## 1. GENERAL INFORMATION

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|---|---|
| **Device Generic Name:** | Real-time HIV-1/HIV-2 PCR test |
| **Device Trade Name:** | **cobas**® HIV-1/HIV-2 Qualitative |
| **Device Product Code:** | MZF |
| **Applicant Name and Address:** | Roche Molecular Systems, Inc.<br>4300 Hacienda Drive<br>Pleasanton, CA 94588 |
| **Establishment Registration Number:** | 3004141078 |
| **Premarket Approval Application (PMA) Number:** | BP190360 |
| **Date of Panel Recommendation:** | Not Applicable |

- □ **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.**

| | |
|---|---|
| **Office's Signatory Authority:** | Nicole Verdun, M.D.<br>Director, OBRR/CBER |
| **Date of FDA Notice of Approval:** | August 12, 2020 |

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

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

| Discipline Reviewed | Reviewer Names |
|---|---|
| Scientific Lead | Iwona Fijalkowska |
| Clinical | Viswanath Ragupathy |
| Chemistry/Manufacturing/Controls (CMC) | Ranadhir Dey |
| Preclinical/Analytical | Nitin Verma<br>Jiangqin Zhao |
| Instrumentation and Software | Nick Anderson<br>Lisa Simone |
| Statistician | Linye Song |
| Bioresearch Monitoring Inspection (BIMO) | Anthony Hawkins |
| DMPQ/Pre-approval Inspection | Deborah Trout |
| Product and Promotional Labeling | Dana Jones |
| Scientific and Programmatic Aspects | Pradip Akolkar<br>David Leiby<br>Julia Lathrop<br>Indira Hewlett |

## 2. INDICATIONS FOR USE

The **cobas**® HIV-1/HIV-2 Qualitative for use on the **cobas**® 6800/8800 Systems is an *in vitro* nucleic acid amplification test for the qualitative detection and differentiation of human immunodeficiency virus type 1 (HIV-1) and type 2 (HIV-2) in human serum and plasma.

The test is intended to be used as an aid in diagnosis of HIV-1/HIV-2 infection. Detection of HIV-1 or HIV-2 nucleic acid is indicative of HIV-1 or HIV-2 infection, respectively. The presence of HIV-1 or HIV-2 nucleic acid in the plasma or serum of individuals without antibodies to HIV-1 or HIV-2 is indicative of acute or primary infection. The **cobas**® HIV-1/HIV-2 Qualitative may also be used as an additional test to confirm the presence of HIV-1 or HIV-2 infection in an individual with specimens reactive for HIV-1 or HIV-2 antibodies or antigens. The assay may also be used as an aid in the diagnosis of infection with HIV-1 and/or HIV-2 in pediatric subjects and pregnant women.

This assay is not intended to be used for monitoring patient status, or for screening donors of blood, plasma, or human cells, tissues, and cellular and tissue-based products (HCT/Ps) for HIV.

## 3. DEVICE DESCRIPTION

The **cobas**® HIV-1/HIV-2 Qualitative assay is based on a fully automated sample preparation (nucleic acid extraction and purification) followed by PCR amplification and detection. The assay discriminates between HIV-1 and HIV-2 by using probes that are labeled with different fluorescent dyes and are detected in separate channels during amplification.

Nucleic acid from patient plasma or serum samples and added armored RNA internal control (IC) molecules are simultaneously extracted by lysing the virus particles followed by purification of the released RNA using magnetic glass beads. The amplification is carried out using target specific primers for the HIV-1 gag gene and the HIV-1 LTR region (dual target for HIV-1) and the HIV-2 LTR. Amplification of the IC is achieved with the sequence-specific forward and reverse primers which are selected with no homology to HIV-1 or HIV-2 genomes. A thermostable DNA polymerase enzyme is used for both reverse-transcription and amplification. The target and IC sequences are amplified simultaneously utilizing a universal PCR amplification profile with predefined temperature steps and number of cycles.

Two probes are used to detect HIV-1, but do not discriminate between HIV-1 group M, group O, and group N. A third probe is used to detect HIV-2, but not to discriminate between HIV-2 group A and group B. The probes are labeled with target specific fluorescent reporter dyes allowing simultaneous detection of HIV-1 target, HIV-2 target and IC in three different channels. When not bound to the target sequence, the fluorescent signal of the intact probes is suppressed by a quencher dye. Real-time detection and discrimination of PCR products is accomplished by measuring the fluorescence of the released reporter dyes for the viral targets and for the internal control (IC), respectively.

## 4. COMPONENTS OF THE TEST

The **cobas**® HIV-1/HIV-2 Qualitative consists of the test components, each in the 96 test cassette and the additional materials required but sold separately:

4.1 The **cobas**® HIV-1/HIV-2 Qualitative reagents (per kit/96 wells):

- The RNA Internal Control Armored RNA construct (non-infectious RNA encapsulated in MS2 bacteriophage) and synthetic Poly rA RNA in Tris buffer, EDTA, sodium azide; 13 mL
- Proteinase solution in Tris buffer/EDTA, calcium chloride, calcium acetate, glycerol; 13 mL
- Elution Buffer: Tris buffer with methyl-4 hydroxybenzoate; 13 mL
- Master Mix R1: Manganese acetate and potassium hydroxide and 0.1% sodium azide; 5.5 mL
- Master Mix R2: A mix of dATP, dCTP, dGTP, dUTP, upstream and downstream HIV-1, HIV-2 and internal control primers, fluorescent-labeled HIV-1 and HIV-2 probes, fluorescent-labeled internal control probe, oligonucleotide aptamer, Z05D DNA polymerase, AmpErase (uracil-N-glycosylase) enzyme (microbial) in Tricine buffer with potassium acetate, dimethyl sulfoxide, glycerol, Tween 20, EDTA and sodium azide; 6 mL

**Additional materials required but sold separately**

4.2 The **cobas**® HIV-1/HIV-2 Qualitative specific positive controls**:**

- HIV-1 M/HIV-2 Positive Control (HIV-1 M/HIV-2 (+) C); 5.2 mL (8 x 0.65 mL)
- HIV-1 O Positive Control (HIV-1 O (+) C); 5.2 mL (8 x 0.65 mL)

4.3 The **cobas**® Normal Human Plasma Negative Control (NHP-NC); 16 mL (16 x 1 mL)

4.4 The **cobas® omni** reagents for sample preparation (common for all **cobas** assays that are run on the **cobas**® 6800/8800 Systems):

- Magnetic Glass Particles (MGP) Reagent
- Specimen Diluent
- Lysis Reagent
- Wash Reagent

4.5 Additional materials (common to all **cobas®** 6800/8800 assays):

- **cobas® omni** Processing Plate 48 wells
- **cobas® omni** Amplification Plate 96 wells
- **cobas® omni** Pipette Tips
- Solid Waste Bag
- Solid Waste Container

## 5. INSTRUMENTATION AND SOFTWARE

- **cobas**® 6800/8800 Platform Overview

The **cobas**® 6800/8800 Systems are platforms that allow users to perform multiple PCR-based in vitro nucleic acid amplification tests. The **cobas**® 6800 System and the **cobas**® 8800 System both provide fully integrated, automated sample preparation, nucleic acid extraction, and target amplification and detection. The **cobas**® 6800 System can process up to 300 molecular diagnostic tests in 8 hours while the **cobas**® 8800 System can process up to (b) (4) molecular tests in 8 hours.

Automated data management is performed by the **cobas**® 6800/8800 software which assigns test results that can be reviewed directly on the system screen, exported to a Laboratory Information System (LIS), or printed as a report.

- **cobas**® 6800/8800 Systems Software Overview

The **cobas**® 6800/8800 Systems Software is the primary interface for operators to access, control, and manage the **cobas**® 6800/8800 Systems. The **cobas**® 6800/8800 Systems Software includes off the shelf software components (e.g., Microsoft Windows and Oracle Database software) as well as software tools that are used for diagnosis and maintenance of the system. The main system functionality is provided by the **cobas**® 6800/8800 System Software and Assay Specific Analysis Package (ASAP) software.

