# Summary Basis for Regulatory Action

**Date: October 4, 2017**

**From: Caren Chancey**, Chair of the Review Committee <ESIG>

**BLA/ STN#: 125653/0**

**Applicant Name: Roche Molecular Systems Inc.**

**Date of Submission: April 7, 2017**

**MDUFA Goal Date: October 7, 2017**

**Proprietary Name: cobas®** Zika, Nucleic acid test for use on the **cobas®** 6800/8800 systems

**Established Name (common or usual name): cobas®** Zika

**Intended Use/Indications for Use:**

The **cobas**® Zika test for use on the **cobas**® 6800 and **cobas**® 8800 Systems is a qualitative *in vitro* nucleic acid screening test for the direct detection of Zika virus RNA in human plasma.

This test is intended for use to screen donor samples for Zika virus RNA in plasma samples from individual human donors, including donors of whole blood and blood components, and other living donors. This test is also intended for use to screen organ and tissue donors when donor samples are obtained while the donor's heart is still beating. Plasma from all donors should be screened as individual samples.

The test is not intended for use as an aid in diagnosis of Zika virus infection.

This test is not intended for use on samples of other body fluids.

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

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

**Review Office Signatory Authority:** Jay S. Epstein, M.D., Director, OBRR/CBER

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

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

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

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

Table 1. Reviews submitted

| **Document Title** | **Reviewer Name** | **Document Date** |
|---|---|---|
| Product Review(s) (Product Office)<br>• *Clinical*<br>• *Non-Clinical* | <br>Julia Lathrop, Ph.D.<br>Krishna Mohan V. Ketha, Ph.D. | <br>Sep. 21, 2017<br>Sep. 19, 2017 |
| Statistical Review(s)<br>• *Clinical* | <br>Tie-Hua Ng, Ph.D. | <br>Sep. 15, 2017 |
| CMC Review<br>• *CMC (Product Office)*<br>• *Facilities Review (OCBQ/DMPQ)*<br>• *Establishment Inspection Waiver (OCBQ/DMPQ)*<br>• *Bioburden (OCBQ/DBSQC)* | <br>Evgeniya Volkova, M.S., M.B.A<br>Maria Rios, Ph.D.<br>CDR Cecily Jones<br>CDR Cecily Jones<br>Hyesuk Kong, Ph.D. | <br>Sep. 20, 2017<br>Sep. 20, 2017<br>Sep. 28, 2017<br>Sep. 28, 2017<br>Aug. 24, 2017 |
| Labeling Review(s)<br>• *APLB (OCBQ/APLB)* | <br>Dana Jones | <br>Aug. 22, 2017 |
| Lot Release Protocols/ Testing Plans | Kori Francis<br>Marie Anderson<br>Swati Verma<br>Susan Zullo, Ph.D. | Sep. 11, 2017<br>Sep. 5, 2017<br>Sep. 18, 2017<br>Oct. 3, 2017 |
| Bioresearch Monitoring Review | Haecin Chun | Sep. 14, 2017 |
| Software and Instrumentation | Sajjad Syed, Ph.D. | Sep. 18, 2017 |
| HCT/Ps and Organ Donors Review | Michelle McClure, Ph.D. | Sep. 25, 2017 |

# 1. Introduction

Roche Molecular Systems Inc., located in Pleasanton, CA, submitted an original Biologic License Application (BLA) for licensure of the cobas® Zika nucleic acid test for use on the cobas® 6800/8800 Systems. This BLA was for the first blood screening assay for the Zika virus. The FDA granted the request by Roche Molecular Systems for a six-month priority review of the application, on the basis of an unmet public health need created by the Zika virus epidemic in the Americas and especially in the U.S. territory of Puerto Rico.

The application was submitted on April 7, 2017 and filed on June 6, 2017. The mid-cycle meeting was held on July 6, 2017. Information requests were sent to the sponsor on May 2, May 15, July 25, August 18, September 25 and September 26, 2017,

and teleconferences were held on July 13, August 9, August 28 and September 12, 2017. Fourteen amendments were received from the sponsor in support of the application.

## 2. Background

Zika virus (Zika) is an enveloped, icosahedral, single-stranded ribonucleic acid (RNA) arbovirus of the family Flaviviridae, genus *Flavivirus*. Zika is transmitted by *Aedes* mosquitoes, and was known to have circulated in Africa and Asia between 1954 and 2007. In 2013-14, Zika caused a large outbreak in French Polynesia and first appeared in South America in 2014. In addition to vector-borne transmission by mosquito, routes for Zika transmission include from an infected woman to her fetus in utero and at birth, sexual transmission, laboratory exposure and blood transfusion. Most Zika infections are asymptomatic, and symptomatic infections usually present with non-specific influenza-like signs and symptoms which are difficult to distinguish clinically from other arboviruses such as dengue, chikungunya and West Nile viruses. However, Zika infection can also cause severe outcomes such as congenital microcephaly and other birth defects in fetuses of Zika-infected mothers, and Guillain-Barré syndrome.

In February 2016, the U.S. FDA issued recommendations to reduce the risk of transfusion-transmitted Zika, which included cessation of blood collections in areas of active Zika transmission unless donations were screened with either a licensed or investigational Zika nucleic acid test (NAT) or were subjected to FDA approved pathogen reduction technology (PRT). This resulted in cessation of blood collection in Puerto Rico because no FDA-approved NAT was available and PRT was available only for plasma and platelet products. In the guidance of August 26, 2016, the FDA expanded the requirement for screening of blood donations with a NAT or using PRT to all blood donations collected in all 50 U.S. states, which was implemented by mid-December, 2016. The cobas® Zika was developed as a response to the need for an FDA-approved NAT-based blood donor screening assay.

The cobas® Zika was designed for use on the cobas® 6800 and cobas® 8800 Systems. These systems integrated fully automated total nucleic acid isolation directly from primary and secondary sample tubes, automated PCR setup, and real time PCR. The systems consisted of a sample supply module for loading and unloading samples, a transfer module for sample identification and transfer of samples and controls to processing plates, a processing module for sample preparation and PCR setup and an analytic module for amplification and detection. Each system was connected to an instrument gateway for data management, scheduling, and workflow control. The instrument gateway was the sole interface to the laboratory information system.

Two other blood screening assays have previously been approved for use with the cobas® 6800/8800 systems, the cobas® WNV (BL125575, approved November 2, 2016) and the cobas® MPX assay (BL125576, approved October 20, 2016). The principles of the assay procedure are similar to the other approved screening assays on the cobas® 6800/8800 systems. RNA from the sample and added internal control are extracted from lysed plasma samples using magnetic glass particles, followed by washing, elution and RT-PCR, using specific probes and primers to discriminate target and controls. Accordingly, many components of the cobas® Zika are common to the cobas® WNV and cobas® MPX assay (omni Reagents and Common Components), with their manufacture,

composition and performance having been reviewed in detail as part of those applications and provided again in this submission for completeness. Review of the cobas® 6800/8800 systems was focused primarily on the assay-specific analysis package (ASAP) and updates made to the system software since approval of the other two screening assays.

Pre-submission discussions were conducted with FDA under BQ160101, with internal meetings held on October 27, 2016 (BQ160101/0) and February 15, 2017 (BQ160101/1), and communications with the sponsor on August 22, 2016 (telecon prior to pre-BLA submission), November 17, 2016 (BQ160101/0 response) and April 3, 2017 BQ160101/1 response). A summary of communications with FDA associated with this pre-submission was provided with the application. Pre-submission discussions are summarized in Table 2 below.

