distilled or deionized water (dilute household bleach 1:10) or follow appropriate site procedures.
- The cobas HBV/HCV/HIV-1 Control Kit and the cobas NHP Negative Control Kit contain plasma derived from human blood. The source material has been tested by licensed antibody tests and found non-reactive for the presence of antibody to HCV, antibody to HIV-1/2, HBsAg, and antibody to HBc. Testing of normal human plasma by PCR methods also showed no detectable HIV-1 (Groups M and O) RNA, HIV-2 RNA,

HCV RNA, HBV DNA, HEV RNA, WNV RNA, and CMV DNA. No known test method can offer complete assurance that products derived from human blood will not transmit infectious agents.

- Do not freeze whole blood or any samples stored in primary tubes.
- Use only supplied or specified required consumables to ensure optimal test performance.
- Safety Data Sheets are available on request from your local Roche representative.
- Closely follow procedures and guidelines provided to ensure that the test is performed correctly. Any deviation from the procedures and guidelines may affect optimal test performance.
- Handle all samples according to good laboratory practice in order to prevent carryover of samples.
- The detection rate of HIV-1 group O at 20 cp/mL (claimed LLoQ for the cobas HIV- 1) was observed to be 90.5% which is less than was determined for all other genotypes evaluated.

Procedural Limitations

- The cobas HIV-1 has been evaluated only for use in combination with the cobas HBV/HCV/HIV-1 Control Kit, cobas NHP Negative Control Kit, cobas omni MGP Reagent, cobas omni Lysis Reagent, cobas omni Specimen Diluent, and cobas omni Wash Reagent for use on the cobas 6800/8800 Systems.
- Reliable results depend on proper sample collection, storage and handling procedures.
- Quantitation of HIV-1 RNA is dependent on the number of virus particles present in the samples and may be affected by sample collection methods.
- Though rare, mutations within the highly conserved regions of a viral genome covered by cobas HIV-1 may affect primers and/or probe binding resulting in the under-quantitation of virus or failure to detect the presence of virus.
- Due to inherent differences between technologies, it is recommended that, prior to switching from one technology to the next, users perform method correlation studies in their laboratory to qualify technology differences. Users should follow their own specific policies/procedures.

VIII. CONTRAINDICATIONS

There are no known contraindications for use for this test.

IX. ALTERNATIVE PRACTICES AND PROCEDURES

There are other FDA approved alternative devices for the in vitro quantitation of HIV-1. These assays, including the cobas HIV-1 provide a means of measuring baseline HIV-1 level and to monitor the effects of antiretroviral therapy by measuring changes in HIV-1 RNA levels during the course of antiretroviral treatment.

X. POTENTIAL ADVERSE EFFECTS OF THE DEVICE ON HEALTH

The possibilities of erroneous results may occur due to device malfunction or operator error. An erroneously high test result may indicate therapeutic failure and/or a higher likelihood of progression to AIDS. A false result may also result in unnecessary treatment and/or psychological trauma to a patient. An erroneously low test result may lead to lack of appropriate treatment and/or instill a false sense of security in a patient which could lead to worsening of the

patient's condition. The risks of erroneous test results are inherent in all in vitro diagnostic products. However, if appropriate directions are followed as stated in the package insert, the likelihood of erroneous results are minimal from the use of this device.

XI. MARKETING HISTORY

The cobas HIV-1 was CE marked on December 3, 2014.

XII. SUMMARY OF PRE-CLINICAL STUDIES

Analytical Sensitivity – Limit of Detection (LOD)

The limit of detection of the cobas HIV-1 was determined by analysis of serial dilutions of the 2nd WHO International Standard for HIV-1 RNA for Nucleic Acid Amplification Technology Assays group M subtype B obtained from the National Institute for Biological Standards and Control (NIBSC), in HIV- negative human EDTA plasma. Panels consisting of five concentration levels plus a negative were tested over three lots of cobas HIV-1 test reagents, multiple runs, days, operators, and instruments. A total of 189 replicates per concentration level were tested and combined results were shown in Table 6.

Table 6: HIV-1 RNA Limit of Detection (LoD) in EDTA Plasma

Input Titer Concentration (HIV-1 RNA cp/mL)	Input Titer Concentration (HIV-1 RNA IU/mL)	Number of Valid Replicates	Number of Positives	Detection Rate (%)
40.0	66.7	189	189	100.0%
20.0	33.3	189	186	98.4%
10.0	16.7	189	171	90.5%
5.0	8.3	189	125	66.1%
2.5	4.2	189	67	35.4%
0.0	0.0	189	0	0.0%
LoD by PROBIT at 95% detection rate		13.2 cp/mL; 95% confidence interval: 11.4, 15.9 cp/mL 22.0 IU/mL; 95% confidence interval: 19.0, 26.5 IU/mL		

Summary: The LoD study as designed demonstrated that the cobas HIV-1 detected HIV-1 RNA at a concentration of 13.2 cp/mL (22.0 IU/mL), with a rate of detection of 95% as estimated by PROBIT analysis. The IU and cp/ml conversion used in this assay is based the on the 2nd WHO International Standard for HIV-1 RNA for Nucleic Acid Amplification Technology Assays group M subtype B obtained from the National Institute for Biological Standards and Control (NIBSC) where one IU of HIV-1 RNA is equivalent to 0.6 cp. The claimed Lower Limit of Quantitation (LLoQ) for the cobas HIV-1 is 20 cp/mL (33.3 IU/mL) and has been demonstrated to generate positive results with a rate of detection of 98.4% (186/189).