## 6. TEST PROCEDURE

### Specimen Collection, Preparation and Storage

- The **cobas**® HIV-1/HIV-2 Qualitative can be performed on plasma specimens collected in tubes with EDTA as the anticoagulant or in PPT™ Plasma Preparation Tubes, or on serum specimens collected in SST™ Serum Separation Tubes.
- Plasma or serum samples are prepared by centrifugation, according to manufacturer's instructions; a minimum required sample volume for the **cobas**® HIV-1/HIV-2 Qualitative is 650 µL.
- If using frozen samples in secondary tubes, the samples should be placed at room temperature (15-30ºC) until completely thawed and then briefly mixed (e.g., vortexed for 3-5 seconds) and centrifuged to collect all sample volume at the bottom of the tube.
- Upon separation EDTA plasma or serum samples may be stored in secondary tubes for up to 24 hours at 30°C followed by up to 5 days at 2°C to 8°C or up to 6 weeks at ≤ -20°C. For long-term storage, temperatures at ≤ -60°C are recommended.
- Plasma and serum samples are stable up to 2 days at temperature 2°C–30°C; up to 7 days if stored at 2°C–8°C and up to 6 weeks if frozen at temperature ≤ -20°C.

- Plasma samples are stable for up to three freeze/thaw cycles when frozen at ≤ -20°C.
- If samples are to be shipped, they should be packaged and labeled in compliance with applicable country and/or international regulations covering the transport of samples and etiologic agents.

### 6.1 Running the cobas® HIV-1/HIV-2 Qualitative

The user loads the samples on the instrument.

- **cobas®** HIV-1/HIV-2 Qualitative can be run with a minimum required sample volume of 650 µL. The test procedure is described in detail in the **cobas®** 6800/8800 Systems Operator's Manual.
- Refill reagents and consumables as prompted by the system.
- Load wash reagent, lysis reagent and diluent.
- Load processing plates and amplification plates.
- Load Magnetic Glass Particles.
- Load test specific reagents.
- Load control cassettes.
- Load tip rack.
- Replace rack for clotted tips.
- Load rack with samples.
- The sample is then processed automatically without any further user interaction until results are generated. More than one test can be ordered for the same sample. The system identifies the orders and manages the process automatically
- Review and export results.
- Unload consumables: remove amplification plates from the analytic module; unload empty control cassettes; empty solid waste and empty liquid waste.
- For a detailed description of how to run an assay, refer to the **cobas®** 6800/8800 Systems Operator's Manual.

Reagents loaded onto the **cobas®** 6800/8800 Systems are stored at appropriate temperatures, and their expiration is monitored by the system. The **cobas®** 6800/8800 Systems allow reagents to be used only within their stability period. The system automatically prevents use of expired reagents.

### 6.2 Procedural Notes

- Do not use **cobas®** HIV-1/HIV-2 Qualitative reagents, **cobas®** HIV-1/HIV-2 Qualitative 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.

## 7. RESULTS

### 7.1 Calculation

The **cobas**® 6800/8800 Systems automatically detect and discriminate HIV-1 and HIV-2 simultaneously for the samples and controls, displaying test validity, overall results, as well as individual target results.

### 7.2 Quality Control Procedures

An internal control (IC) is included in the assay that ensures PCR integrity for every sample. The IC uses specific non-competitive pairs of primers, probes and dyes reporting into a separate channel and allows monitoring the sample preparation process and the workflow on the system from nucleic acid extraction through detection.

External controls, one Normal Human Plasma Negative Control [(–) C] and two positive controls [HIV-1M/HIV-2 (+) C and HIV-1 O (+) C] are processed with each batch. Batch validity is confirmed if no flags appear for all three controls in the **cobas**® 6800/8800 report. If a flag (Q02) appears with any control, the entire batch is designated invalid.

## 8. INTERPRETATION OF RESULTS

Results and their corresponding interpretation for detecting HIV-1 and HIV-2 are shown below in Table 1.

**Table 1. Interpretation of Results**

| Valid | Overall Results | Target 1 | Target 2 | Interpretation |
|---|---|---|---|---|
| Yes | Reactive | HIV-1 Reactive | HIV-2 Reactive | All requested results were valid. Target signal detected for HIV-1 and HIV-2. |
| Yes | Reactive | HIV-1 Reactive | HIV-2 Non-Reactive | All requested results were valid. Target signal detected for HIV-1. No target signal detected for HIV-2. |
| Yes | Reactive | HIV-1 Non-Reactive | HIV-2 Reactive | All requested results were valid. No target signal detected for HIV-1. Target signal detected for HIV-2. |
| Yes | Non-Reactive | HIV-1 Non-Reactive | HIV-2 Non-Reactive | All requested results were valid. No target signal detected for HIV-1 or HIV-2. |
| No | Invalid | Invalid | Invalid | Both HIV-1 and HIV-2 results are invalid. Original specimen should be re-tested to obtain valid HIV-1 and HIV-2 results. If the results are still invalid, a new specimen should be obtained. |

## 9. WARNINGS AND PRECAUTIONS

As with any test procedure, good laboratory practice is essential to 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.

- For *in vitro* diagnostic use only.
- This test is not intended for use in screening blood or plasma donors.
- 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/HIV-2 Qualitative 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 following appropriate site procedures.
- **cobas**® HIV-1/HIV-2 Qualitative Control Kit and **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 HIV-1/2. Testing of normal human plasma by PCR methods also showed no detectable HIV-1 (Groups M and O) RNA and HIV-2 RNA. 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 (SDS) 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.
- False positive results may occur if carryover of samples is not adequately controlled during sample handling and processing.

## 10. LIMITATIONS

- **cobas**® HIV-1/HIV-2 Qualitative has been evaluated only for use in combination with the **cobas**® HIV-1/HIV-2 Qualitative 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 type (EDTA plasma or serum) and sample collection (venipuncture). Use of the assay with other types of specimens may not yield accurate results.
- Detection of HIV-1 and HIV-2 nucleic acid is dependent on the number of virus particles present in the sample and may be affected by sample collection,

storage and handling. Sample storage and handling procedures must be followed as stated in the product insert, to avoid risk of inaccurate results.

- False negative (non-reactive) results may be obtained when testing individuals undergoing anti-retroviral therapy (ART) or taking pre-exposure prophylaxis (PrEP) or post-exposure prophylaxis (PEP).
- Though rare, mutations within the highly conserved regions of a viral genome covered by **cobas**® HIV-1/HIV-2 Qualitative may affect primers and/or probe binding resulting in the failure to detect the presence of virus.
- Invalid test results could occur due to interference with triglycerides at concentrations higher than 25 g/L and with human DNA at concentrations higher than 1.5 mg/L.
- Non-reactive test result does not exclude the possibility of infection with HIV. A comprehensive risk history and clinical judgement should be considered before concluding that an individual is not infected with HIV.
- Due to inherent differences between technologies, it is recommended that, prior to switching from one technology to the another, users perform method correlation studies in their laboratory to qualify technology differences. Users should follow their own specific policies/procedures.
- The device is intended to be used as an aid in diagnosis and should not be used in isolation but in conjunction with clinical status, history and risk factors of individuals being tested. AIDS and AIDS-related conditions are clinical syndromes and their diagnosis can only be established clinically.
- **cobas**® HIV-1/HIV-2 Qualitative is not intended for use in screening blood, blood products, tissue or organ donors for HIV

## 11. CONTRAINDICATIONS

There are no known contraindications for use for this test.

## 12. ALTERNATIVE PRACTICES AND PROCEDURES

There is only one FDA approved alternative in vitro nucleic acid amplification test for the qualitative detection of HIV-1 intended for use as an aid in diagnosis of HIV-1 infection, including acute or primary infection.