Table 2. Discussion and recommendations from pre-submission BQ160101

| **Meeting and Response Date** | **Topic** | **Sponsor Proposal** | **FDA recommendation** |
|---|---|---|---|
| **BQ160101/0 (11/17/2016)** | Kit size and configuration | 480 test kit, analogous to other cobas testing kits | Acceptable |
| **BQ160101/0 (11/17/2016)** | Formulation of positive and negative controls | Positive control (PC) of (b) (4) LoD armored RNA, buffer negative control (NC) | Reduce PC concentration and use normal human plasma as NC |
| **BQ160101/0 (11/17/2016)** | Software version intended for use at product launch | ASAP version for launch will be most current at time of BLA submission | Provide risk justification if ASAP version higher than that used for clinical testing |
| **BQ160101/0 (11/17/2016)** | Reagent stability testing | Use prior studies for omni common reagent stability; use MPX and WNV data for Zika-specific reagents; use data from accelerated stability and comparable reagents to support shelf life claim | Use of prior study data for omni reagents acceptable; Zika-specific reagents should be tested in real-time and claim may be updated through annual reporting |
| **BQ160101/0 (11/17/2016)** | Supplemental non-clinical testing | Include IND #16926 studies plus exogenous interference, specimen stability, supplemental cross-reactivity with Japanese encephalitis virus complex, SW version 1.1.09 or higher | Acceptable provided that impact analysis provided for higher software versions; include yellow fever virus in cross-reactivity study per agreement during IND #16926 |

| | | | |
|---|---|---|---|
| | | to be used | discussion |
| **BQ160101/0 (11/17/2016)** | FDA-CBER Zika panel | Requests that panel be provided as available | Panel under development and would be provided when complete |
| **BQ160101/0 (11/17/2016)** | Clinical Reproducibility study design | Testing across 3 lots x 3 sites x 5 days x 2 batches x 2 replicates, using "pilot lots" | Recommended use of 3 replicates, "pilot lots" acceptable |
| **BQ160101/0 (11/17/2016)** | Clinical Specificity Study dataset/lock date | Use ~150,000 samples from continental US under IND #16926 from May 22- August 27, 2016 | Acceptable |
| **BQ160101/0 (11/17/2016)** | Clinical Sensitivity study | No Clinical Sensitivity study planned | Data from ongoing IND may be evaluated to determine clinical sensitivity, RMS to provide statistical analysis plan and acceptance criteria |
| **BQ160101/1 (4/3/2017)** | Response to FDA re: PC design | Justification for use of (b) (4) LoD PC | Acceptable |
| **BQ160101/1 (4/3/2017)** | Clinical Sensitivity Study design | Test reactive, alt NAT+ samples from specificity study as known positives, with two repeat tests using the sensitivity from the repeat with lowest result | Acceptable |

## 3. Chemistry Manufacturing and Controls (CMC)

### a) Manufacturing Summary

The cobas® Zika consists of the cobas® Zika Kit, cobas® Zika Control Kit, omni reagents, and common reagents. The test was developed to be available in a 480-test kit format only.

The assay-specific components of the cobas® are the Zika primers and probes, contained in the cobas® Zika Kit's Master Mix Reagent 2 (MMX-R2), and the Zika Positive Control (PC) that constitutes the cobas® Zika Control Kit. The RNA Internal Control is shared with the licensed cobas® MPX (BL125576) and cobas® WNV (BL125575) tests, and the rest of the reagents were common to most cobas 6800/8800 tests. Manufacturing of all components takes place at the sponsor's (b) (4) facility. Since the RNA Internal Control, omni reagents and common reagents were the

same as those used with the approved cobas® WNV and cobas® MPX kits, supporting documentation from those prior submissions was used to describe CMC for these components. The review for the cobas® Zika focused primarily on the assay-specific kit components.

Table 3. Description of assay-specific and common components of the cobas® Zika

| Kit Name | Kit Component | Type of component |
|---|---|---|
| cobas® Zika (480T), 7972466190 | Master Mix-R2, 7972555001 | Assay Specific |
| | RNA Internal Control, 433090001 | Used for cobas® Zika, MPX, and WNV |
| | Protease, 6433081001 | common Component |
| | Elution Buffer, 6433073001 | common Component |
| | Master Mix-R1, 6433103001 | common Component |
| cobas® Zika Control Kit, 8129690190 | Zika Control, 8129738001 | Assay Specific |
| cobas® 6800/8800 NHP Negative Control, 7002220190 | LBLD COBAS 6800/8800 NHP NEG CTL 1.0 mL, 5831334001 | Used for cobas® Zika, MPX, and WNV |
| | cobas omni Wash Reagent, 6997503190 | omni Reagent |
| | cobas omni Specimen Diluent, 6997511190 | omni Reagent |
| | cobas omni Lysis Buffer, 6997538190 | omni Reagent |
| | cobas omni Magnetic Glass Particles (MGP) Reagent, 6997546190 | omni Reagent |

The formulation and manufacturing of all individual kit components, as well as packaging of the kits, was described in the In Vitro Product Report. The assay-specific components of the MMX-R2 reagent were the Zika-specific primers (b) (4) and (b) (4), and the Zika-specific probe (b) (4). The purified Zika-specific primers and probes were combined with bulk cobas® 6800/8800 generic MMX buffer, dNTPs, purified IC primers and probes, aptamer, Enz Z05-D, Enz UNG and PCR grade water. The Zika PC was formulated first as an intermediate stock mixing the armored RNA PC with bulk normal human plasma (NHP) by (b) (4)

(b) (4) prior to vial filling. The cobas® Zika Control kit consisted of 16 vials of the manufactured PC.

The to-be-marketed formulation of the cobas® Zika Control Kit and the cobas® NHP Negative Control Kit was modified, and the revised formulation was used in two non-clinical studies and in the clinical reproducibility study. The changes included replacing the (b) (4) as the positive control with an armored RNA, (b) (4) the PC concentration from (b) (4) LoD to (b) (4) LoD, formulating the controls in negative human plasma matrix and aligning the formulation with the licensed cobas tests. These changes were demonstrated to have no effect on assay performance. The In Vitro Product Report for the omni reagents and common components described the formulation and manufacturing of the omni Reagents and common components used with all tests run on the cobas 6800/8800 Systems. There were no differences in formulation between the investigational product and the to-be-marketed product. The product was not sterile, and the sponsor validated (b) (4) for omni reagents and common components by presenting a study done for the cobas® MPX test components.

The In Vitro Substance Report contained the Initial Performance Report as well as separate reports on synthesis/purification (where applicable), chemical formula/structure (where applicable), characterization, and stability of the following test components:

a. Aptamer
b. Uracil-N-Glycosylase (UNG) and Z05D DNA Polymerase
c. Generic Master Mix Buffer
d. MMR2 Internal Control (IC) Primers and Probe
e. RNA Internal Control Stock
f. Zika Positive Control Stock
g. Zika Primers and Probe

The Initial Performance Report described the design process for primers and probes used in the kit. Using Roche proprietary software, sets of primers and probes located in the most conservative regions of the ZIKV genome were chosen and assessed for exclusivity and inclusivity. The top (b) (4) assay candidates were picked for evaluation in lab screening, one of which was curtailed due to manufacturing issues. (b) (4) an armored RNA-(b) (4) were created to serve as positive controls. Preliminary testing revealed poor performance of one of the candidates, and further analysis and characterization demonstrated that the (b) (4) Zika assay was suitable for development as a cobas® Zika test.

The In Vitro Substance Report section for the Omni Reagents and Common Components only consisted of reports on MGP Reagent and Protease, including information on chemical formulas/structures and characterization of the materials.

Test specifications and validation records for the common components such as aptamer, RNA IC, IC primers and probes, MMX buffer, bulk and stock enzymes UNG and Z05 D were provided in the original submission based on documentation prepared for the previously licensed cobas® MPX and cobas® WNV assays. Test specifications and validation records for the Zika-specific components and the assembled cobas® Zika 480t kit were provided as a series of amendments on July 11, August 4 and August 14, 2017. The assembled cobas® Zika 480T test kit underwent visual inspection of the assembled

5 reagent vessels and their order and readability, testing of the functionality of the RFID label, and functional testing of the contained reagents. The Zika PC underwent visual inspection of the assembled reagent vessels and their readability, testing of the functionality of the RFID label, and functional testing (performed at the vial level). Additional sections described the raw materials and bulk and fill container and closure systems and contained manufacturing flowcharts, batch production records and SOPs.