Verification of Limit of Detection (LoD) for Group M Subtypes, Group O and Group N

Cultured HIV-1 samples for HIV-1 group M (A, C, D, F, G, H, CRF01_AE, CRF02_AG), HIV-1 group O, and HIV-1 group N were diluted to three different levels (i.e., 0.5x LoD, 1.0x LoD and 2.0x LoD) in EDTA plasma bracketing the claimed LoD of 20 cp/mL. The rate of detection

was determined using 63 replicates for each level. The proposed LoD for all HIV-1 genotypes with the detection rate of >95% was confirmed to be 20 cp/mL, with the exception of HIV-1 Group O. Testing was conducted with 1 kit lot of the cobas HIV-1 reagents.

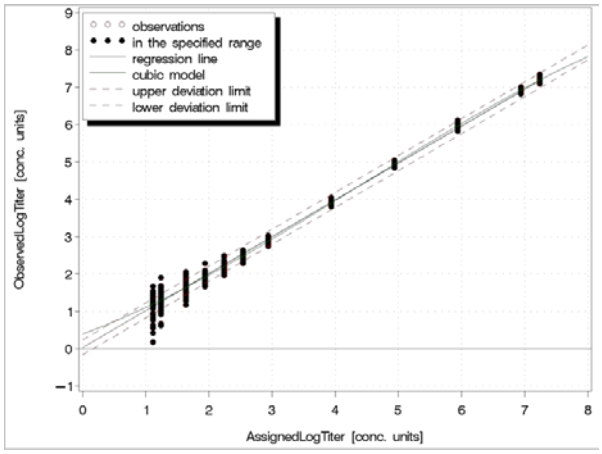
Summary: The cobas HIV-1 detected HIV RNA from HIV-1 group M (A, C, D, F, G, H, CRF01_AE, CRF02_AG) and HIV-1 group N at the claimed LoD of 20 cp/mL except for HIV-1 group O. The detection rate of HIV-1 group O at 20 cp/mL was 90.5%. The performance of the cobas HIV-1 is acceptable across the Group M and N subtypes evaluated in the study. The performance of the cobas HIV-1 assay for the detection of Group O was found to be lesser than with other subtypes, with a detection rate of 90.5% at 20 cp/mL. The performance for Group O is acceptable as it is a rare variant of HIV-1, predominately found in West Africa. However, the reduced performance of the cobas HIV-1 for Group O will be included in the warnings and limitations sections of the labelling.

Linear Range

The linearity of the cobas HIV-1 was evaluated using a dilution series consisting of a 12 member panel spanning the linear range of the assay for HIV-1 group M subtype B. The linearity panel design included one concentration below the LLoQ (1.30E+01 cp/mL, 21.7IU/mL) to one concentration above the ULoQ (1.73E+07 cp/mL, 2.89E+07 IU/mL). The expected linear range of the cobas HIV-1 is from 20 cp/mL to 1.00E+07 cp/mL (33.3 IU/mL to 1.67E+07 IU/mL). The panel members used in this study were prepared from a high titer HIV-1 RNA positive specimen (cell culture supernatant). All titers were calculated and converted into $\log_{10}$ titer. The evaluation was performed according to CLSI Guideline EP06-A using 3 cobas HIV-1 kit lots. The data were analyzed for maximum deviation between the linear regression and the better fitting non-linear regression. In addition, the LLoQ was determined for the lowest concentration level (20 cp/mL) in the LOD panel with a detection rate $\geq$ 95 %. To determine LLoQ a statistical approach described in CLSI EP21-A was used. Testing was conducted using three reagent lots and analyzed on three cobas 6800 Systems with three operators.

Summary: As shown in the Fig.2, the linear range of the cobas HIV-1 test is 1.30E+01 to 1.73E+07 cp/mL observed from 3 combined kit lots and shows absolute deviation from the better fitting non-linear regression of less than $0.2\log_{10}$. Based on the analysis, the cobas HIV-1 demonstrated linearity from 20 cp/mL to 1.00E+07 cp/mL (33.3 IU/mL to 1.67E+07 IU/mL) and the LLoQ was determined as 20cp/mL. The linear range and the LLoQ of the cobas HIV-1 as analyzed and presented is acceptable.

Figure 2: Linear Range Determination in EDTA Plasma



Verification of the Linear Range for Group M Subtypes, Group O and Group N

The dilution series used in the verification of the linear range for various subtypes analyzed on the cobas HIV-1 consisted of seven panel members spanning the linear range of 20 cp/mL to 1.00E+07 cp/mL. This range covers the medical decision points. The panel members were prepared from high titer HIV-1 culture supernatants of the respective subtypes. Testing was conducted with two lots of the cobas HIV-1 reagent using14 replicates per level in EDTA plasma.

Summary: The linear range of the cobas HIV-1 was evaluated for HIV-1 group M subtypes, HIV-1 group O and HIV-1 group N and was confirmed to be linear from 20 cp/mL to 1.00E+07 cp/mL. For each subtype analyzed the maximum deviation between the linear regression and the better fitting non-linear regression was equal to or less than 0.2 $\log_{10}$.

Precision – Within Laboratory

The precision of the cobas HIV-1 was determined by analysis of serial dilutions of an HIV-1 high positive sample (Group M Subtype B; cultured virus) in HIV negative EDTA plasma. Eight dilution levels were tested using 48 replicates for each level and across three lots of the cobas HIV-1 test reagents using three cobas 6800 systems and three operators over 12 days. The precision determination was based on CLSI EP5-A2 and results of each lot and all three lots combined for different concentration levels are listed in Table 7. The precision of the cobas HIV-1 was assessed by calculating the SD of the observed $\log_{10}$ titer at each dilution level of an HIV-1 positive sample (Group M Subtype B). In addition the contribution of components to within lot precision (kit lot, Instrument, Day, Run and within Run) and to total precision was evaluated.

