



To: Administrative File(s): STN 125653/0, cobas[®] Zika Test, Nucleic Acid Test for Use on the cobas[®] 6800/8800 Systems

From: Cecily Jones, CMC Facility Reviewer, OCBQ/DMPQ/MRBI

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Through: Carolyn Renshaw, Branch Chief, OCBQ/DMPQ/MRBI

Subject: *Final Review Memo*: Original BLA

Applicant: Roche Molecular System, Inc.
(b) (4)

Action Due Date: October 6, 2017

Background

In response to FDA recommendations, Roche Molecular Systems, Inc. (hereafter referred to as RMS) developed the cobas[®] Zika Test, a qualitative PCR NAT assay, to enable the simultaneous detection of Zika RNA in plasma from donors of whole blood and blood components and the internal control in a single test of an infected, individual donation. The cobas[®] Zika was approved under IND 16926 on Mar. 30, 2016 to screen blood donations. On August 26, 2016, the US FDA expanded the requirement for screening of blood donations with a NAT or using PRT to extend to all blood donations collected in all the 50 U.S. states, as of November 18, 2016. Twelve US testing sites are currently enrolled under IND 16926. The approval of the cobas[®] Zika BLA will offer novel capability to detect Zika RNA so that infected Zika RNA; and thereby, provide heightened protection from transfusion-transmitted Zika infection for recipients of donated blood components or products and enable the use of a licensed test for the screening blood donation supply.

There are no differences between the investigational product and the to-be-marketed product in the formulation of the cobas[®] Zika Test kit. The cobas[®] Zika Control Kit and the cobas Negative Control Kit were modified based on the pre-submission feedback received from FDA BQ 160101. The revised formulation was used to complete investigational testing in the reviewed studies and will remain the

same in the to-be-marketed finished products.

cobas[®] Zika is run on the **cobas**[®] 6800/8800 Systems, which have been cleared by CBER. The **cobas**[®] 6800/8800 Systems also support licensed **cobas**[®] MPX (BL 125576) and **cobas**[®] WNV (BL 125575) tests. A list of common reagents and components shared between **cobas**[®] Zika and **cobas**[®] MPX and **cobas**[®] WNV tests are provided and outlined.

Roche Molecular Systems, Inc. requested a Priority Review of the subject Original BLA, which was granted by the Agency; and therefore, would be reviewed under a six-month review timeframe. The key milestone dates for the BLA are as follows:

- First Committee Meeting: 1 May 2017
- Filing Meeting: 22 May 2017
- Filing Action Letter with Deficiencies Issued: 6 June 2017
- Mid-Cycle Committee Meeting: 6 July 2017
- Action Letter Issued: 6 October 2017

Proposed Intended Use Statement

The **cobas**[®] Zika test for use with the **cobas**[®] 6800/8800 System, is a non-sterile, single-use, qualitative *in vitro* nucleic acid screening test for the direct detection of Zika virus RNA in plasma specimens from individual human donors, including donors of whole blood and blood components, and other living donors. It is also intended for use in testing plasma specimens to screen organ donors when specimens are obtained while the donor's heart is still beating. The test is not intended for use as an aid in diagnosis. The test is not intended for screening other body fluids. This test is not intended for use on samples of cord blood.

Product Information

Background

Zika virus can be transmitted via transfusion. Most (about 80%) Zika infections are asymptomatic, so questioning donors about recent symptoms suggestive of Zika infection is an effective way to identify infected donors. Like other infectious diseases for which blood donations are screened, blood donations must be screened with a sensitive assay to detect Zika RNA so that infected units may be interdicted and discarded.