The Stability Report for the Zika, omni, and common components described stability studies that have been performed or were still ongoing on the test components to confirm the initial shelf life claim and support future shelf life. Studies performed on any lots other than Manufacturing Lots had no differences in batch records, fill volume, type/volume of container, closure type and intended manufacturing environment. The kit components were stored at the recommended storage conditions and evaluated visually and in functional tests at predetermined time points using predefined acceptance criteria. The stated shelf life claims were as follows:

Table 4. Shelf Life of cobas® Zika assay components

| Kit | Material Number | Claimed Shelf Life |
|---|---|---|
| cobas® Zika (480T) | 7972466190 | 8 months |
| cobas® Zika Control Kit | 8129690190 | 3 months |
| cobas® NHP Negative Control Kit | 7002220190 | 24 months |
| cobas omni MGP Reagent | 6997546190 | 24 months |
| cobas omni Lysis Reagent | 6997538190 | 24 months |
| cobas omni Wash Reagent | 6997503190 | 24 months |
| cobas omni Specimen Diluent | 6997511190 | 24 months |

The sponsor is using stability data produced with other cobas® tests (MPX, WNV, HEV, MBC, HIV-1 O, HIV-2) to support shelf life claims for the omni reagents and the Negative Control Kit.

Bioburden qualification tests (bacteriostatic and fungistatic qualification) were performed on one of the bulk Zika MMX-R2 (material number: 7972440990), one of the bulk Zika positive control (material number: 8129681990), and the vialed component samples listed in the table below for (b) (4) to demonstrate that the cobas Zika test matrix does not inhibit bacterial and fungal growth (Table 5).

Table 5. cobas® Zika Test Kit Matrixes for Bioburden Qualification

| Kit component | Batch | Bulk Material |
|---|---|---|
| Proteinase Solution (PASE) | (b) (4) | |
| Elution Buffer (EB) | | |
| Master Mix Reagent 1 (MMX-R1) | | |
| Zika Master Mix Reagent 2 (MMX-R2) | | |
| Internal Control | | |
| Zika Positive Control | | |
| Normal Human Plasma Negative Control (NHP-NC) | | |
| cobas omni MGP Reagent | | |
| Cobas omni Specimen Diluent | | |

The test methods for (b) (4) were reviewed and were found to be compliant with (b) (4), and the test results indicate there is no product inhibition on microorganism growth, indicating their matrixes are suitable for the intended test method.

(b) (4) were performed on three lots of Zika master mix reagent ((b) (4)) and three lots of Zika positive control ((b) (4)) including cobas omni reagents in the cobas® Zika to demonstrate effectiveness of sodium azide is adequate in preventing microbial growth using (b) (4) indicator microorganisms (b) (4)]. The test was performed based on (b) (4) products as described in (b) (4). The results for the cobas® Zika component lots at all time points were within the acceptance criteria for product (b) (4) as per (b) (4); that is: there was no increase ((b) (4)) from the initial calculated count at (b) (4). Thus, the preservative sodium azide included in the formulation of the *in vitro* diagnostic reagents was shown to have effective anti-microbial properties in accordance with (b) (4).

b) **CBER Lot Release**

A lot release testing plan was developed by CBER and will be used for routine lot release. The results of testing of three conformance lots of cobas® Zika were evaluated and found to be acceptable by CBER.

Table 5: Lot Release Testing

| Batch ID | Expiration Date | Kit Lot Pass/Fail |
|---|---|---|
| Y12974 | 2018/07 | Pass |
| YD2970 | 2018/08 | Pass |
| YD2971 | 2018/08 | Pass |

c) Facilities review/inspection

Table 6. Manufacturing information

| Manufacturing Facility | Field Establishment Identifier (FEI) number | Inspection Dates | Inspection Waiver Justifications |
|---|---|---|---|
| Roche Molecular Systems, Inc., (b) (4) | (b) (4) | Waived | Team Biologics (b) (4) VAI |

Team Biologics conducted a surveillance inspection of the Roche Molecular Systems, Inc. (b) (4) facility from (b) (4). The inspection was classified VAI and all inspectional issues have been resolved.
DMPQ recommends waiver of the pre-license inspection for Roche Molecular Systems, Inc (b) (4). This waiver recommendation is based on criteria outlined in CBER SOPP 8410 "Determining When Pre-Licensing/Pre-Approval Inspections are Necessary."

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

e) Container Closure
N/A

# 4. Software and Instrumentation

## 4.1 Summary

The Roche cobas® Zika donor screening assay operates on the cobas® 6800/8800 Systems.

The Roche cobas® 6800/8800 Systems and p680 pooler instrument were reviewed under the pre-market notifications (510ks) BK140195 and BK140196. These submissions were cleared in October of 2016. The cobas® 6800/8800 Systems currently support the licensed cobas® MPX (BL 125576) and cobas® WNV (BL 125575) donor screening assays. The sponsor states that the cobas® Synergy software (cleared under BK160113) can also be optionally utilized to manage individual donor

Zika testing results between the cobas® 6800/8800 Systems and a Laboratory Information System (LIS).

Since the cobas® 6800/8800 Systems, associated p680 instrument and software have been previously reviewed, the subject software/instrumentation review primarily focused on the Assay Specific Analysis Package (ASAP) related to the cobas® Zika assay. This pathway of concentrating the instrumentation/software review on the cobas® Zika specific ASAP module was discussed and agreed upon between CBER and the sponsor in the pre-submission BQ160101.

The ASAP module contains the assay-specific parameters that are utilized by the cobas® 6800/8800 Systems to perform the cobas® Zika test and obtain final results.

The points listed below are a summary of information provided by the Sponsor in the original BLA 125653 and its subsequent amendments.

- ***Versioning:*** The Sponsor states that the cobas® Zika assay will operate on cobas® 6800/8800 System Software (SW) Version 1.02.13. The cobas® Zika-specific ASAP module version is listed as c-v10.1.0.

- ***Device Description:*** The cobas® 6800/8800 Systems Software (SW) provides basic functionality such as a Graphical User Interface (GUI), instrument management, database functionality, report engines, and LIS interfaces. These basic functions do not change when a new ASAP is added onto the system. An ASAP module encompasses information related to an individual assay such as cobas® WNV, cobas® MPX, or cobas® Zika.

  The ASAP modules are deployed on the Instrument Gateway (IG) and on the Instrument Manager (IM) for each cobas® 6800/8800 System. The ASAPs are built using a common software framework and include assay (test) specific software configuration. An individual ASAP consists of Instrument Operational Parameters, Assay Curve-fitting Algorithms, Result Calculation, Result Detail View, Test and Process Definitions, Analysis Workflow Rules, Configuration Presentation.

  To perform a specific test (cobas® Zika, WNV, etc.), a user selects the test from the cobas® 6800/8800 SW GUI, which in turn, loads the ASAP module and initiates the hardware/software procedures pertaining to sample transfer, specific sample preparation, amplification and detection of the specified analyte.

- ***Risk Analysis:*** A Risk Analysis was performed by the sponsor on the ASAP software. The sponsor provided "cobas® Zika for use on cobas® 6800/8800 Systems" Risk Management Report in their submission. The report provided a summary of the risk management activities, identified product risks, and implemented mitigations for the cobas® Zika test. The identified risks included those associated with the product safety and performance. The

mitigation measures that reduced the risks related to the impacts of potential failure modes and hazards were also provided. The sponsor has claimed that no risk events concerning the cobas® Zika test have occurred during all the testing. Overall, the Risk Management and mitigating features are in place to reduce the risks posed by the ASAP software failure.