Summary: The cobas HIV-1 results showed acceptable precision across a concentration range of 100 cp/mL to 1.00E+07 cp/mL and the SD of each concentration range is less than 0.2 $\log_{10}$ cp/mL when tested with three lots of reagents (Table 7).

Table 7: Within Laboratory Precision of cobas HIV-1 (EDTA Plasma Samples)

Nominal	Assigned	EDTA plasma			
		Lot 1	Lot 2	Lot 3	All Lots

Concentration (cp/mL)	Concentration (cp/mL)*	SD	SD	SD	Pooled SD
1 00E+07	8 67E+06	0 04	0 06	0 03	0 05
1 00E+06	8 67E+05	0 06	0 05	0 04	0 05
1 00E+05	8 67E+04	0 05	0 07	0 04	0 05
1 00E+04	8 67E+03	0 06	0 06	0 04	0 05
1 00E+03	8 67E+02	0 07	0 06	0 07	0 07
4 00E+02	3 47E+02	0 09	0 10	0 09	0 09
2 00E+02	1 73E+02	0 15	0 08	0 14	0 11
1 00E+02	8 67E+01	0 15	0 11	0 10	0 12

* Titer data are considered to be log-normally distributed and are analyzed following $\log_{10}$ transformation. Standard deviations (SD) columns present the log-transformed titer for each of the three reagent lots and the combined lots.

Performance with HIV-1 Negative Specimens

Performance of the cobas HIV-1 was determined by analyzing 600 EDTA plasma samples from healthy HIV negative individuals. Each of these samples was tested with two lots of the cobas HIV-1 reagents. All samples tested negative for HIV-1 RNA. In the test panel, the cobas HIV-1 "Target Not Detected" was 100% (95% confidence limit: 99.5, 100%).

Summary: Testing of 600 known negative specimens using the cobas HIV-1 generated acceptable results as the test did not yield any false positive results.

Potentially Interfering Microbial Contaminants

The analytical sensitivity and specificity of the cobas HIV-1 assay in the presence of microbial contaminants was evaluated by adding microbes (Summarized in Table 8) into three replicates of HIV-1 RNA positive EDTA plasma containing at LLoQ) or HIV RNA negative EDTA plasma. Each microorganism was added at a concentration of particles/mL or CFU/mL. In addition a panel member was evaluated without any potentially interfering microorganism for HIV-1 RNA positive and HIV-1 RNA negative EDTA plasma.

Summary: The results demonstrate that none of the non-HIV pathogens interfered with the cobas HIV-1 performance. Negative results were obtained with the cobas HIV-1 for all microorganism samples without HIV-1 target and positive results were obtained for all of the microorganism samples with HIV-1 target spiked. Furthermore, the mean $\log_{10}$ titer of each of the positive HIV-1 samples containing potentially cross-reacting organisms was within ± 0.3 log10 of the mean $\log_{10}$ titer of the respective positive spike control. The results from the microorganisms tested demonstrate acceptable performance with no cross-reactivity of the cobas HIV-1 observed.

Table 8: Microorganisms Tested for Cross-Reactivity

Viruses		Bacteria	Yeast
Adenovirus type 5	Varicella-Zoster Virus	Propionibacterium	Candida albicans

Cytomegalovirus	West Nile Virus	Staphylococcus aureus	
Epstein-Barr Virus	St. Louis encephalitis Virus		
Hepatitis A Virus	Murray Valley encephalitis Virus		
Hepatitis B Virus	Dengue virus types 1, 2, 3, and 4		
Hepatitis C Virus	Tick-Borne Encephalitis Virus (strain HYPR)		
Hepatitis D Virus	Influenza Virus A		
Human T-Cell Lymphotropic Virus types 1 and 2	Zika Virus		
Human Herpes Virus Type-6	Human Papillomavirus		
Herpes Simplex Virus Type 1 and 2	Yellow Fever Virus		

Potentially Interfering Endogenous and Exogenous Substances

The effect of potentially interfering endogenous substances on the cobas HIV-1 quantitation of HIV-1 was evaluated. Three replicates of EDTA plasma samples with (LLoQ) and without HIV-1 were spiked with elevated levels of triglycerides (up to 34.5 g/L), conjugated bilirubin (0.252 g/L), unconjugated bilirubin (0.253 g/L), albumin (58.7 g/L), hemoglobin (2.9 g/L) and human DNA (2 mg/L). In addition, samples with an elevated level of auto antibodies from clinical conditions including systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), and antinuclear antibody (ANA) were tested in presence and absence of HIV-1 RNA. The selection of interfering substances was based on the CLSI guideline EP7-A2.
In addition, drug compounds (exogenous) listed in Table 9 were tested at three times the C_{max} (based on published plasma peak levels) with cp/mL of EDTA plasma LLoQ) and without HIV-1 viral target. The evaluation was performed using a single reagent lot and two different cobas 6800 systems.

Summary: All potentially interfering substances evaluated in this study have been shown not to interfere with the test performance. Positive results were obtained on all samples with HIV-1 target. The mean $\log_{10}$ titer of each of the positive HIV-1 samples containing potentially interfering substances was within ± 0.3 $\log_{10}$ of the mean $\log_{10}$ titer of the respective positive spike control. Furthermore, negative results were obtained with the cobas HIV-1 for all samples without HIV target.