There are no FDA approved in vitro nucleic acid amplification tests for the qualitative detection of HIV-2.

## 13. POTENTIAL ADVERSE EFFECTS OF THE DEVICE ON HEALTH

Potential adverse effects of the **cobas**® HIV-1/HIV-2 Qualitative relate to the risk of false positive and false negative results. While performance studies indicate that this risk is likely to be very small, the potential for inaccurate results exists. The risk of incorrect results is minimized by following the procedures and instructions outlined in the Package Insert.

## 14. MARKETING HISTORY

The **cobas**® HIV-1/HIV-2 Qualitative was CE certified under the European Union's In Vitro Diagnostics Directive (IVDD) on June 7, 2017 and is commercially available in the European Union. The product is also approved in Australia. The device has not been withdrawn to date from the market in any country for reasons relating to safety and effectiveness of the device.

## 15. SUMMARY OF NONCLINICAL STUDIES

All non-clinical studies for **cobas**® HIV-1/HIV-2 Qualitative were performed at Roche Diagnostics Laboratories using the **cobas**® 6800/8800 Systems. All the non-clinical and clinical studies were performed on either a **cobas**® 6800 System or **cobas**® 8800 System. Both Systems have been approved for interchangeable use as described in the System description.

### 15.1 Limit of Detection

The limit of detection (LoD) of **cobas®** HIV-1/HIV-2 Qualitative was determined by 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

No international standard is currently available for HIV-1 group O RNA. The Roche Primary Standard for HIV-1 group O RNA was derived from commercially available cultured virus stocks and is traceable to the CBER HIV-1 Subtype RNA Reference Panel #1 Lot 01.

One copy of HIV-1 RNA is equivalent to 1.7 International Units (IU) and one copy of HIV-2 RNA is equivalent to 0.2 IU.

Serial dilutions of the standards in HIV-negative human EDTA plasma and serum were prepared. Panels of five or six concentration levels plus a negative (unspiked) sample were tested using three lots of **cobas**® HIV-1/HIV-2 Qualitative reagents.

To estimate LoD, PROBIT analysis was applied to the data combined across dilution series, multiple runs, days, operators and reagent lots, including calculation of the lower and upper limit of the 95% confidence interval. The claimed LoD values are summarized in Table 2.

**Table2. Limit of Detection Values of cobas® HIV-1/HIV-2 Qualitative**

| Matrices | Analyte | LoD [copies/mL] (95%CI) |
|---|---|---|
| EDTA Plasma | HIV-1 group M | 12.8 (10.2;18.0) |
| | HIV-1 group O | 15.4 (11.9; 22.2 |
| | HIV-2 | 35.4 (24.1; 72.3) |
| Serum | HIV-1 group M | 12.8 (10.1; 18.2) |
| | HIV-1 group O | 13.3 (10.6; 18.7) |
| | HIV-2 | 26.3 (20.1; 40.8) |

## 15.2 Inclusivity of HIV Groups and Subtypes

### a. Verification of LoD

Verification of LoD was performed using clinical or cultured samples of HIV-1 group M subtypes: A, C, D, F, G, H and circulating recombinant forms CRF01_AE, CRF02_AG; of HIV-1 group N and of HIV-2 group B, both in plasma and in serum. Each sample was diluted to LoD of **cobas**® HIV-1/HIV-2 Qualitative, based on the assigned titers using HIV quantitative assay. Each diluted sample was tested in (b) (4) replicates. One reagent lot was used in the study. The data confirmed that **cobas**® HIV-1/HIV-2 Qualitative detects the analyte subtypes at concentrations equal to claimed LoDs, with an upper 95% CI equal to or greater to the expected reactivity rate of 95%.

### b. Verification of Inclusivity

Verification of inclusivity for HIV-1 group M subtypes A, C, D, F, G, H, J, K and circulating recombinant forms (CRF01_AE, CRF02_AG, CRF12_BF, CRF14_BG), HIV-1 group O, HIV-1 group N, HIV-2 group A and HIV-2 group B were performed using undiluted samples or samples diluted to about 5x LoD or 3x LoD of **cobas**® HIV-1/HIV-2 Qualitative. The results demonstrate 100% reactive rates for all the genotypes and subtypes used for HIV-1 and HIV-2.

## 15.3 Precision

The precision of **cobas**® HIV-1/HIV-2 Qualitative was determined using Roche Secondary Standard for HIV-1 group M and Roche Primary Standard for HIV-2.

A panel of individually formulated HIV-1 group M and a panel of HIV-2 were used, each panel comprising of six members at concentrations of approximately 0.6x LoD, 1x LoD and 3x LoD, both in serum and in plasma. Testing was performed over 4 days, with three reagent lots and three instruments. The assessed variability components were run, day, lot and instrument (combined with operator). Assay precision was assessed based on the percentage of reactive test results at each concentration level for each of

the variability components analyzed. The acceptance criterion was an overlap between the 95% confidence intervals, within each testing level evaluated.

The acceptance criteria were met for all samples at levels 1x LoD and 3x LoD, for all tested precision parameters.

Lot-to-lot precision data are summarized in Table 3 below.

**Table 3. cobas® HIV-1/HIV-2 Qualitative Reagent Lot-to-Lot Precision**

| Analyte | Concentration | Reagent Lot | % Reactive (95% CI) | |
|---|---|---|---|---|
| | | | Plasma | Serum |
| HIV-1 group M | 0.6x LoD | 1 | 77.4% (67.0%; 85.8%) | 85.7% (76.4%; 92.4%) |
| HIV-1 group M | 0.6x LoD | 2 | 76.2% (65.7%; 84.8%) | 70.2% (59.3%; 79.7%) |
| HIV-1 group M | 0.6x LoD | 3 | 82.1% (72.3%; 89.6% | 78.6% (68.3%; 86.8%) |
| HIV-1 group M | 1x LoD | 1 | 98.8% (93.5%; 100% | 98.8% (93.5%; 100%) |
| HIV-1 group M | 1x LoD | 2 | 98.8% (93.5%; 100%) | 98.8% (93.5%; 100%) |
| HIV-1 group M | 1x LoD | 3 | 100% (95.7%; 100%) | 98.8% (93.5%; 100%) |
| HIV-1 group M | 3x LoD | 1 | 100% (95.7%; 100%) | 100% (95.7%; 100%) |
| HIV-1 group M | 3x LoD | 2 | 100% (95.7%; 100%) | 100% (95.7%; 100%) |
| HIV-1 group M | 3x LoD | 3 | 100% (95.7%; 100%) | 100% (95.7%; 100%) |
| HIV-2 | 0.6x LoD | 1 | 90.5% (82.1%; 95.8%) | 81.0% (70.9%; 88.7%) |
| HIV-2 | 0.6x LoD | 2 | 85.7% (76.4%; 92.4%) | 81.0% (70.9%; 88.7%) |
| HIV-2 | 0.6x LoD | 3 | 86.9% (77.8%; 93.3%) | 85.7% (76.4%; 92.4%) |
| HIV-2 | 1x LoD | 1 | 97.6% (91.7%; 99.7%) | 95.2% (88.1%; 98.7%) |
| HIV-2 | 1x LoD | 2 | 96.4% (89.9%; 99.3%) | 98.8% (93.5%; 100%) |
| HIV-2 | 1x LoD | 3 | 97.6% (91.7%; 99.7%) | 97.6% (91.7%; 99.7%) |
| HIV-2 | 3x LoD | 1 | 100% (95.7%; 100%) | 100% (95.7%; 100%) |
| HIV-2 | 3x LoD | 2 | 100% (95.7%; 100%) | 100% (95.7%; 100%) |
| HIV-2 | 3x LoD | 3 | 100% (95.7%; 100%) | 100% (95.7%; 100%) |

### 15.4 Seroconversion

The effectiveness of **cobas**® HIV-1/HIV-2 Qualitative in detecting the analytes during the pre-seroconversion period was evaluated by comparison with the FDA licensed 4th generation (b) (4) serology test.