- ***Testing:*** The sponsor provided a description of Validation and Verification activities specific to the cobas® Zika assay. The sponsor outlined testing activities that were performed to ensure that the cobas® Zika assay on the cobas® 6800/8800 Systems conforms to the requirements defined in table 3.1 of DH-04482.01-031B, "cobas® Zika Verification Report of requirements related to Zika Assay Specific Analysis Package (ASAP) for use on the cobas 6800/8800 systems". The sponsor provided a verification report that details verification/validation activities conducted. Some of the requirements tested and passed include: "the ASAP shall assign the following targets to the parameters: Target 1: ZIKA", "the ASAP shall assign the following targets to channels: Target 1: channel 3 (HEX)", "the ASAP shall report the target results for an RMC as valid or invalid". The sponsor has also provided verification activities to support Zika ASAP module internal testing before deploying it on the cobas® Systems.

  In addition, the sponsor conducted analytical and clinical studies that validate the Systems and ASAP Zika module functionality in actual screening laboratories. The sponsor utilized the cobas® 6800/8800 Systems and the Zika-specific ASAP module to conduct a feasibility study, cross reactivity supplemental study, clinical specificity and sensitivity study.

  The sponsor requested licensure in BL125653 for the following software versions: for the cobas® 6800/8800 system software version SW v1.02.13; and for the Zika ASAP module, c-v10.1.0. The previously-licensed version of the cobas® 6800/8800 system software (SW) in use with the cobas® MPX and cobas® WNV assays was SWv1.01.09. The sponsor provided a list of key new features and anomaly fixes associated with the subsequent version update to SW v1.02.13. Some of these revisions related to assay functions such as (b) (4) liquid level detection; under-aspiration surveillance (which detects insufficient volume transfer of patient samples); prevention of "cycler timeout" run aborts (by increasing timeout from 30 seconds to 40 seconds); and installation of a Microsoft Service Patch.

- ***Anomalies:*** The sponsor stated that they do not have any unresolved anomalies associated with the cobas® Zika ASAP module.

- ***Development Management:*** The sponsor provided a summary of their software development life cycle plan, describing the processes that have been put into place to manage the various software development life cycle activities, including a summary of the configuration management and maintenance activities. The sponsor also provided a description of the

software system partitioned into its functional subsystems. The sponsor has included a Traceability Matrix (TM), which details the links between the requirements, design, implementation, validation and testing. The sponsor provided an ASAP specific TM that pertains to their cobas® Zika assay.

The cobas® 6800/8800 Systems and associated instrument and software have been previously reviewed and assessed for their safety and effectiveness in prior submissions. Hence, the subject submission (BL125653) instrumentation / software review focused on the Zika specific ASAP module and its ability to apply the parameters defined, characterize the Curve, and calculate the final results that should be either Reactive, Non-Reactive or Invalid. Based on the software documentation provided and supporting analytical and clinical studies conducted with the cobas® 6800/8800 Systems using the Zika ASAP module, the instrumentation and software areas of the subject submission BL125653 are adequate. Specific issues pertaining to exact anomaly mitigation, (b) (4) sub-module revision and its impact on the sensitivity/specificity, and SW/ASAP versioning were addressed by the sponsor during the review.

### 4.2 Review Issues

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

1. Conflict between system software versions requested for approval for different assays on the cobas® 6800/8800 Systems. During software review, it was noted that different versions of the cobas® 6800/8800 Systems software were submitted for approval in this BLA (SW v1.02.12) and BL125575/6 for cobas® WNV (SW v1.02.13). In the Information Request sent to the sponsor on July 25, 2017, the sponsor was requested to provide a comparison table between v1.02.12 and v1.02.13 and to explain how the later software version would impact performance of the cobas® Zika test. In their response of August 8, 2017, the sponsor stated that they intend to launch the cobas® Zika with SW v1.02.13 and that the differences between SW v1.02.13 and SW v1.02.12 affect cobas® multiplex assays only and not the cobas® Zika. The sponsor also intends to launch cobas® Zika ASAP Software v10.1.0, which enables compatibility with cobas Synergy Software (BK160113). The response was considered acceptable.

2. Conflict between possible results reported by the (b) (4) and the final results reporting. During software review, it was noted that while the (b) (4) The response was considered acceptable.

3. Updates to (b) (4) in Zika Calculation Package. In an Information Request sent to the sponsor on July 25, 2017, the sponsor was requested to explain (b) (4) functionality, version update history and analysis of data using different versions during the non-clinical specificity study. Additionally, in the Information request sent to the sponsor on August 18, 2017, the sponsor was asked to further clarify whether the revisions to the (b) (4) parameters would affect the sensitivity and specificity of the assay.

The sponsor responded on August 29, 2017 that sensitivity and specificity of the cobas® Zika would not be affected by the (b) (4) changes because (b) (4) The response was considered acceptable.

4. Mitigations for invalid batches and results between system software and ASAP version changes. During software review, it was noted that hardware and software issues occurred that led to invalidation of batches, and also to invalid results within valid batches during the clinical studies which were performed using System Software Version 01.01.09 and ASAP 9.1.0. The sponsor then requested approval for the cobas® Zika assay on the 6800/8800 systems using SW v01.2.12 and ASAP nc-10.2.0 (c-10.0.0) without noting whether updates were made to the SW and ASAP to mitigate the issues detected during clinical testing. In the Information Request sent to the sponsor on July 25, 2017, the sponsor was requested to describe all of the new features/safeguards implemented to reduce the occurrences of the errors/issues observed during the studies, and to provide verification and validation testing pertaining to these safeguards, updated hazard/risk analysis, and any new anomalies introduced.

The sponsor responded on August 17, 2017 and provided tables describing the mitigations for invalid batches and invalid samples in valid batches in SWv.1.02.12. The sponsor also noted that a tip handling error was being caused by loose stop disks in the sample pipettor; their suggested mitigation was as follows: “The periodic maintenance procedure was adapted to exclude routine weekly cleaning of the sample pipettor by the operator.”

The software and instrumentation reviewer noted that this proposed mitigation was a change to the instrument maintenance routine that could adversely affect the performance of all assays run on the cobas® 6800/8800 instrument. Therefore, in the Information Request sent to the sponsor on August 18, 2017, the sponsor was asked to provide further information on the possible effect of this change to the maintenance routine. The sponsor responded on August 29, 2017 that no negative effect is expected or has been observed because the risk of contamination was mitigated by the stop disk itself and not the weekly cleaning; the weekly cleaning was causing the errors. The response was considered acceptable.

## 5. Analytical Studies

The sponsor performed non-clinical/analytical studies to investigate and describe the functionality of the cobas® Zika assay under certain conditions.

### 5.1 Limit of Detection/Repeatability

Since no Zika International Standard was available at the time this study was conducted, the Roche Zika Secondary Standard ((b) (4) cp/ml), a heat-inactivated virus culture supernatant, in target-negative pooled-EDTA plasma was used to produce panels of testing material for this study. A total of three independent panels of five concentrations (16, 12, 8, 4, 2 cp/mL) and a blank were prepared by three different operators, and tested with 2 reagent lots and 3 runs per lot per day on different systems, and a minimum of 20 replicates per concentration divided between lots. Based on the results of the Probit analysis performed by the sponsor, the 95% LoD was determined to be 8.1 copies/ml (95% confidence range 6.1-13.6 copies/ml). Results were consistent across lots, testing days/operators and instruments as determined by overlapping 95% CIs calculated for each variable.

Table 7. Combined LoD Results for the cobas® Zika assay over 2 reagent lots

| Zika RNA concentration (copies/ml) | Number of reactive results | Number of valid replicates | % reactive | 95% CI lower bound (one-sided) |
|---|---|---|---|---|
| 16.0 | 190 | 190 | 100.0% | 98.4% |
| 12.0 | 188 | 190 | 98.9% | 96.7% |
| 8.0 | 180 | 189 | 95.2% | 91.8% |
| 4.0 | 135 | 189 | 71.4% | 65.5% |
| 2.0 | 94 | 190 | 49.5% | 43.3% |
| 0.0 | 0 | 190 | 0.0% | 0.0% |
| 95% LoD by Probit analysis | 8.1 copies/ml<br>95% CI: 6.1-13.6 copies/ml | | | |

### 5.2 Analytical specificity

The sponsor tested (b) (4) EDTA-plasma clinical specimens from healthy blood donors, collected in the northeastern U.S., with the investigational assay to determine analytical specificity. The results showed 100% specificity, with all negative samples non-reactive by the cobas® Zika assay, which met the study acceptance criteria of specificity (b) (4) .