Table 9: Drugs Tested for Interference with the Quantitation of HIV-1 RNA by the cobas HIV-1 assay

Class of Drug	Generic Drug Name	
Immune Modulators	Peginterferon alfa-2a Peginterferon alfa-2b Ribavirin	
HIV Entry Inhibitor	Maraviroc	
HIV Integrase Inhibitors	Elvitegravir/Cobicistat	Raltegravir
Non-nucleoside HIV Reverse Transcriptase Inhibitors	Efavirenz Etravirine	Nevirapine Rilpivirine
	Atazanavir	Lopinavir

HIV Protease inhibitors	Tipranavir Darunavir Fosamprenavir	Nelfinavir Ritonavir Saquinavir
HCV Protease Inhibitors	Boceprevir Simeprevir	Telaprevir
Reverse Transcriptase or DNA	Abacavir	Tenofovir
Polymerase Inhibitors	Emtricitabine Entecavir Foscarnet Cidofovir Lamivudine Ganciclovir	Adefovir dipivoxil Telbivudine Zidovudine Aciclovir Valganciclovir Sofosbuvir
Compounds for Treatment of Opportunistic Infections	Azithromycin Clarithromycin Ethambutol Fluconazole Isoniazid	Pyrazinamide Rifabutin Rifampicin Sulfamethoxazole (SMX) Trimethoprim (TMP)

Cross Contamination

The cross contamination rate for the cobas HIV-1 was determined by testing 240 replicates of HIV negative human EDTA-plasma samples and 225 replicates of a high titer HIV-1 sample at 4.00E+06 cp/mL. The positive and negative samples were placed in rows and columns in alternate positions as a checker board configuration. The study was performed using the cobas 6800 System. In total, five runs were performed with positive and negative samples and two runs were performed before and after cross contamination using 93 negative samples and 3 RMC controls to assess carry over from previous runs.

Summary: All 240 replicates of the negative sample were negative; resulting in a cross contamination rate of 0% indicating the cobas 6800 is not impacted by carry over contamination from previous runs or from the high positive samples analyzed in the same run.

Equivalence of Collection Tubes

The cobas HIV-1 test uses EDTA Plasma samples collected in either EDTA Lavender Top tubes (LT) and EDTA-Plasma samples collected in BD Vacutainer® Plasma Preparation Tubes (PPT). Tube equivalency was evaluated by testing 87 HIV-1 RNA positive matched blood samples (collected in LT and PPT) that were prospectively collected (Table 10A). The viral loads of these samples were spread across the linear range of the assay and included 11 subtypes of HIV-1. In addition, matched blood samples from 118 HIV-1 negative healthy (un-infected) individuals were also prospectively collected in LT and PPT tubes and analyzed (Table 10B).

Summary: The cobas HIV-1 test demonstrated comparable performance (87/87) for matched HIV-1 positive RNA plasma collected in EDTA-plasma tubes (LT and PPT). All 118 matched HIV-1 negative samples collected from un-infected individuals in LT EDTA tested negative and 117/118 negative samples collected in PPT tested negative. One sample collected in PPT

generated a false positive result during the initial analysis and was negative with repeat testing. Vial loads were comparable for HIV-1 positive paired samples collected in LT and PPT. The tube equivalence study demonstrated acceptable performance between the LT and PPT tubes.

Table 10A: Summary of Results for Equivalence of Collection Tubes using HIV-1 Positive Samples

	No. of samples tested	Positive	Negative	Mean $\log_{10}$ difference	95% CI
No. of HIV-1 RNA positive samples	87	87	0	0.02	-0.02, -0.07

Table 10B: Summary of Results for Equivalence of Collection Tubes using HIV-1 Negative Samples

	No. of samples tested	Positive	Negative	%Agreement
No. of HIV-1 RNA Negative samples	118	1[#]	117	LT 100%
				PPT 99.96%

[#] False positive in PPT but not in LT.
LT: Lavender top
PPT: Plasma Preparation Tube

Internal Quantitation Standard (QS) and Control Failure Rate Study

The purpose of this study was to evaluate the failure rate of HIV-1 RNA-QS and Roche Manufactured Controls used in the cobas HIV-1 assay as HIV-1 low positive, HIV-1 high positive and negative controls. The analysis was performed using the data obtained from 11 non-clinical technical performance validation studies. There were a total of 14 invalid runs not related to HIV-1 RNA-QS or controls that were excluded for analysis.

Summary: The analysis indicates that the overall RNA-QS Failure Rate was 0.00% (0/10146) in positive samples, 0.00% (0/1887) in negative samples and 0.00% (0/12033) for both combined. The overall Control Failure Rate was 0.00% (0/573) and the overall sample reliability was 99.87% (11460/11474) based on a 95% Pearson Clopper CI (99.81, 99.87) in this study. The 14 invalid runs were mostly related to sample volume or operator error such errors would not affect the safety and effectiveness of the device.

Real-Time Reagent Stability

Expiration dating of the cobas test kit reagents and the omni reagents was evaluated using 3 kit lots. Real-time and accelerated stability studies were evaluated from date of manufacture of kit lots. On board stability was evaluated using [redacted] replicates of High Positive Control (HPC) and Low Positive Control (LPC) after [redacted] hours ([redacted] minutes). The differences in measurements of positive controls should be within ± 0.5 $\log_{10}$ and negative controls should be 100% negative. Negative Control was tested after [redacted] hours ([redacted] minutes) incubated at [redacted] along with non-stressed RMC controls. In addition, on board open kit stability and the number of times that test

specific reagent cassettes can be used after the 1st loading was evaluated for [redacted] days over [redacted] runs. For this study a panel of test specimens consisting of 3 HIV-1 RNA positive linear range ([redacted] cp/mL) and negative plasma ([redacted] replicates of each member) were used.