Twenty-five commercially available seroconversion panels for HIV-1 group M comprising 230 panel members were tested. Each panel member was tested undiluted with **cobas**® HIV-1/HIV-2 Qualitative and with an FDA approved 4th generation HIV Ag/Ab serology test. The data indicated that **cobas**® HIV-1/2 Qualitative detected the presence of HIV on the same or an earlier draw than the reference serology method, in all panels (Table 4).

**Table 4. Performance of cobas® HIV-1/HIV-2 Qualitative on HIV Seroconversion panels**

| HIV Sero-conversion Panel | Number of Panel Members Tested | Number of Panel Members with Reactive Result | | Days to First Reactive Result | | Days Earlier Detection* |
|---|---|---|---|---|---|---|
| | | cobas® HIV-1/ HIV-2 Qualitative | HIV Ag/Ab Assay | cobas® HIV-1/ HIV-2 Qualitative | HIV Ag/Ab Assay | |
| HIV6243 | 10 | 6 | 4 | 18 | 25 | 7 |
| HIV9011 | 11 | 3 | 2 | 30 | 38 | 8 |
| HIV9012 | 8 | 5 | 3 | 9 | 16 | 7 |
| HIV9013 | 7 | 3 | 2 | 18 | 23 | 5 |
| HIV9018 | 10 | 5 | 3 | 21 | 28 | 7 |
| HIV9020 | 21 | 5 | 3 | 83 | 90 | 7 |
| HIV9022 | 9 | 3 | 2 | 23 | 25 | 2 |
| HIV9030 | 16 | 6 | 3 | 40 | 47 | 7 |
| HIV9031 | 19 | 8 | 4 | 120 | 146 | 26 |
| HIV9034 | 13 | 4 | 3 | 41 | 46 | 5 |
| HIV9076 | 9 | 3 | 3 | 66 | 66 | 0 |
| HIV9089 | 6 | 5 | 3 | 7 | 16 | 9 |
| HIV12008 | 13 | 7 | 5 | 21 | 28 | 7 |
| PRB954 | 7 | 5 | 2 | 7 | 17 | 10 |
| PRB956 | 5 | 4 | 2 | 40 | 47 | 7 |

| | | | | | | |
|---|---|---|---|---|---|---|
| PRB958 | 6 | 6 | 4 | 0 | 7 | 7 |
| PRB961 | 9 | 4 | 2 | 19 | 27 | 8 |
| PRB962 | 6 | 4 | 2 | 7 | 14 | 7 |
| PRB963 | 7 | 4 | 2 | 9 | 17 | 8 |
| PRB967 | 6 | 5 | 3 | 3 | 17 | 14 |
| PRB968 | 10 | 6 | 4 | 15 | 26 | 11 |
| PRB969 | 10 | 7 | 3 | 53 | 70 | 17 |
| PRB973 | 4 | 4 | 2 | 0 | 7 | 7 |
| PRB976 | 4 | 4 | 2 | 0 | 7 | 7 |
| PRB977 | 4 | 4 | 2 | 0 | 13 | 13 |
| Total | 230 | 120 | 70 | - | - | - |

* Days Earlier Detection with **cobas**® HIV-1/HIV-2 Qualitative than with HIV Ag/Ab Assay

Additionally, low and mixed titer early infection and HIV-2 panels were tested with the **cobas**® HIV-1/2 Qualitative and the reference serology assay. The study showed an acceptable concordance between **cobas**® HIV-1/HIV-2 Qualitative and the results, based on the Certificate of Analysis.

### 15.5 Potentially Interfering Microbial Contaminants

- Microbial interference

The analytical specificity of **cobas®** HIV-1/HIV-2 Qualitative was evaluated by testing a panel of microorganisms at $10^5$ or $10^6$ particles, copies, or PFU/mL, for viral isolates, bacterial strains and yeast isolates, respectively (Table 5). The microorganisms were added to HIV negative human EDTA plasma and tested with and without HIV-1 and HIV-2 virus added to a concentration of approximately 3x LoD of **cobas®** HIV-1/HIV-2 Qualitative for each virus. Non-reactive results were obtained for all microorganism samples without HIV-1 and HIV-2 target and reactive results were obtained for all microorganism samples with HIV-1 and HIV-2 targets. The study confirmed that tested microorganisms do not cross-react or interfere with **cobas**® HIV-1/HIV-2 Qualitative.

**Table 5. Microorganisms Tested for Potentially Interfering Microbial Contaminants**

| Viruses | | Bacteria | Yeast |
|---|---|---|---|
| Adenovirus type 5 | Varicella-Zoster Virus | *Propionibacterium acnes* | 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 | TBE Virus (strain HYPR) | | |
| Hepatitis D Virus | Influenza A Virus | | |
| 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 | | |

- Medical conditions interference

EDTA plasma samples from each of the disease states listed in Table 6 (one from Adenovirus type 5 and ten from each of the other disease states) were tested with and without HIV-1 and HIV-2 added to a concentration of approximately 3x LoD of **cobas®** HIV-1/HIV-2 Qualitative for each virus. The disease states listed did not cross-react or interfere with **cobas®** HIV-1/HIV-2 Qualitative.

**Table 6. Disease State Samples Tested for Potentially Interfering Microbial Contaminants**

| Disease State | | |
|---|---|---|
| Adenovirus type 5 | Hepatitis B Virus | Herpes Simplex Virus type 2 |
| Cytomegalovirus | Hepatitis C Virus | Human T-cell lymphotropic Virus type I |
| Dengue Virus | Hepatitis E Virus | Human T-cell lymphotropic Virus type II |
| Epstein-Barr Virus | Herpes Simplex Virus type1 | West Nile Virus |

- HIV-1 and HIV-2 interference

Potential interference between HIV-1 and HIV-2 was investigated by testing panels consisting of HIV-1 or HIV-2 at a concentration of approximately 3x LoD in the presence of potentially interfering high titers of HIV-2 (up to approximately 2E+05 cp/mL) or HIV-1 (up to approximately 2E+07 cp/mL), respectively. Furthermore, the reactive rates of single and co-formulated panel for both HIV targets were assessed with approximately 3x LoD and high titers.

The reactive rates for HIV-1 and HIV-2 were both 100%. Single formulated panels were reactive in the target channel and non-reactive in the non-target channel. HIV-1 and HIV-2 showed no cross reactivity or competitive interference on **cobas**® HIV-1/HIV-2 Qualitative.

### 15.6 Potentially Interfering Endogenous and Exogenous Substances

- Endogenous substances

The following endogenous substances were tested for potential interference with **cobas**® HIV-1/HIV-2 Qualitative: triglycerides (b) (4) g/L); conjugated bilirubin (0.2 g/L); unconjugated bilirubin (0.2 g/L); albumin (60 g/L); hemoglobin (2 g/L); human DNA (b) (4) mg/L). No interference was observed at the indicated concentrations.

Interference was detected at concentrations higher than 25 g/L triglycerides and higher than 1.5 mg/L human DNA, resulting in invalid results.

Other potentially interfering substances tested showed no interference at the concentrations tested with the **cobas**® HIV-1/HIV-2 Qualitative performance. Non-reactive results were obtained with **cobas**® HIV-1/HIV-2 Qualitative for all samples without HIV target and reactive results were obtained on all samples with HIV-1 and HIV-2 targets.

- Autoimmune diseases

Performance of the assay was tested in presence of the following autoimmune diseases: systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), and antinuclear antibody (ANA). All potentially interfering disease states showed no interference.

- Drug compounds

Potential interference of drug compounds listed in Table 7 with performance of the **cobas**® HIV-1/HIV-2 Qualitative were tested at the level of 3x $C_{max}$. All tested drug components showed no interference.