### 5.3 Analytical Sensitivity using Clinical Specimens

The sponsor identified Zika NAT-positive specimens by screening (b) (4)

(b) (4)

(b) (4)

(b) (4)

(b) (4)

All (b) (4) contrived samples were reactive using the cobas Zika test.

**5.4 Detection of Zika Virus at LoD**

To assess detection using the cobas® Zika assay in specimens close to the 95% LoD of the assay (8.1 copies/ml, 95% CI 6.1, 13.6), the sponsor singly diluted five Zika NAT-positive specimens to ~13.6 copies/ml, the upper bound of the LoD 95% CI. Twenty-one (21) replicates were tested for each diluted sample; all were reactive for Zika and had valid Internal Controls (ICs), for a 100% reactivity rate.

**5.5 Endogenous Interferences**

The sponsor assessed performance of the cobas® Zika assay in the presence or absence of potential endogenous interferents, by testing (b) (4) normal negative EDTA-plasma specimens both spiked with interferents and unspiked (controls). Both interferent-spiked and unspiked specimens were tested with and without the addition of ~3x LoD of Zika target, to assess sensitivity and specificity respectively. All interferents were tested according to (b) (4) guidelines, except for human DNA, which was tested at 2.0 mg/L in the absence of existing (b) (4) guidelines.

Table 9. Endogenous Interferents tested

| Potential Interferent | Concentration tested |
|---|---|
| Albumin | (b) (4) 61.4 g/L |
| Bilirubin | (b) (4) 0.28 g/L |
| Hemoglobin | (b) (4) 2.9 g/L |
| Human DNA | 2.0 mg/L |
| Triglycerides | (b) (4) 33.2 g/L |

All of the 3x LoD Zika-spiked samples had reactive results for Zika and valid ICs and all of the unspiked samples had negative results with valid ICs, regardless of the presence or absence of the interferents, indicating that the interferents tested did not affect the sensitivity or specificity of the cobas® Zika.

### 5.6 Cross-Reactivity

The analytical specificity of the cobas® Zika was evaluated by testing cross reactivity with clinical specimens including HIV-positive, HBV-positive, or HCV-positive samples ((b) (4) ) and 6 cultured microorganisms at 1E+06 copies/mL including Chikungunya virus, Dengue virus serotype 1-4 and West Nile virus (3 replicates each). The clinical specimens were tested with and without Zika virus added to a concentration of approximately 3x LoD of the cobas® Zika. The cultured microorganisms were added separately to normal, virus-negative, human pooled plasma and tested with and without Zika virus. Zika-positive and Zika-negative spiked specimens, without potential cross-reactants, were also included to confirm the performance of the Zika spiking. All of the 3x LoD Zika-spiked samples had reactive results for Zika and valid ICs and all of the unspiked samples had negative results for Zika with valid ICs, regardless of the presence or absence of the potential cross reactants.

Table 10. Cross-reactivity testing of the cobas® Zika assay

| Microorganism | Sensitivity (Zika-spiked) | | | | Specificity (unspiked) | | | |
|---|---|---|---|---|---|---|---|---|
| | Zika Reactivity | | IC Reactivity | | Zika Reactivity | | IC Reactivity | |
| | # | % | # | % | # | % | # | % |
| HIV | (b) (4) | 100 | (b) (4) | 100 | (b) (4) | 0 | (b) (4) | 100 |
| HBV | (b) (4) | 100 | (b) (4) | 100 | (b) (4) | 0 | (b) (4) | 100 |
| HCV | (b) (4) | 100 | (b) (4) | 100 | (b) (4) | 0 | (b) (4) | 100 |
| Chikungunya virus | 3/3 | 100 | 3/3 | 100 | 0/3 | 0 | 3/3 | 100 |
| Dengue virus serotype 1 | 3/3 | 100 | 3/3 | 100 | 0/3 | 0 | 3/3 | 100 |
| Dengue virus serotype 2 | 3/3 | 100 | 3/3 | 100 | 0/3 | 0 | 3/3 | 100 |
| Dengue virus serotype 3 | 3/3 | 100 | 3/3 | 100 | 0/3 | 0 | 3/3 | 100 |
| Dengue virus | 3/3 | 100 | 3/3 | 100 | 0/3 | 0 | 3/3 | 100 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| serotype 4 | | | | | | | | |
| West Nile virus | 3/3 | 100 | 3/3 | 100 | 0/3 | 0 | 3/3 | 100 |
| Positive spike control | (b) (4) | 100 | (b) (4) | 100 | (b) (4) | N/A | (b) (4) | N/A |
| Negative spike control | | N/A | | N/A | | 0 | | 100 |

Syphilis (*Treponema pallidum*) cross-reactivity testing was not included at the time of the initial testing of the investigational assay due to a lack of sample availability. During the pre-submission process (BQ160101), FDA requested that supplemental cross-reactivity testing for related flaviviruses of the Japanese Encephalitis virus (JEV) serogroup (JEV, Usutu virus, Murray Valley Encephalitis virus, St. Louis Encephalitis virus, and Yellow Fever virus) also be performed in addition to the flaviviruses previously assessed. The cultured microorganisms (syphilis and flaviviruses) were added separately to normal, virus-negative, human pooled plasma, and tested using the method described above. All of the 3x LoD Zika-spiked samples had reactive results for Zika and valid ICs and all of the unspiked samples had negative results for Zika with valid ICs, regardless of the presence or absence of syphilis or JEV serogroup flaviviruses. The presence of potentially cross-reactive pathogens had no effect on the sensitivity or specificity of the cobas® Zika assay.

Table 11. Supplementary cross-reactivity testing of the cobas® Zika

| **Microorganism** | **Sensitivity (Zika-spiked)** | | | | **Specificity (unspiked)** | | | |
|---|---|---|---|---|---|---|---|---|
| | **Zika Reactivity** | | **IC Reactivity** | | **Zika Reactivity** | | **IC Reactivity** | |
| | **#** | **%** | **#** | **%** | **#** | **%** | **#** | **%** |
| Japanese Encephalitis virus | 3/3 | 100 | 3/3 | 100 | 0/3 | 0 | 3/3 | 100 |
| Murray Valley Encephalitis virus | 3/3 | 100 | 3/3 | 100 | 0/3 | 0 | 3/3 | 100 |
| St. Louis Encephalitis virus | 3/3 | 100 | 3/3 | 100 | 0/3 | 0 | 3/3 | 100 |
| Usutu virus | 3/3 | 100 | 3/3 | 100 | 0/3 | 0 | 3/3 | 100 |
| Yellow Fever virus | 3/3 | 100 | 3/3 | 100 | 0/3 | 0 | 3/3 | 100 |
| Syphilis (*Treponema pallidum*) | 3/3 | 100 | 3/3 | 100 | 0/3 | 0 | 3/3 | 100 |
| Positive spike control | 3/3 | 100 | 3/3 | 100 | N/A | N/A | N/A | N/A |
| Negative spike control | N/A | N/A | N/A | N/A | 0/3 | 0 | 3/3 | 100 |

### 5.7 Matrix Equivalency

Performance of the cobas® Zika in (b) (4) negative specimens collected in different sample matrices (EDTA, (b) (4) and PPT Plasma Separation Tubes) was assessed by (b) (4) The specificity and sensitivity of the cobas® Zika was not affected by the plasma matrix tested.