Summary: The real-time stability data of 3 kit lots of the cobas HIV-1, omni reagents and controls was demonstrated for [redacted] months at their intended storage temperature (2°C – 8°C). These data indicate that the cobas HIV-1 kit components are stable for up to [redacted] months. The labelling will include kit stability to 16 months. The on-board stability of reagent components demonstrated that the reagents are stable until completion of the run. In addition after the first use, the cobas HIV-1 assay reagents are stable for up to 30 days at 4°C and reagents can be used for a maximum of [redacted] intervals if <96 samples are tested. The on-board stability in the labelling will indicate a maximum of 10 intervals.

Reproducibility

Multisite reproducibility of the cobas HIV-1 was evaluated in EDTA plasma at 3 clinical sites using 3 cobas 6800 systems and 3 reagent lots. This study was designed in accordance with CLSI document EP5-A2. The study was performed using panels constructed from well characterized HIV-1 group M, subtype B cultured virus stock and from EDTA plasma that was negative for HIV-1 RNA and HIV-1/2 antibodies. The panel consisted of 7 positives within the linear range of the assay and one negative sample. Two operators at each site tested each reagent lot for 6 days (each operator tested for 3 days). There were two runs performed per day for each of panel member (ranging from 0-5,000,000 cps/ml) in triplicate. The selected viral load ranges cover the key medical decision points as indicated in the 2015 Department of Health and Human Services Guidelines for the Use of Antiretroviral Agents in HIV-1-Infected Adults and Adolescents. A total of 324 tests (3 lots x 3 instruments x 2 operators x 3 replicates x 6 days) were performed per panel for a total of 2592 tests (324 per panel member x 8 panels). Each site produced 864 test results. A total of 2,585/2,592 (99.7%) tests were valid. There were 7 invalid results, 6 were due to clot detected during aspiration and 1 sample had low sample volume. Assay precision was evaluated using a random effects model comparing lots, sites, operators and days.

Summary: Table 11 shows the HIV-1 positive panel members total variance, SDs, and lognormal CVs for the cobas HIV-1 as determined by analysis of variance. There were 2263/2268 valid results obtained. Each positive panel member result was log transformed and results indicated that the observed mean viral load of each panel member differed by no more than $0.17 \log_{10}$ cps/ml. In addition the range of the CVs among positive panel members was from 18.85% to 46.25%. The CVs were calculated using a log normal model. The variations between site and operator each contributed to < 5% of total variance across all panel members, and day and run each contributed to ≤ 10% of total variance across all panel members. The estimated detectable viral load differences were well below $0.5 \log_{10}$ in accordance with current US treatment guidelines where a threshold difference between two viral load levels $>0.5 \log_{10}$ should be a difference which is above the variability of the assay. The within-run component contributed the most variability for the majority of the panel members.

Table 11: Multisite Reproducibility

HIV RNA Concentration (log_{10} copies/mL)			Percent of Total Variance (CV(%))						
Expected	**Mean[a] (SD)[c]**	**No. of Tests[b]**	**Lot**	**Site**	**Operator**	**Day**	**Run**	**Within-Run**	**CV(%)[d]**
1.70	1.69 (0.191)	323	17% (18.23)	0% (0.00)	0% (0.00)	1% (3.42)	5% (9.66)	78% (40.32)	46.25
2.30	2.22 (0.116)	321	32% (15.15)	0% (0.00)	1% (2.26)	4% (5.60)	0% (0.00)	63% (21.55)	27.27
2.60	2.48 (0.102)	323	34% (13.84)	4% (4.85)	3% (3.75)	0% (0.00)	1% (2.74)	58% (17.99)	23.86
3.00	2.84 (0.092)	324	39% (13.30)	0% (0.00)	1% (2.01)	0% (0.00)	7% (5.67)	52% (15.37)	21.33
4.00	3.86 (0.081)	324	43% (12.33)	1% (1.94)	3% (3.34)	10% (5.82)	6% (4.54)	37% (11.39)	18.85
5.00	4.92 (0.084)	324	43% (12.64)	0% (0.00)	3% (3.56)	6% (4.65)	6% (4.52)	42% (12.60)	19.44
6.70	6.63 (0.087)	324	45% (13.60)	0% (0.00)	2% (3.00)	3% (3.42)	0% (0.00)	50% (14.23)	20.32

Note: This table only includes results with detectable viral load.

[a] Calculated using SAS MIXED procedure.

[b] Number of valid tests with detectable viral load.

[c] Calculated using the total variability from the SAS MIXED procedure.

[d] Lognormal model used for CV(%) = sqrt(10^[SD^2 * ln(10)] - 1) * 100.

CV(%) = percent coefficient of variation; HIV = human immunodeficiency virus; No. = number; RNA = ribonucleic acid; SD = standard deviation; sqrt = square root.

Validation of Viral Load Quantitation

The objective of this study was to compare the cobas HIV-1 with the previously approved cobas AmpliPrep/cobas TaqMan HIV-1 Test, version 2.0(CAP/CTM v2.0) at viral load levels spanning the linear range of both tests.

The evaluation was performed at 5 clinical sites using a total of 410 retrospectively collected frozen samples. Each of these samples was tested using both the cobas HIV-1 and CAP/CTM v2.0. The test samples were obtained from 321 (78.3%) males and 89 (21.7%) females, with an age range of 19-72 years. Sample characteristics were shown in Table 12.