**Table 7. Drug Compounds Tested for Interference with cobas® HIV-1/HIV-2 Qualitative**

| Class of Drug | Generic Drug Name |
|---|---|
| Immune Modulators | Peginterferon α-2a; Ribavirin; Peginterferon α-2b |
| HCV Inhibitors | Simeprevir; Sofosbuvir |

| Class of Drug | Generic Drug Name |
|---|---|
| Reverse Transcriptase or DNA Polymerase Inhibitors | Aciclovir; Adefovir dipivoxil; Cidofovir; Emtricitabine; Entecavir; Foscarnet; Ganciclovir; Lamivudine; Telbivudine; Tenofovir; Valganciclovir |
| Compounds for Treatment of Opportunistic Infections | Azithromycin; Clarithromycin; Ethambutol; Fluconazole; Isoniazid; Pyrazinamide; Rifabutin; Rifampicin; Sulfamethoxazole; Trimethoprim |
| Statin | Atorvastatin |
| Selective Serotonin Reuptake Inhibitor | Fluoxetine; Paroxetine; Sertraline |
| Antihistamine | Loratadine |
| Beta-blocker | Nadolol |
| Decongestant | Phenylephrine HCl |
| Nonsteroidal Anti-inflammatory drug | Naproxen; Ibuprofen |
| Pain reliever | Acetaminophen; Acetylsalicylic Acid |
| Vitamins | Ascorbic Acid |

### 15.7 Cross Contamination/ Carryover

Cross contamination rate was determined by testing 240 replicates of HIV negative human plasma sample and 225 replicates of a HIV-1 positive sample at high titer 4.00E+06 cp/mL. Overall five runs were performed with positive and negative samples in a checkerboard configuration. No cross-contamination was detected.
HIV-1 RNA was not detected in any of the HIV-1 negative samples. The **cobas**® HIV-1/HIV-2 Qualitative was shown not to be affected by carryover contamination from previous runs or from the high positive samples analyzed in the same run.

### 15.8 Animal Studies

Not applicable.

### 15.9 Additional Studies

- PPT/EDTA Tube Equivalency

The sample collection tubes equivalency study was performed to demonstrate the equivalency between EDTA plasma tubes and plasma preparation tubes (PPTs). Specimens used in the study were pre-characterized, with 28 specimens having HIV-1 viral loads between 250 and 75,000 copies/mL, and 26 specimens with HIV-1 viral loads between 100 and less than 250 copies/mL. Tube equivalency was confirmed by the overall percent agreement (OPA) of 100% with a 95% Confidence Interval of 93.4% to 100%.

- Matrix Equivalency

Effect of (b) (4) anticoagulant on the assay performance was evaluated using (b) (4) EDTA-Plasma and (b) (4)-Plasma individual donors. The samples were spiked at approximately (b) (4) LoD with the HIV-1 Group M Roche secondary Standard ((b) (4) and with the HIV-2 Intermediate Stock Solution. Samples were spiked (b) (4) HIV-1 and HIV-2, each at the appropriate level. The non-spiked samples served as negative controls for each donor. A 100% agreement between EDTA and (b) (4) samples was observed for both reactive and non-reactive samples, confirming matrix equivalency.

### 15.10 Real-Time Reagent Stability

**Shelf-life stability:** The **cobas**® HIV-1/HIV-2 Qualitative and the **cobas**® HIV-1/HIV-2 Qualitative Control Kit and its components are assigned a shelf life of 24 months at 2-8°C.
The real-time and accelerated stability studies were performed to assess stability of the HIV-1/2 MMX-R2 component of the **cobas**® HIV-1/ HIV-2 Qualitative. The shelf life of HIV-1/2 MMX-R2 component of the **cobas**® HIV-1/ HIV-2 Qualitative was determined by testing three lots using a real time stability protocol with predefined acceptance criteria. The real-time reagent stability period was calculated from (b) (4) date of manufacturing (DOM) to the actual date on which the test was performed. The shelf life of each kit is assigned based on the component with the shortest stability data.

**On-board stability:** The **cobas**® HIV-1/2 Qualitative test-specific reagent cassettes are stable for up to (b) (4) days at 4°C (Open Kit stability) and remain stable for 8 hours at (b) (4) (on board stability).

## 16. SUMMARY OF CLINICAL STUDIES

To assess clinical performance of **cobas**® HIV-1/2 Qualitative when used as aid in diagnosis for detection of HIV-1 and HIV-2 RNA in human plasma and serum, the applicant performed following three clinical studies to establish a reasonable assurance of safety and effectiveness:

- Clinical Specificity
- Clinical Sensitivity
- Reproducibility

All clinical studies were performed at three US clinical sites.

### 16.1 CLINICAL SPECIFICITY

#### 16.1.1 HIV Low-Risk Population

The clinical specificity of **cobas**® HIV-1/HIV-2 Qualitative on the **cobas**® 6800/8800 Systems was determined by comparison to the CDC HIV Laboratory testing algorithm. Following the CDC guidelines, if a specimen was positive on the HIV

Ag/Ab test but indeterminate or negative on the FDA-approved HIV-1/HIV-2 differentiation assay, additional testing was done with an FDA-approved HIV-1 NAT.

The overall HIV-1 specificity and HIV-2 specificity of **cobas®** HIV-1/HIV-2 Qualitative test was 100%, with no difference between plasma and serum specimens (Table 8).

**Table 8. Specificity of cobas® HIV-1/HIV-2 Qualitative by Target Analyte and Sample Type in the HIV Low-Risk Population**

| Target Analyte, Sample Type | Total cobas®HIV-1/HIV-2 Qualitative Nonreactive Subjects | Total Status-Negative by CDC HIV Testing Algorithm | Specificity (95% CI) |
|---|---|---|---|
| HIV-1, Overall | 5902 | 5902 | 100% (99.94%; 100%) |
| HIV-1, Plasma | 3608 | 3608 | 100% (99.90%; 100%) |
| HIV-1, Serum | 2294 | 2294 | 100% (99.84%; 100%) |
| HIV-2, Overall | 5914 | 5914 | 100% (99.84%; 100%) |
| HIV-2, Plasma | 3618 | 3618 | 100% (99.84%; 100%) |
| HIV-2, Serum | 2293* | 2293 | 100% (99.84%; 100%) |

* Three additional serum specimens were not included because serology discordant resolution HIV-2 NAT testing was not done due to insufficient specimen volume.

### 16.1.2 HIV-1 High-Risk Population

### A. Comparison to FDA approved HIV-1 NAT

- HIV-1 Reactivity

Specimens used in this study were collected from subjects at high risk of HIV infection. Among 519 evaluable samples, 5 tested HIV-1 reactive and 514 tested non-reactive with **cobas®** HIV-1/HIV-2 Qualitative test as well as with FDA-approved HIV-1 Qual NAT as shown in (Table 9).

**Table 9. Performance of cobas® HIV-1/HIV-2 Qualitative in HIV-1 High-Risk Population**

| Target Analyte | Specimen Type | Total Number of Samples | Specificity Estimate (95% CI) |
|---|---|---|---|
| HIV-1 | Overall | 519 | 100% (99.3%, 100%) |
| | Plasma | 341 | 100% (98.9%, 100%) |
| | Serum | 178 | 100% (97.9%, 100%) |

- HIV-2 Reactivity

One plasma sample was HIV-2 reactive on the **cobas®** HIV-1/HIV-2 Qualitative test but HIV-2 RNA was not detected by the RUO HIV-2 Plasma RNA quantitative assay, and was confirmed HIV negative by the HIV-1/HIV-2 differentiation assay.

**B. Comparison to the CDC HIV Laboratory Testing Algorithm**

A total number of 585 samples from HIV-1 individuals at high-risk of HIV infection were tested with the **cobas®** HIV-1/HIV-2 Qualitative and with the CDC HIV testing algorithm. This algorithm was used for comparing the performance of **cobas®** HIV-1/HIV-2 Qualitative as currently this is the only CDC recommended method for HIV diagnosis.