### 5.8 Internal Control and RMC Failure Rates

The failure rate of the Internal Control and of the Positive and Negative Roche Manufactured Controls (RMC) and Sample Reliability of the cobas® Zika was assessed using data generated during other non-clinical studies (Limit of Detection, Specificity, Analytical Sensitivity in Clinical Specimens, Detection of Isolates Near LoD, Cross Reactivity, Matrix Equivalency).

The IC Failure was calculated as the (number of IC Failures/number of RMC + samples) x 100. The Control Failure rate was calculated as the (number of invalid controls/number of RMC) x 100. The Sample Reliability was calculated as [1-(number of invalid and not processed samples/number of samples)] x 100. The IC and RMC Control Failure Rate was 0.00% and the Sample Reliability Rate was 99.23%.

### 5.9 Exogenous Interferences

The sensitivity and specificity of the cobas® Zika were evaluated in the presence or absence of exogenous interfering substances commonly found in blood donations. The tested interferents were prepared as (b) (4) working solutions in an appropriate solvent ((b) (4) ) and solvent controls were included for each specimen. Zika-spiked and unspiked samples for each tested potential interferent were prepared for each of (b) (4) individual virus-negative EDTA plasma specimens, along with positive and negative solvent controls and spiking controls. For all of the potential exogenous interfering substances and solvent controls tested, all Zika-spiked specimens were cobas® Zika -reactive and all non-spiked specimens were non-reactive with valid ICs, showing that the tested interferents and solvents did not affect the sensitivity and specificity of the assay.

Table 12. Medications Tested for Interference with cobas® Zika

| Name of Drug Tested | Concentration |
|---|---|
| Acetaminophen | 1337.4 µmol/L |
| Acetylsalicylic Acid | 3656.6 mmol /L |
| Ascorbic Acid (vitamin C) | 345.5 µmol/L |
| Atorvastatin | 606.1 µg Eq/L |
| Fluoxetine | 11.3 µmol/L |
| Ibuprofen | 2449.5 µmol/L |
| Loratadine | 0.8 µmol/L |
| Nadolol | 3.9 µmol/L |
| Naproxen | 2191.9 µmol/L |
| Paroxetine | 3.1 µmol/L |

| | |
|---|---|
| Phenylephrine HCL | 496 µmol/L |
| Sertraline | 2.0 µmol/L |

**5.10 Clinical Specimen Stability**

In addition to the plasma matrix equivalency testing performed, the sponsor conducted additional testing to determine the stability of Zika specimens in whole blood samples collected in different anticoagulants (EDTA, EDTA-PPT, (b) (4) ) under conditions that simulated the handling, transporting and processing of donated blood samples in a blood bank prior to the testing of the separated plasma with the cobas® Zika. To prepare the testing specimens, whole blood was collected from each of (b) (4) donors in EDTA, (b) (4) blood bags as well as PPT tubes. The different blood donations were (b) (4)

.

Samples collected in PPT tubes were (b) (4)

(b) (4)

(b) (4)

(b) (4)

(b) (4) The product insert recommends testing of samples using only the anticoagulants and storage conditions which met the acceptance criteria for this study.

**5.11 On-board and Open Kit stability**

The on-board and open kit stability of the cobas® Zika was tested to confirm that the kits met the same stability specifications as other cobas blood screening assays: open kit stability of 30 days since loading and max 20 hours on-board stability for the cobas® Zika 480-test kit, and max 10 hours on-board stability for the cobas® Zika control kit. The study did not include on-board and open kit stability for the cobas omni reagents and the cobas® NHP Negative Control kit, since stability for those reagents was established in prior regulatory submissions for the cobas® WNV and cobas® MPX kits (BL125575 and BL125576).

To assess on-board and open-kit stability of the 480-test cobas® Zika kit, (b) (4)

(b) (4) The sponsor concluded that both the cobas® Zika 480-test kit and the cobas® Zika Control Kit met the stated required specifications.

**5.12 Cross-contamination**

A new study to determine the cross-contamination rate for the cobas® Zika kit used on the cobas® 6800/8800 Systems was not provided for this application. To determine the cross-contamination rate, which is a function of the instrument, data from the study supporting cross-contamination for the cobas® MPX (BL125576) were provided. The results demonstrated no cross-contamination with a rate of 0%.

**5.13 Review issues**

During the review of this section, the following issues were raised and resolved:

a) *Both the LoD and Repeatability Studies were performed using only* (b) (4) *lots. RMS had proposed using three lots for these studies in the Pre-submission.*

The above comment was resolved during the Mid-cycle Meeting (07/06/17) and the data for (b) (4) lots was found acceptable.

*b) Cross-reactivity testing was performed with only 3 replicates for each potentially cross-reactive agent.*

The above comment was clarified and resolved during the Internal Meeting (07/06/17). The use of only 3 replicates met the requirement for three replicates which had been conveyed to the sponsor during the pre-IND stage.

## 6. Clinical Studies

The clinical studies supporting this application were performed under IND #16926. Testing under this IND was initiated on April 4, 2016 in the U.S. territory of Puerto Rico; collection sites for the clinical studies were added later in states considered to be at high risk for ZIKV outbreaks, and expanded throughout the U.S. following the FDA guidance issued August 26, 2016 which recommended universal ID-NAT testing. Although testing under the IND is ongoing, the dataset for the clinical specificity and sensitivity studies was closed on October 9, 2016, per agreement with FDA under pre-submission BQ160101.

**Clinical specificity**

Under the clinical specificity study, 358,266 donations collected in U.S. states (i.e., excluding the U.S. territory of Puerto Rico) were tested as individual donations using cobas® Zika. Since no FDA-approved comparator assay was available, samples reactive on cobas® Zika were tested with an alternative NAT (using index plasma) and anti-Zika IgM serology (using index serum) to confirm the Zika status of each donation. Donations that were non-reactive on cobas® Zika were not tested further. Donors with Zika-reactive donations were invited to participate in a follow-up study, and follow-up samples were also tested by cobas® Zika, alternative NAT, and IgM serology, which could be used to determine the Zika status of the original donation by detecting later Zika seroconversion or increasing viral loads. The clinical specificity of cobas® Zika was calculated as the percentage of Zika RNA-negative donations from the U.S. states that were non-reactive on cobas® Zika divided by the total number of donations tested. IRB approvals were obtained for the protocol by the sponsor, testing sites, and in some cases, individual collection sites prior to the start of testing under the IND clinical specificity protocol. Donors were consented under the collection site's standard donor consent process for study participation, and donors with reactive results were consented separately for participation in follow-up studies.

A total of 358,266 eligible donations were collected from enrolled donors in the U.S. states; of these, 228 (0.06%) were not evaluable, leaving 358,038 evaluable specimens in the study. Testing was conducted at five sites: Creative Testing Solutions (CTS) in Tempe, AZ; Gulf Coast Regional Blood Center (GCR) in Houston, TX; QualTex Laboratories (QTX) in Norcross, GA; The Blood Connection, Inc. (BCN) in Piedmont, SC; and The Blood Center (BCT) in Hammond, LA. The samples were not distributed evenly between the clinical testing sites.

Table 13. Evaluable samples tested by clinical site.

| Site | Samples tested | Percent of total |
|---|---|---|
| CTS | 214,015 | 59.77 |
| GCR | 83,400 | 23.29 |
| QTX | 32,801 | 9.16 |
| BCN | 23,515 | 6.57 |
| BCT | 4,307 | 1.20 |
| Total | 358,038 | 100.00 |

During initial testing for the specificity study, 4,919 batches (samples tested together on a single instrument run) were performed, of which 4,796 (97.5%) were valid and 123 (2.5%) were invalid. The majority of invalid batches were due to positive and negative control failure (103/123, 83.7%). The frequencies of invalid batches varied between test sites, ranging from 1.3% at GCR to 5.7% at QTX. One cobas® 6800 instrument at QTX had a failure rate of 9.8% (20/205 batches); all of the invalid batches on this instrument were due to positive and negative control failure. Within valid batches, 358,344 donations were tested, of which 358,038 results were valid and 306 (0.09%) were invalid, leaving a total of 358,038 evaluable donations. The majority of the 306 invalid results within valid batches were due to detection of a clot within the sample during aspiration (170, 55.6%); 41 invalid results (13.4%) were because of anomalies that occurred during calculations. The 306 invalid results represented a total of 228 non-evaluable specimens since some non-evaluable specimens were tested more than once.