Table 12: Sample Characteristics

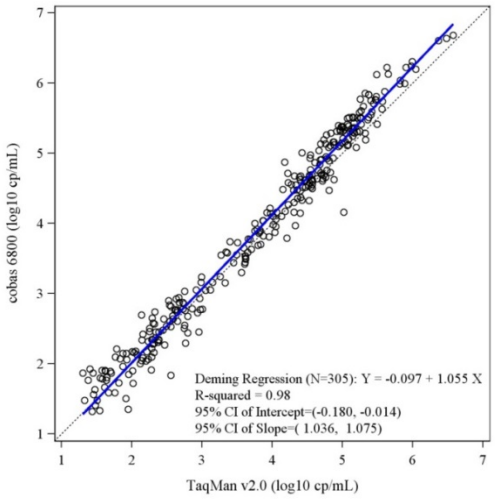
Antiviral Medication	Statistics, n (%)
	(N = 410)
Yes	208 (50.7%)
No	137 (33.4%)
Unknown	65 (15.9%)
CD4 Cell Count (cells/µL)	
N	391
Mean (Standard Deviation)	438.1 (267.7)

Median	401
Range	0 – 1548

Of the 410 samples, 305 samples had viral load measurements within the linear range (20 cp/mL to 1.00E+07 cp/mL) for both assays and were used for method comparison analysis. In addition the viral load levels of these samples were spread across the medical decision points. The remaining 105 samples had viral load levels outside of the quantifiable ranges for both assays and could not be used for the validation study.

The results demonstrate that the cobas HIV-1 and CAP/CTM v2.0 assays are comparable based on the Deming regression estimate (Fig 3) (R^2=0.98) and the mean paired viral load difference between the two assays is 0.112 $\log_{10}$ cp/mL at 95% CI (0.086, 0.137). These differences are lower than the clinically meaningfully difference of 0.5 $\log_{10}$ cp/ml.

Figure 3: Deming Regression Analysis between the cobas HIV-1 on the cobas 6800 System and the TaqMan HIV-1 Test, v2.0.



Summary: Based on the method comparison study results the cobas HIV-1 demonstrates a high level of agreement with the cobas AmpliPrep/cobas TaqMan HIV-1 Test v2.0 (R^2 = 0.98) across the linear range and clinically meaningful viral load thresholds of the test.

Viral Load Discrimination at Clinically Meaningful Thresholds

In the method comparison study between the cobas HIV-1 and CAP/CTM v2.0 for 410 subjects, concordance analysis was evaluated using two viral load cutoffs and two outcomes for each threshold level <200 or >200 and <50 or >50 cp/mL. Table 13 shows that at the 200cp/mL viral load cut off, there were 398 concordant and 12 discordant results observed. As per McNemar's test these discordances were not statistically significant (p value 0.772) and overall percent agreement between both assays was 96.8%.

Table 13: Viral Load Discrimination at the Clinically Meaningful Threshold of 200 copies/mL

	TaqMan v2.0		
cobas 6800	**< 200 cp/mL[a]**	**≥ 200 cp/mL**	**Total**
< 200 cp/mL [a]	156	5	161
>= 200 cp/mL	7	242	249
Total	163	247	410
Overall Agreement (95% CI)	96.8% (94.6%, 98.3%)		
p-value [b]	0.772		

[a] Target Not Detected and Below the Limit of Detection results were categorized as < 200 cp/mL.
[b] Calculated using McNemar's Test.
CI = confidence interval; cp/mL = copies per milliliter.

Similarly, Table 14 shows that at the 50 cp/mL viral load cutoff, there were 390 concordant and 20 discordant results observed. Overall percent agreement between the assays was 95.4%. As per McNemar's test the discordant results were statistically significant (p value 0.0442). In this study 15/125 (12%) samples previously found to have a viral load <50 cp/mL with CAP/CTM v2.0 gave results >50 cp/mL with the cobas HIV-1. Conversely, 1.7% (5/285) of samples previously found to have >50 cp/mL, were shown to have <50 cp/mL by the cobas HIV-1. As per the DHHS treatment guideline, discrepant results above and below 50 cp/mL are not uncommon in successfully treated HIV-1 positive patients and may not be predictive of virologic failure. Therefore the performance is acceptable.

Table 14: Viral Load Discrimination at the Clinically Meaningful Threshold of 50 copies/mL

	TaqMan v2.0		
cobas 6800	**< 50 cp/mL[a]**	**≥ 50 cp/mL**	**Total**
< 50 cp/mL [a]	110	5	115
>= 50 cp/mL	15	280	295
Total	125	285	410
Overall Agreement (95% CI)	95.4% (92.9%, 97.2%)		
p-value [b]	0.0442		

[a] Target Not Detected and Below the Limit of Detection results were categorized as < 50 cp/mL.
[b] Calculated using McNemar's Test.
CI = confidence interval; cp/mL = copies per milliliter.

Performance Equivalence of the cobas 6800 and 8800 Systems

The cobas 6800 and 8800 systems are also used to measure the viral load of HBV and HCV in addition to HIV. A full study of the cobas systems performance equivalency was performed with the cobas HCV for LoD, LLoQ, Linearity, Precision, and Reproducibility on samples from HCV clinical studies. The results presented in the cobas HCV package insert support equivalent performance of the cobas 6800 and the cobas 8800. Based on the evaluation using a subset of

HCV clinical samples from treatment plan 1 (infected with HCV genotype 1) or treatment plan 2 (infected with HCV genotype 2 or 3), the Deming linear regression analysis (Fig 4A) showed a high level of concordance (R^2 = 0.997).

Performance equivalence of the cobas systems for HIV-1 was further evaluated using 123 HIV-1 RNA positive samples (spanning the linear range 20 to1.0E+07 cp/mL) and is summarized in Figure 4B. Results indicate a high level of concordance (R^2 = 0.99) and the mean paired differences at 95% CI (-0.024, 0.022) were -0.001 $\log_{10}$ cp/mL. These differences are lower than the clinically meaningfully difference of 0.5 $\log_{10}$ cp/mL.