Samples that tested reactive by the **cobas®** HIV-1/HIV-2 Qualitative and positive by CDC algorithm, or non-reactive by the **cobas®** HIV-1/HIV-2 Qualitative and negative by the CDC algorithm were not further tested. In case of discrepancy between the **cobas®** HIV-1/HIV-2 Qualitative results and the results of the CDC HIV testing algorithm, additional resolution testing was carried out by using Alt HIV-1 NAT and Alt HIV-2 NAT. All samples tested in this study were HIV-2 nonreactive by the **cobas®** HIV-1/HIV-2 Qualitative test and/or CDC HIV testing algorithm.

There were seven specimens (2 plasma and 5 serum) positive for HIV-1 with the **cobas®** HIV-1/HIV-2 Qualitative and the CDC algorithm and one sample tested positive by the CDC algorithm but negative by the **cobas®** HIV-1/HIV-2 Qualitative; this sample was found negative by FDA-approved HIV-1/HIV-2 differentiation assay and was further confirmed as target not detected following resolution testing with HIV-1 NAT. There were 577 specimens that tested non-reactive (or negative) by both assays. The specificity of **cobas®** HIV-1/HIV-2 Qualitative in this study was 99.8% (95% CI: 99.0%; 100%).

When stratified by the specimen type, 378 plasma specimens were negative by **cobas®** HIV-1/HIV-2 Qualitative resulting in specificity of 100% (95% CI: 99.0%; 100%) and 199 serum specimens (of 200 tested) were negative by **cobas®** HIV-1/HIV-2 Qualitative in HIV-1 Chanel resulting in a specificity of 99.5% (95%CI: 97.2%; 100%), as presented in Table 10.

**Table 10. The Specificity of the cobas® HIV-1/HIV-2 Qualitative and the CDC HIV Testing Algorithm, by Analyte and Type, in the HIV-1 High-Risk Population**

| Target Analyte | Specimen Type | Total Number of Samples | Specificity* |
|---|---|---|---|
| HIV-1 | Overall | 585 | 99.8% [577/578] / (99.0%; 100.0%) |
| | Plasma | 380[a] | 100% [378/378] / (99.0%; 100.0%) |

| | | | |
|---|---|---|---|
| | Serum | 205[b] | 99.5% [199/200] / (97.2%; 100.0%) |
| HIV-2 | Overall | 585 | 100% [585/585] / (99.4%; 100.0%) |
| | Plasma | 380 | 100% [380/380] / (99.0%; 100.0%) |
| | Serum | 205 | 100% [205/205] / (98.2; 100.0%) |

* Specificity: [samples tested by **cobas®** HIV-1/HIV-2 Qualitative / samples tested by CDC] / (95% Exact CI)

[a] Two plasma specimens were confirmed positive by both assays

[b] Five Serum specimens were confirmed positive by both assays

All samples were nonreactive for HIV-2 RNA by the **cobas®** HIV-1/HIV-2 Qualitative and CDC HIV testing algorithm Resulting is a specificity of 100% (95% CI: 99.4%; 100%) in HIV-2 Chanel (Table 10).

### 16.1.3 HIV-2 High-Risk Population

Samples were collected from individuals living in HIV-2 endemic areas. Overall, 499 evaluable samples from a HIV-2 high-risk population were tested for reactivity with the **cobas®** HIV-1/HIV-2 Qualitative and with the CDC HIV testing algorithm; discordant resolution testing was carried out by using Alt HIV-1 NAT and Alt HIV-2 NAT. There were 497 HIV-1 evaluable samples and 499 HIV-2 evaluable samples. The performance of **cobas®** HIV-1/HIV 2 Qualitative in the HIV-2 high-risk population is summarized in Table 11.

**Table 11. Summary of Agreement Between the cobas® HIV-1/HIV-2 Qualitative and the CDC HIV Testing Algorithm, by Analyte and Specimen Type, in the HIV-2 High-Risk Population**

| Target Analyte | Specimen Type | Total Number of Samples | PPA Estimate* | NPA Estimate** |
|---|---|---|---|---|
| HIV-1 | Overall | 497 | 79.0% (79/100) | 99.5% (395/397) (98.2%, 99.9%) |
| | Plasma | 330 | 79.7% (59/74) | 100% (256/256) (98.6%, 99.9%) |
| | Serum | 167 | 76.9% (20/26) | 98.6% (139/141) (95.0%, 99.8%) |
| HIV-2 | Overall | 499 | 44.4% (4/9) | 100% (490/490) (99.2%, 100.0%) |
| | Plasma | 332 | 66.7% (2/3) | 100% (329/329) (98.9%, 100.0%) |
| | Serum | 167 | 33.3% (2/6) | 100% (161/161) (97.7%, 100.0%) |

* PPA Estimate: [samples tested with **cobas®** HIV-1/HIV 2 Qualitative/samples tested by CDC algorithm]

** NPA Estimate: [samples tested with **cobas®** HIV-1/HIV 2 Qualitative/samples tested by CDC algorithm] / (95% Exact CI)

## 16.2 CLINICAL SENSITIVITY

The performance of the **cobas®** HIV-1/HIV-2 Qualitative on the **cobas®** 6800/8800 Systems (two **cobas®** 6800 Systems and one **cobas®** 8800 System) was compared to an FDA approved HIV-1 NAT diagnostic assay and RUO HIV-2 Assay.

### 16.2.1 HIV-1 Known Positive Population

- HIV-1 Reactivity

1030 subjects known to be infected with HIV-1 of a viral load > 100 copies/mL were tested in this study. All samples were reactive by the **cobas®** HIV-1/HIV-2 Qualitative and one sample was negative by an FDA-approved HIV-1 Qual NAT; this sample was confirmed positive with a viral load 152 cp/mL, by the HIV-1 Quantitative assay applied for resolution of discordance.

The sensitivity of the **cobas®** HIV-1/HIV-2 Qualitative, stratified by the HIV-1 subtype and by the sample matrix, is presented in Table 12.

**Table 12. Sensitivity of cobas® HIV-1/HIV-2 Qualitative for the HIV-1 Known Positive Population**

| Population/Subtype | Specimen Type | Number Reactive by cobas® HIV-1/HIV 2 Qualitative/ Total Tested | Sensitivity (95% CI) |
|---|---|---|---|
| HIV-1 Overall | Overall | 1030/1030 | 100% (99.6%, 100.0%) |
| | Plasma | 712/712 | 100% (99.5%, 100.0%) |
| | Serum | 318/318 | 100% (98.8%, 100.0%) |
| HIV-1 B subtype | Overall | 736/736 | 100% (99.5%, 100%) |
| | Plasma | 502/502 | 100% (99.3%, 100%) |
| | Serum | 234/234 | 100% (98.4%, 100%) |
| HIV-1 Non-B subtype | Overall | 294/294 | 100% (98.7%, 100%) |
| | Plasma | 210/210 | 100% (98.3%, 100%) |
| | Serum | 84/84 | 100% (95.7%, 100%) |

- HIV-2 Reactivity

Two of 1030 samples were found reactive for HIV-1 and HIV-2. One plasma sample tested HIV-1 positive by an FDA-approved HIV-1/HIV-2 differentiation assay but was not tested for HIV-2 due to insufficient volume. The second plasma sample tested HIV positive-untypable by the HIV-1/HIV-2 differentiation assay but was not detected by HIV-2 quantitative assay.

### 16.2.2 HIV-2 Known Positive Population

- HIV-2 Reactivity

Overall 183 HIV-2 known positive samples with HIV-2 viral loads ≥ 100 copies/mL by (ROU or LDT) HIV-2 Plasma RNA Quantitative Assay were tested. Samples were tested with the **cobas**® HIV-1/HIV-2 Qualitative followed by comparator testing with the HIV-2 LDT quantitative protocol developed by the site.