Of the 358,038 evaluable specimens, 358,015 had non-reactive cobas® Zika results. These specimens were not tested further and the donors were not enrolled in follow-up studies. Twenty-three specimens had reactive cobas® Zika results and were eligible for the follow up study. Of these 23, 13 enrolled in follow-up, 5 declined participation in follow-up, and 5 were lost to follow-up. Of the 13 donors who did enroll in follow-up, 11 returned for both scheduled visits to complete the study, and 2 were lost to follow-up after the first visit. No donor status was changed by the follow-up results.

Samples found to be reactive were further characterized into 3 categories as noted in Table 14. Reactivity category 4 reflects those samples which were negative and not tested further.

Table 14. Reactivity of samples tested by cobas® Zika

| Reactivity Category | Number of donations in category | cobas® Zika reactivity | Alternative NAT | Zika IgM | Donation Status |
|---|---|---|---|---|---|
| 1 | 7 | + | + | variable | Positive |
| 2 | 7 | + | - | + | Positive |
| 3 | 9 | + | - | - | Negative |
| 4 | 358,015 | - | Not tested | Not tested | Negative |

Of the 23 donations found to be reactive, the 7 donations classified into Reactivity Category 1 were considered Zika-positive based on positive alternative NAT results on the index donation. Two out of these seven donations were also IgM-positive; three out of the seven donors participated in follow-up studies, in which all three were IgM-positive on at least one follow-up sample and one was also cobas ®Zika-reactive on follow-up. Seven other donations were classified into Reactivity Category 2; these donations were negative when tested by alternative NAT, but were considered Zika-positive because they were positive for Zika IgM. Four of the seven Reactivity Category 2 donors participated in follow-up studies; three of those four were also Zika IgM-positive on follow-up and the fourth was equivocal. None of the four was cobas Zika-reactive on follow-up. The remaining nine donations were classified into Reactivity Category 3 and considered negative for Zika on the basis of negative results on alternative NAT and Zika IgM.

The sponsor calculated the clinical specificity of cobas® Zika as the percentage of status-negative donations that had non-reactive cobas® Zika results. The true specificity of the assay is difficult to determine because there is no available FDA-approved comparator. The clinical specificity of cobas® Zika for donations tested individually is 99.997% (358,015/358,024; 95% CI: 99.995% to 99.999%). Specificity estimates were similar between the five sites, ranging from 99.994% (95% CI 99.798 to 99.998) to 100.000% (95% CI 99.984 to 100.000). The results of the clinical specificity test support the use of cobas® Zika as a blood donation screening test for Zika virus RNA.

Table 15. Clinical specificity of the cobas® Zika

| Test site | Total number of status-negative donations | Cobas® Zika reactivity | | Specificity estimate (95% Exact CI) |
|---|---|---|---|---|
| | | Reactive | Non-reactive | |
| Overall | 358,024 | 9 | 358,015 | 99.997 (99.995,99.999) |
| CTS | 214,006 | 6 | 214,000 | 99.997 (99.994,99.999) |
| GCR | 83,400 | 1 | 83,399 | 99.999 (99.993,100.000) |
| QTX | 32,796 | 2 | 32,794 | 99.994 (99.978,99.998) |
| BLC | 23,515 | 0 | 23,515 | 100.000(99.984, 100.000) |
| BCT | 4,307 | 0 | 4,307 | 100.000(99.911, 100.000) |

## Clinical Performance Evaluation

### Evaluation of the Sensitivity of cobas® Zika

The evaluation of the sensitivity of cobas® Zika was done using 25 confirmed Zika-positive clinical samples at an internal testing site. The cobas® Zika test detected 100% (95% CI 86.2%-100%).

### Evaluation of the Yield and PPV of cobas® Zika in a Zika Outbreak

Since an insufficient number of samples from clinically confirmed Zika cases were available for clinical sensitivity testing, the positive predictive value (PPV) for cobas® Zika was calculated for an area with a low prevalence of Zika cases (the U.S. states excluding the U.S. territory of Puerto Rico) and an area with

a high prevalence of Zika cases (the U.S. territory of Puerto Rico), using data collected under the clinical specificity protocol (cX8-ZIKA-412) for IND #16926. The PPV was calculated as the percentage of initially reactive specimens which were confirmed as Zika-positive by alternative NAT or IgM testing. In the U.S. states, 23 out of 358,038 evaluable donations tested were initially reactive by cobas® Zika, and 14/23 were confirmed positive, yielding a PPV of 60.9% (95% exact CI 38.5-80.3). In Puerto Rico, 286 out of 37,042 evaluable donations tested were initially reactive by cobas® Zika, and 272/286 were confirmed positive, yielding a PPV of 95.1% (95% exact CI 91.9-97.3). The results of the evaluation support the use of cobas® Zika as a blood donation screening test for Zika virus RNA.

**Clinical Reproducibility**

The clinical reproducibility study for cobas® Zika was performed under protocol cX8-ZIKA-427 in IND #16926. Testing was performed at three sites, one internal and two external, American Red Cross (Gaithersburg, MD) and Mississippi Valley Regional Blood Center (Davenport, IA). Reproducibility was assessed across the following factors: 3 reagent lots x 3 test sites (1 instrument per site) x 5 days of testing x 2 batches x 3 replicates of each concentration tested. A coded test panel consisting of 3 Zika-positive panel members was prepared by spiking cultured Zika into Zika-negative EDTA plasma at three different concentrations (0.5x LoD, 1-2x LoD, and 3x LoD) along with 1 Zika-negative member (EDTA plasma only). Estimated amounts of RNA expected to be present within each panel member were not provided by the sponsor. Each testing batch consisted of 12 panel members (3 replicates of each concentration) plus one positive and one negative control, both of which had to be valid for a batch to be considered valid. Within valid batches, a test result could be considered invalid due to an invalid IC with a non-reactive test result or due to an incident/protocol deviation.

The results showed 100% agreement with expected results for the negative, positive 1-2X LoD and positive 3X LoD panel members (95% CI 98.6, 100.0). Although the positive 0.5X LoD panel member was designed to contain less Zika virus RNA than the 95% LoD that was calculated for the assay (8.1 copies/ml, see LoD studies in the non-clinical section), the expected result for that panel member was positive, and the results showed 76.1% agreement with that expected result (95% CI 70.6, 81.1). At test levels at or above the LoD the cobas® Zika did not vary significantly across sites, lots, days or batches tested.

Table 16. Reproducibility study

| Viral Target | Expected viral concentration | Results in agreement with Viral Target | Percent agreement | 95% Exact CI |
|---|---|---|---|---|
| Negative | 0 | 268/268 | 100.0 | (98.6, 100.0) |
| Zika-positive | ~0.5x LoD | 204/268 | 76.1 | (70.6, 81.1) |
| | ~1-2x LoD | 269/269 | 100.0 | (98.6, 100.0) |
| | ~3x LoD | 270/270 | 100.0 | (98.6, 100.0) |

The sponsor also calculated the overall mean, standard deviation and coefficients of variation (%) of Ct values for positive panel members with reactive cobas® Zika results, both overall and attributed to individual variance components (site, lot, day, batch and within-batch) by expected viral concentration. Within-batch variation accounted for most of the variance observed: 92.6% for 0.5X LoD, 89.3% for 1-2X LoD and 91.4% for the 3x LoD positive panel members. The overall analytical specificity was 100% (95% CI 98.6, 100.0) because no reactive results were observed for the negative panel member in any tests. The results met the study acceptance criterion set by the sponsor of a 95% CI lower limit equal to or greater than (b) (4) for the percent agreement for the panel members with concentrations at or above LoD. The results supported the intended use for the cobas® Zika and demonstrate adequate reproducibility.