Figure 4A: Deming Linear Regression Plot of HCV Viral Load ($\log_{10}$ IU/mL)

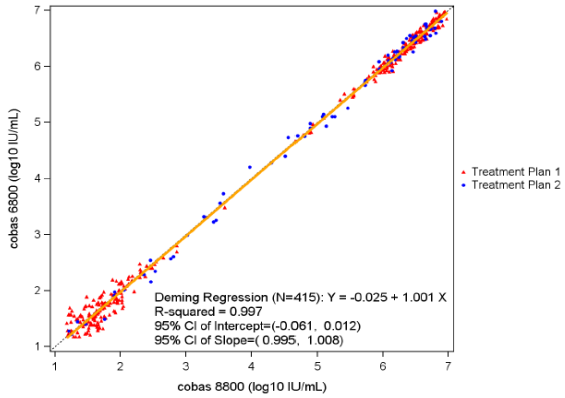
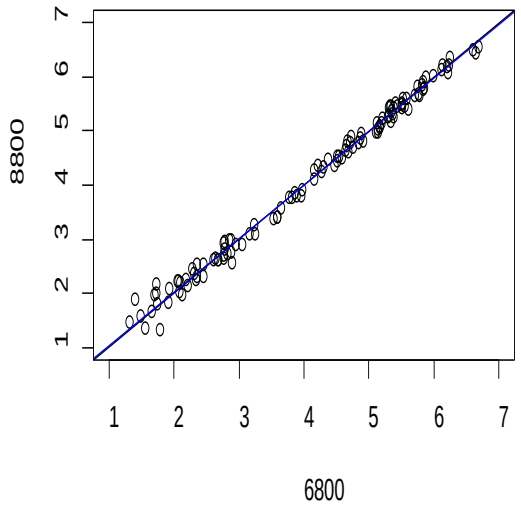


Figure 4B: Deming Linear Regression Plot of HIV Viral Load ($\log_{10}$ cp/mL)



Summary: Based on the study results for HIV-1 and in conjunction with the results obtained with HCV, both the cobas 6800 and 8800 demonstrate equivalent quantitation.

XIII. SUMMARY OF CLINICAL STUDY

Correlation of Virological Outcomes from Patients on Therapy using the DHHS Guideline for Viral Load Levels

The objective of this study was to validate that viral loads below 50 cp/mL and 200 cp/mL measured by the cobas HIV-1 test using the cobas 6800/8800 systems at specific time points (24 and 48 weeks) are predictive of clinical and virological therapeutic outcomes.

Specimens for this study were selected from a completed phase III clinical trial (Boehringer Ingelheim Pharmaceuticals, Inc VERxVE) from trial participants who were followed up to 144 weeks. The selected samples were previously characterized with the FDA approved CAP/CTM v2.0 and retested with the cobas HIV-1 test. Results were compared to thresholds used in current clinical guidelines. The following viral load thresholds were evaluated;

a) Guideline based threshold of 50 cps/ml at 24 weeks and 48 weeks of treatment.
b) Guideline based threshold of 200 cps/ml at 24 weeks and 48 weeks of treatment.

In the VERxVE trial, using the CAP/CTM v2.0 assay, virological failure was defined as the inability to achieve or maintain suppression of viral replication to an HIV RNA level < 50 copies/mL at visit 20 or at the final visit if there was no visit 20.

In this study ~6000 tests from 355 specimens (longitudinally collected and evaluated) of VERxVE trial participant's blood collected at multiple time point(s) were performed at 4 clinical sites. Of the 355 enrolled subjects, the virological failure status could not be determined for one subject and was excluded from analyses. Thus, for virological failure, 354 subjects were evaluated. Demographic characteristics of these study samples were shown in Table 15. Samples from subjects under 19 years of age were not evaluated.

Table 15: Demographic Characteristics of the 355 Subjects Included in the Study

Demographic Characteristics	Statistics
	(N=355)
Age (years)	
Mean (Standard Deviation)	38 (9.3)
Median	38
Range	19 – 68
Sex, n (%)	
Male	322 (90.7%)
Female	33 (9.3%)
Race/Ethnicity, n (%)	
Asian	5 (1.4%)
Black / African-American	45 (12.7%)
White / Caucasian	303(85.4%)
Other	2(0.6%)

CD4 Count at Screening (cells/µL), n (%)	
50 to < 200	117 (33.0%)
200 to < 350	209 (58.9%)
350 to < 400	17 (4.8%)
≥ 400	9 (2.5%)
Unknown	3 (0.8%)

The associations between two virological thresholds and two therapeutic outcomes (virological) at key treatment time points (weeks 24 and 48) were demonstrated by calculating the positive predictive value (PPV), negative predictive value (NPV) and odds ratio (OR). Briefly, the PPV determines the probability of virological failure given that the subject's viral load was above the virological threshold (50 or 200 cp/mL) at a specific visit. The NPV determines the probability of no virological failure given that subject's viral load was below the virological threshold (50 or 200 cp/mL) at a specific visit. Of the 355 baseline samples analyzed, one sample had missing virological failure information and was excluded from the analysis.

The analyses of the 50 copies/mL virological threshold with virological failure (at Week 24 and Week 48) are shown in Table 16. At Week 24, the PPV was 15.6% (10/64, 95% CI: 7.8%, 26.9%), and, at Week 48, the PPV was 25.7% (9/35, 95% CI: 12.5%, 43.3%). At Week 24, the NPV was 90.9% (251/276, 95% CI: 86.9%, 94.1%), and, at Week 48, the NPV was 91.1% (285/313, 95% CI: 87.3%, 94%). At Week 24, the OR was 1.86 (95% CI: 0.75, 4.29), which was not statistically significant (p = 0.191). At Week 48, the OR was 3.51 (95% CI: 1.31, 8.71) which was statistically significant (p = 0.012).