Among samples tested, 182 were reactive by the **cobas**® HIV-1/HIV 2 Qualitative and the HIV-2 RNA quantitative assay (Table 13). One sample tested non-reactive by **cobas**® HIV-1/HIV 2 Qualitative and was confirmed reactive by the HIV-2 plasma RNA quantitative assay, with a viral load of 198 cp/mL.

**Table 13. Performance of cobas® HIV-1/HIV-2 Qualitative in HIV-2 Known Positive Population**

| Population | Specimen Type | Number Reactive by cobas® HIV-1/HIV 2 Qualitative/Total Tested | Sensitivity (95% CI) |
|---|---|---|---|
| HIV-2 | Overall | 182/183 | 99.5% (97.0%, 99.99%) |
| | Plasma | 115/115 | 100% (96.8%, 100%) |
| | Serum | 67/68 | 98.5% (92.1%, 99.96%) |

- HIV-1 reactivity

Two specimens were reactive for HIV-1 and HIV-2 by the **cobas**® HIV-1/HIV-2 Qualitative and confirmed reactive by Alt HIV-1 NAT and Alt HIV-2 NAT.

### 16.3 Pregnant Women

The samples from pregnant women were collected in the first (9.0%), second (43.3%) and third (47.1%) trimester of pregnancy. The samples consisted of 344 specimens that included 35 contrived samples. The following groups were tested:

- HIV-1 Known Positive: 25 commercially available natural samples and 35 samples prepared from HIV-negative plasma collected from HIV-negative pregnant women and spiked with HIV-1 tissue culture supernatant at 3 concentrations ranging from 37.8 copies/mL to 10,000 copies/mL

- HIV-1 High-risk: 236 samples
- HIV Low-risk: 48 samples.

Samples were tested with the **cobas®** HIV-1/HIV-2 Qualitative and the results were compared to the CDC HIV testing algorithm results. Table 14 summarizes PPA and NPA of **cobas®** HIV-1/HIV-2 Qualitative in pregnant women populations. For the high-risk pregnant population, there was no discordant resolution performed with a second NAT test. Causes of the observed rate of discordance may include sample integrity, nucleic acid test negative/ positive serology samples, treatment to prevent infection.

**Table 14. Performance of cobas® HIV-1/HIV-2 Qualitative in the Pregnant Women**

| Target Analyte | Populations of Pregnant Women | Total Number of Specimens | PPA* (95% CI) | NPA** (95% CI) |
|---|---|---|---|---|
| HIV-1 | HIV-1 Known Positive | 60 | 100% [60/60] (94.0%, 100%) | NA |
| | HIV-1 High-risk | 236 | 48.8% [20/41] | 100% [195/195] (98.1%, 100%) |
| | HIV Low-risk | 48 | NA | 100% [48/48] (92.6%, 100%) |
| HIV-2 | HIV-1 High-risk | 236 | NA | 100% [236/236] (98.4%, 100%) |
| | HIV Low-risk | 47[a] | NA | 100% [47/47] (92.5%, 100%) |

* PPA: [Reactive with **cobas®** HIV-1/HIV-2 Qualitative/Positive with CDC testing algorithm] / (95% CI)
** NPA: [Non-reactive **cobas®** HIV-1/HIV-2 Qualitative/Negative with CDC testing algorithm]
NA: Not Applicable

[a] One sample was not included due to insufficient sample volume for HIV-2 NAT testing

### 16.4 Pediatric Population

In this study 328 samples were tested: 302 samples were collected from pediatric population (ages 1 to 21 years) and 26 were contrived HIV-1 positive samples. Table 15 summarizes performance of **cobas®** HIV-1/HIV-2 Qualitative test as compared to the reference methods for the pediatric populations tested in the study.

**Table 15. Performance of cobas® HIV-1/HIV-2 Qualitative in the Pediatric Population**

| Target Analyte | Populations of Pediatric Subjects | Total Number of Specimens | PPA* / (95% CI) | NPA** / (95% CI) |
|---|---|---|---|---|
| HIV-1 | HIV-1 Known Positive | 76 | 100% [76/76] / (95.3%, 100%) | NA |
| | HIV-1 High-risk | 199 | 100% [2/2] | 100% [197/197] / (98.1%, 100%) |
| | HIV Low-risk | 52 | NA | 100% [52/52] (93.2%, 100%) |
| HIV-2 | HIV-1 High-risk | 200 | NA | 100% [200/200] / (98.2%, 100%) |
| | HIV Low-risk | 52 | NA | 100% [52/52] / (93.2%, 100%) |

* PPA: [Reactive with **cobas**® HIV-1/HIV-2 Qualitative / Positive by CofA or with CDC testing algorithm]
** NPA: [Non-reactive **cobas**® HIV-1/HIV-2 Qualitative / Negative by CofA or with CDC testing algorithm]
NA: Not Applicable

**16.5 Supplemental Claim**

To support the claim for the **cobas**® HIV-1/HIV-2 as a test to confirm HIV infection (supplemental test), serologically discordant specimens were identified from a blood donor screening clinical study and sourced from commercial vendors. Some samples from a single donor were diluted up to 1:4 with HIV-1 negative plasma to obtain the required volume. In order to use clinical samples from blood donors, equivalency between EDTA and (b) (4) plasma was demonstrated. The following populations were represented:

- HIV Ag/Ab test repeatedly reactive and HIV-1/HIV-2 differentiation assay negative specimens
- HIV Ag/Ab test repeatedly reactive and HIV-1/HIV-2 differentiation assay indeterminate specimens.

For HIV-1, **cobas**® HIV-1/HIV-2 Qualitative results were compared to an FDA approved HIV-1 Qualitative NAT assay. For HIV-2, the **cobas**® HIV-1/HIV-2 Qualitative results were compared to the RUO HIV-2 Plasma RNA quantitative assay results.

Among 61 HIV Ab/Ag repeatedly reactive/ HIV-1/HIV-2 differentiation assay negative samples, 56 were negative for HIV-1 RNA by both the **cobas**® HIV-1/HIV-2 Qualitative test and by an FDA approved HIV-1 Qualitative NAT assay. Four samples tested positive with both assays. One sample tested HIV-1 non-reactive on the **cobas**® HIV-1/HIV-2 Qualitative test and HIV-1 positive with the HIV-1 Qualitative NAT. This sample was confirmed negative (< 30 copies/mL) with an FDA-approved HIV-1 Quantitative NAT. None of the tested samples were reactive for HIV-2 RNA by **cobas**®HIV-1/HIV-2 Qualitative or by RUO HIV-2 plasma RNA quantitative assay.

Among 57 HIV Ab/Ag repeatedly reactive and indeterminate on HIV-1/HIV-2 differentiation assay, 50 were negative for HIV-1 RNA by both assays. Among 7 HIV-1 reactive samples by **cobas®** HIV-1/HIV-2 Qualitative, one sample was found negative by comparator assays and confirmed negative (viral load <30cp/mL) by the FDA-approved HIV-1 Quantitative NAT. None of the samples were reactive for HIV-2 RNA by **cobas®** HIV-1/HIV-2 Qualitative or RUO HIV-2 plasma RNA quantitative assay.

### 16.6 Reproducibility

Reproducibility of the **cobas®** HIV-1/HIV-2 Qualitative was evaluated across reagent lots, test site/instrument systems, operators, days, batches, and within batch. Sample panels consisting of one negative and six positive members were tested at three sites using three reagent lots, two **cobas®** 6800 Systems and one **cobas®** 8800 System, and two operators over 6 days; three replicates of each panel member were performed for each batch. The negative percent agreement was estimated as 100%, with a corresponding 95% exact confidence interval CI of (98.9%, 100.0%) and the positive percent agreement was 100% for each panel member, for both HIV-1 and HIV-2.