**Review Issues**

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

1. Clinical performance evaluation and labeling:
   Due to the cobas® Zika being a first-of-its-kind assay, there was no FDA licensed or approved assay available to use as either a comparator for clinical specificity or a qualifier for clinical sensitivity. After discussion with the review committee and OBRR management it was concluded that the sensitivity of any unapproved alternate assay used to qualify samples would be undetermined and likely to be lower than the cobas® Zika, and that as a first-of-a-kind assay, approval could proceed without a clinical sensitivity study performed on clinical samples obtained outside the clinical specificity study. The sponsor was asked to include in the PI both the results of the analytical sensitivity study involving clinically confirmed samples (see section 5.3) and the positive predictive value calculated from the initially reactive and confirmed samples from cobas® Zika testing in U.S. states and the U.S. territory of Puerto Rico. The sponsor updated the PI as requested and the issue was considered resolved.
2. High invalid run rate observed on cobas® 6800 instrument at QualTex testing site. During testing of donor samples for the clinical specificity study, one cobas® 6800 instrument at QualTex, serial number (b) (4), had a failure rate of 9.8% (20/205 batches); all of the invalid batches on this instrument were due to positive and negative control failure. This issue was referred for investigation by BIMO during the inspection of the testing site. The inspector did not identify any issues that would indicate a systemic problem with the 6800 instrument and the issue was considered resolved.
3. Use of EDTA (index) plasma vs. unit plasma for repeat testing in sensitivity study. Protocol cX8-ZIKA-412 specified that repeat testing may be performed using plasma from the unit rather than index (EDTA) plasma. This was a concern to the review team because of the Clinical Stability study results which suggested that plasma from blood collected with (b) (4) anticoagulants may not perform as well as plasma collected

with EDTA. In a teleconference on August 9, 2017, the sponsor was asked to provide additional data on whether EDTA or unit plasma was used for repeat testing. Data provided by the sponsor on August 16, 2017 indicated that of the 206 specimens that underwent repeat testing, 199 were tested with unit plasma. The sponsor also noted that there was typically a delay in testing these samples of several days while the plasma bag was shipped from the collection site. The reviewers concluded that use of unit plasma could not be confirmed to have had an impact on the sensitivity study results, and the use of unit plasma was considered acceptable.

4. Clinical Reproducibility Statistical Analysis. The statistical reviewer noted that tests of the 0.5x LoD Zika-positive panel member which generated non-reactive results on the cobas Zika assay were not included in the statistical analysis of variability attributable to different study factors. The sponsor was asked in the Information Request dated July 25, 2017, to redo the analysis to include these non-reactive samples to more accurately describe the variability of the assay. The sponsor responded on August 7, 2017 that since the cobas® Zika generates Ct values for reactive samples only, that it was not possible to include non-reactive samples for which no Ct value was generated. The response was considered acceptable.
5. Clinical Specificity Statistical Analysis. The statistical reviewer requested in the Information Request dated July 25, 2017 that the sponsor clarify two points of data provided for the clinical specificity study in regards to invalid batches and invalid results generated by the cobas® Zika assay. For the first point, the sponsor was asked to clarify whether the 123 invalid batches generated during testing for the specificity study were retested. The sponsor responded on August 8, 2017 that 6,919/6,940 (99.7%) of the unique donations within the 123 invalid batches were retested, and that the remaining samples were not available for retesting for a variety of reasons. For the second point, in the July 25 IR, the sponsor was also asked to reconcile the number of invalid results within valid batches (306) with the number of non-evaluable donations (228) described in the Clinical Specificity Study report. The sponsor responded on August 8, 2017 and clarified that while 306 was the number of invalid test results within valid batches, 228 was the number of unique donations that never generated a valid result on the cobas® Zika (i.e., some of the 306 were valid upon retest, and some of the 228 were part of invalid batches). Because the Sponsor included the correct invalid rate in the analysis, the review team found the responses to both points of the information request acceptable.
6. Reagent Lots used during the Clinical Specificity Study. The statistical reviewer noted a discrepancy between the Clinical Specificity Study Report, which stated that 5 reagent lots were used for testing, and the provided draft Summary Basis for Approval, which stated that 4 reagent lots were used for testing. This question was included in the Information Request dated July 25, 2017, and the sponsor responded on August 4, 2017 that while 5 lots were used under the Clinical Specificity Study Protocol cX8-ZIKA-412, only 4 lots were used for testing samples collected in the

U.S. states excluding the U.S. territory of Puerto Rico, which were used in the data analysis for the Clinical Specificity Study. The 5th lot was used in initial testing of specimens in Puerto Rico only. The response was considered acceptable.

7. Qualification regarding plasma testing for living organ/tissue donors. The Office of Tissues and Advanced Therapies (OTAT) reviewed this application. The intended use includes testing of organ and tissue donors when samples were collected while the donor's heart was still beating only. OTAT requested that the sponsor include a qualification in the PI that ZIKV RNA may persist longer in organs and in other body fluids than it does in plasma; thus, a negative result obtained in testing plasma may not mean that other cells or tissues recovered are not infected with ZIKV. The sponsor revised the procedural limitations section of the product insert to add this qualification and the matter was considered resolved.

**Label Considerations**

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

**BIMO**

Bioresearch Monitoring (BIMO) inspection assignments were issued for three clinical sites that participated in the conduct of Study cX8-ZIKA-412. The inspections did not reveal problems that impact the data submitted in this BLA.

**a) Pediatrics**

N/A

**b) Other Special Populations**

N/A

## 7. Advisory Committee Meeting

It was determined that this regulatory submission did not require presentation at an Advisory Committee meeting prior to approval.

## 8. Other Relevant Regulatory Issues

None

**Post Marketing Requirements and Post Marketing Commitments**

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

## 9. Labeling

**Proprietary name:** cobas® Zika, Nucleic acid test for use on the cobas® 6800/8800 systems

**APLB Review:** The Advertising and Promotional Labeling Branch (APLB) found the proposed Instructions for Use (IFU), carton and container labels to be acceptable from a promotional and comprehension perspective.

**Carton and immediate container labels:** In a teleconference on August 28, 2017, the sponsor notified the FDA of a discrepancy in the labeling of the negative control. Since this negative control (prepared from Negative Human Plasma (NHP)) is used in several assays on the cobas® 6800/8800 system, the carton does not state that the NHP has tested negative for Zika. This information is in the PI of the cobas® Zika assay but not on the carton. The sponsor will revise the carton labeling but may not be able to implement this change before ADD. The sponsor will communicate this information to the U.S. customers through a letter. FDA agreed to the proposed interim mitigation.

## 10. Recommendations and Risk/ Benefit Assessment

### a) Recommended Regulatory Action

The Review Committee reviewed the original submission and related amendments submitted by RMS. All review issues have been resolved; therefore, the Review Committee recommends licensure of the cobas® Zika for use on the cobas® 6800/8800 Systems.

The major review issue which required resolution from the committee was the structure and proper labeling of the data provided by the sponsor to support the clinical sensitivity claim for the cobas® Zika assay. This issue was resolved by consultation among the lead reviewer, clinical and statistical reviewers with DETTD and OBRR management. It was concluded that as a first-of-its-kind product, review and licensure could proceed without a sensitivity study with samples screened by an FDA-licensed comparator assay, and that the structure of the study could be conveyed in the product insert.

### b) Risk/ Benefit Assessment

The cobas® Zika is the first assay of its kind intended for detection of Zika virus nucleic acid in blood donations. The assay has an estimated 95% LoD of 8.1 copies of Zika RNA/ml and a high specificity (99.997%) demonstrated in the clinical studies supporting this submission. Given the possible negative clinical outcomes for those contracting Zika virus through blood donations, the cobas® Zika is likely to offer a significant public health benefit.

### c) Recommendation for Postmarketing Activities

No postmarketing activities have been proposed for this application.