Table 16: Comparison of a 50 copies/mL Virological Threshold with Virological Failure

		Virological Failure[a]		
On-Treatment Visit	Virological Threshold	Yes	No	Total
Week 24	≥ 50 cp/mL	10	54	64
	< 50 cp/mL	25	251	276
	Total	35	305	340[+]
Week 48	≥ 50 cp/mL	9	26	35
	< 50 cp/mL	28	285	313
	Total	37	311	348[+]

[+] Valid results obtained by cobas HIV-1.

[a]Virological Failure is classified as 'Yes' if the viral load of a specimen was greater than or equal to 50 cp/mL at visit 20 or at the final visit if there was no visit 20. Final visit had to be Visit 12 or later.

When 200 cp/mL thresholds were used to define virological failure (Table 17), at Week 24, the PPV was 22.2% (2/9, 95% CI: 2.8%, 60%), and, at Week 48, the PPV increased to 100% (2/2, 95% CI: 15.8%, 100%). At Week 24, the NPV was 90.0% (298/331, 95% CI: 86.3%, 93%), and, at Week 48, the NPV was 89.9% (311/346, 95% CI: 86.2%, 92.9%). At Week 24, the OR was

2.57 (95% CI: 0.25, 14.27), which was not statistically significant (p = 0.469). At Week 48, the OR was 20.76 (95% CI: 2.46, Not Calculable) which is statistically significant (p = 0.022).

Table 17: Comparison of a 200 copies/mL Virological Threshold with Virological Failure

		Virological Failure[a]		
On-Treatment Visit	Virological Threshold	Yes	No	Total
Week 24	≥ 200 cp/mL	2	7	9
	< 200 cp/mL	33	298	331
	Total	35	305	340[+]
Week 48	≥ 200 cp/mL	2	0	2
	< 200 cp/mL	35	311	346
	Total	37	311	348[+]

[+] Valid results obtained by cobas HIV-1.

[a] Virological Failure is classified as 'Yes' if the viral load of a specimen was greater than or equal to 50 cp/mL at visit 20 or at the final visit if there was no visit 20. Final visit had to be visit 12 or later.

Summary: The negative cobas HIV-1 results correlate highly with the negative results for virological failure obtained on the previously approved CAP/CTM v2.0 in clinical trials. These results demonstrate that the cobas HIV-1 can be used to monitor patients on antiretroviral therapy (ART) and to predict which patients will respond to treatment regardless of the threshold used (50 copies/ml or 200 copies/mL) The results presented in clinical evaluation study support the intended use for patient prognosis based on monitoring the effect of drug therapy on HIV-1 viral load.

XIV. INSPECTIONS

Manufacturing Facilities Review/Inspection

Roche Molecular System will manufacture the cobas 6800/8800 test kit components in the currently licensed facilities in . A biennial Team Biologics inspection was performed on at the facilities and classified as Voluntary Action Indicated (VAI). Based on the Team Biologics inspection, DMPQ recommends an inspection waiver for this PMA.

Bioresearch Monitoring (BIMO) Inspections

CBER BIMO issued inspection assignments to two sites in United States. These inspections did not reveal any deviations that impacted the data submitted in this PMA. The inspections were classified as No Action Indicated (NAI).

XV. CONCLUSIONS DRAWN FROM THE PRECLINICAL AND CLINICAL STUDIES

Safety Conclusions

Based on the results of the analytical and clinical studies, the safety of the cobas HIV-1, when used according to the directions provided, should be safe and pose minimal risk to patients due to false test results.

Effectiveness Conclusions
The clinical study results, in combination with the non-clinical performance evaluations including validation of viral load quantitation, strongly support the effectiveness of the cobas HIV-1 for the medical intended use to assess patient prognosis by measuring the baseline HIV-1 level or to monitor the effects of antiretroviral therapy by measuring changes in HIV-1 RNA levels during the course of antiretroviral treatment.

Benefit-Risk Conclusions
The benefits outweigh the risks at the level of performance observed in the pivotal clinical study. Complimentary analytical studies strengthen this conclusion. Accurate quantitation of HIV-1 RNA is an essential component of the treatment of HIV-1 infection. In an era of highly active antiretroviral therapy for HIV infection, accurate quantitation of viral load to monitor treatment and assess sustained virological suppression has substantial individual benefit (i.e., reduction of the risk of disease progression). Risk related to inaccurate quantitation was substantially mitigated by device design (i.e., use of controls). Appropriate warnings to address routine risks encountered in the laboratory practice are contained in the labeling and package inserts for the device. Standard good laboratory practices are considered sufficient to mitigate the risks to the end user.

Overall Conclusions
The data in this application support the reasonable assurance of safety and effectiveness of this device when used in accordance with the intended use. The data from the analytical studies demonstrated acceptable performance of the cobas HIV-1 when used according to the instructions for use as stated in the package insert. The clinical studies performed with the cobas HIV-1 and statistical analysis support the use of this device to quantitate HIV-1 RNA and the test can be used to measure the therapeutic effects by measuring both baseline and changes in HIV-1 RNA levels during the course of antiretroviral treatment.

XVI. PANEL RECOMMENDATIONS
Not Applicable – This product was not submitted for review by the Blood Products Advisory Committee.

XVII. FDA/CBER DECISION
The PMA BP150262 is recommended for approval.