For HIV-1 positive panel members, the coefficient of variation (CV(%)) for all panel members was ≤ 1.9%, demonstrating low variability of **cobas®** HIV-1/HIV-2 Qualitative results across reagent lots, sites/instruments, days, operators, and batches (Tables 16 and 17).

**Table 16. Attributable Percentage of Total Variance, Total Precision Standard Deviation (SD) and CV(%) of Cycle Threshold Values from HIV-1 Reactive Results**

| | | Percentage of Total Variance CV% (SD) | | | | | | Total Precision CV% (SD) |
|---|---|---|---|---|---|---|---|---|
| **Panel Member** | **N** | **Lot** | **Site** | **Operator** | **Day** | **Batch** | **Within-Batch** | |
| ~3xLOD (3.78E1) HIV-1, Negative HIV-2 | 324 | 0.0% (0.0) | 0.1% (0.1) | 0.0% (0.0) | 8.7% (0.5) | 0.0% (0.0) | 91.2% (1.8) | 1.9% (0.69) |
| >3xLOD (1.00E5) HIV-1, Negative HIV-2 | 322 | 0.0% (0.0) | 15.5% (0.4) | 0.0% (0.0) | 33.1% (0.5) | 4.5% (0.2) | 46.9% (0.6) | 0.9% (0.24) |
| >3xLOD (1.00E5) HIV-1, ~3xLOD (8.37E1) HIV-2 | 323 | 0.0% (0.0) | 7.8% (0.3) | 0.0% (0.0) | 45.0% (0.6) | 10.9% (0.3) | 36.3% (0.6) | 1.0% (0.25) |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| ~3xLOD (3.78E1) HIV-1, >3xLOD (1.00E5) HIV-2 | 323 | 1.5% (0.2) | 0.0% (0.0) | 0.0% (0.0) | 0.0% (0.0) | 0.0% (0.0) | 98.5% (1.8) | 1.8% (0.67) |

**Table 17. Attributable Percentage of Total Variance, Total Precision Standard Deviation (SD) and CV(%) of Cycle Threshold Values from HIV-2 Reactive Results**

| | | Percentage of Total Variance CV% (SD) | | | | | | Total Precision CV% (SD) |
|---|---|---|---|---|---|---|---|---|
| **Panel Member** | **N** | **Lot** | **Site** | **Operator** | **Day** | **Batch** | **Within-Batch** | |
| Negative HIV-1, ~3xLOD (8.37E1) HIV-2 | 324 | 60.0% (1.3) | 3.3% (0.3) | 0.0% (0.0) | 14.6% (0.7) | 4.5% (0.4) | 17.6% (0.7) | 0.59% (1.7) |
| Negative HIV-1, >3xLOD (1.00E5) HIV-2 | 324 | 31.9% (0.9) | 10.0% (0.5) | 0.0% (0.0) | 32.9% (1.0) | 0.0% (0.0) | 25.1% (0.8) | 0.42% (1.7) |
| >3xLOD (1.00E5) HIV-1, ~3xLOD (8.37E1) HIV-2 | 323 | 26.0% (0.7) | 4.0% (0.3) | 0.0% (0.0) | 9.9% (0.4) | 4.2% (0.3) | 55.9% (1.0) | 0.46% (1.3) |
| ~3xLOD (3.78E1) HIV-1, >3xLOD (1.00E5) HIV-2 | 323 | 38.2% (0.9) | 8.2% (0.4) | 0.0% (0.0) | 24.7% (0.8) | 0.0% (0.0) | 28.9% (0.8) | 0.39% (1.5) |

## 17. INSPECTIONS

### 17.1 Manufacturing Facilities Review/Inspection

Facility information and data provided in the PMA were reviewed by CBER and found to be sufficient and acceptable.

The following information provides justification to support the waiver recommendations:

**Table 18: Manufacturing Facilities cobas® HIV-1/HIV-2 Qualitative**:

| Location | Activity | Most Recent Inspection |
|---|---|---|
| Roche Molecular Systems, Inc. 1080 US Highway 202 South Branchburg, NJ 08876 (FEI 2243471) | Component Manufacture (filling, capping, labeling of kit components) and Final Kit Manufacture | July 2019 Surveillance Team Biologics NAI |

Based on the Team Biologics recent inspection, DMPQ recommends an inspection waiver for this PMA.

### 17.2 Bioresearch Monitoring (BIMO) Inspections

A bioresearch monitoring (BIMO) inspection was issued for one U.S. clinical investigator site that participated in the conduct of Protocol cX8-HIV-319 and Protocol cx8-HIV-320. The inspection did not reveal substantive issues that impact the data submitted in this premarket approval (PMA).

## 18. CONCLUSIONS DRAWN FROM THE STUDIES

### 18.1 Effectiveness Conclusions

Multi-center clinical studies were conducted in the U.S. The **cobas®** HIV-1/HIV-2 Qualitative performed with clinical sensitivity and specificity comparable to an FDA approved HIV-1 NAT

Results from the clinical studies indicate that the **cobas®** HIV-1/HIV-2 Qualitative, together with supplemental testing, can be used effectively for the simultaneous qualitative detection and differentiation of HIV-1 and HIV-2 RNA in human serum and plasma.

### 18.2 Safety Conclusions

The risk of the device is based on data collected in the clinical study conducted to support PMA approval as described above. Based on the results of the clinical studies, the **cobas**® HIV-1/HIV-2 Qualitative, when used according to the labeling and in conjunction with other clinical information, is safe to use and poses minimal risk to the patient due to false test results.

Reactive specimens must be investigated by additional, more sensitive NAT, or supplemental tests. Confirmation of the test result on a freshly drawn specimen and counseling are considered an important part of testing for HIV. A negative test result at any point in the investigation of individual subjects does not preclude the possibility of exposure to or infection with HIV-1 and/or HIV-2. Negative results can occur if the quantity of marker present in the specimen is below the detection limit of the assay, or if the marker is not present during the stage of disease in which a specimen is collected.

## 19. BENEFIT-RISK DETERMINATION

As a diagnostic test the **cobas**® HIV-1/HIV-2 Qualitative involves removal of blood from an individual for testing purposes. This test presents no more of a safety hazard to an individual than is presented to an individual who is having their blood drawn for any other diagnostic evaluation. The benefit to HIV-1 or HIV-2 infected individuals tested by this assay outweighs any potential adverse event or risk to the patient or user due to assay malfunction or operator error. The potential risks encountered with this in vitro diagnostic test are not unusual in the clinical laboratory setting. Appropriate warnings for these risks are contained in the labeling and package inserts for these devices. Standard good laboratory practices are considered sufficient to mitigate the risks to the end user. Potential adverse effects of the **cobas**® HIV-1/HIV-2 Qualitative relate to the risk of false positive and false negative results. While performance studies indicate that this risk is likely to be very small, the potential for inaccurate results exists. The risk of incorrect results is minimized by following the procedures and instructions outlined in the Package Insert.

## 20. OVERALL CONCLUSIONS

The data in this application support the reasonable assurance of safety and effectiveness of this device when used in accordance with the indications for use. The data from the nonclinical studies demonstrated acceptable analytical sensitivity, precision, and analytical specificity of the **cobas**® HIV-1/HIV-2 Qualitative when used according to the instructions for use as stated in the labeling, the warnings and precautions, and limitations sections of the labeling. The clinical studies and the statistical analysis of clinical data in this application has shown that the **cobas**® HIV-1/HIV-2 Qualitative detecting and differentiating HIV-1 and HIV-2 RNA in human serum and plasma and that the assay is safe and effective when used according to the directions for use in the labeling

## 21. APPROVAL SPECIFICATIONS

- Directions for use: See device labeling.
- Hazards to Health from Use of the Device: See Indications, Contraindications, Warnings, Precautions, and Adverse Events in the device labeling.
- Post-approval Requirements and Restrictions: None

## 22. PANEL RECOMMENDATIONS

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

## 23. FDA/CBER DECISION

PMA BP190360 is recommended for approval.