The basis for the subject requested Priority Review is related to the unmet medical, public health or laboratory need in addressing the ongoing Zika epidemic in the U.S./Puerto Rico. Locally-acquired Zika virus cases were first reported in Puerto Rico in December 2015, prompting concern for the safety of the local blood supply. In response to this blood safety concern, FDA issued recommendations in February 2016 to reduce the risk of transfusion-transmitted Zika virus, including cessation of blood collections in areas of active Zika virus transmission, unless donations were screened with a Zika virus nucleic acid testing (NAT) or were subjected to pathogen reduction technology (PRT). Puerto Rico was required to discontinue collections, as no FDA-approved NAT test was available and PRT is available only for plasma and platelet products.

Principles of the Assay

cobas® Zika is based on fully automated sample preparation (nucleic acid extraction and purification) followed by PCR amplification and detection. The **cobas®** 6800/8800 Systems consist of the sample supply module, the transfer module, the processing module, and the analytic module. Automated data management is performed by the **cobas®** 6800/8800 software which assigns test results for all tests as non-reactive, reactive, or invalid. Results can be reviewed directly on the system screen, and printed as a report.

Samples should be tested as individual samples.

Nucleic acids from the sample and added armored RNA internal control (IC) molecules (which serve as the sample preparation and amplification/detection process control) are simultaneously extracted. In addition, the test utilizes two external controls: a positive and a negative control. Viral nucleic acids are released by addition of proteinase and lysis reagent to the sample. The released nucleic acids bind to the silica surface of the added magnetic glass particles. Unbound substances and impurities, such as denatured proteins, cellular debris, and potential PCR inhibitors (such as hemoglobin) are removed with subsequent wash reagent steps and purified nucleic acids are eluted from the glass particles with elution buffer at elevated temperature.

Selective amplification of target nucleic acid from the donor sample is achieved using virus-specific forward and reverse primers which are selected from highly conserved regions of the viral nucleic acid. A thermostable DNA polymerase enzyme is used for both reverse-transcription and amplification. The master mix includes deoxyuridine triphosphate (dUTP), instead of deoxythymidine triphosphate (dTTP), which is incorporated into the newly synthesized DNA (amplicon). Any contaminating amplicons from previous PCR runs are destroyed by the AmpErase enzyme [uracil-N-glycosylase], which is included in the PCR mix, when heated in the first thermal cycling step. However, newly formed amplicons are not destroyed since the AmpErase enzyme is inactivated once exposed to temperatures above 55°C.

The **cobas®** Zika master mix contains detection probes which are specific for Zika virus and IC nucleic acid. The specific Zika virus and IC detection probes are each labeled with one of two unique fluorescent dyes which acts as a reporter. Each probe also has a second dye which acts as a quencher. The two reporter dyes are measured at defined wavelengths, thus permitting detection and discrimination of the amplified Zika virus target and the IC. When not bound to the target sequence, the fluorescent signal of the intact probes is suppressed by the quencher dye. During the PCR amplification step, hybridization of the probes to the specific single-stranded DNA template results in cleavage by the 5' to 3' nuclease activity of the DNA polymerase resulting in separation of the reporter and quencher dyes and the generation of a fluorescent signal. With each PCR cycle, increasing amounts of cleaved probes are generated and the cumulative signal of the reporter dye is concomitantly increased. Since the two specific reporter dyes are measured at defined wavelengths, simultaneous detection and discrimination of the amplified Zika target and the IC are possible.

The **cobas®** Zika Nucleic Acid Test Kit is packaged 7 kits per carton to include 480 tests (14.5ml (1) MMX-R1; 17.5ml (1) MMX-R2; 38ml (1) Proteinase Solution (PASE); 38ml (1) Elution Buffer (EB); 38ml (1) IC), (4) Positive Control Kit MiniRacks ZIKA (+) C (4 vials/mini-rack) and SW **cobas** Zika ASAP CD; Version 10.0. The cobas NHP Negative Control Kit contains the negative control that is used by most cobas 6800/8800 tests. The cobas Omni Reagent kits (Wash Reagent, Specimen Diluent, Lysis Reagent, and MGP Reagent) are common to all cobas 6800/8800 tests.

Note the kit components listed in Tables 1-4 below.

Table 1. cobas® Zika Test Kit

Kit Component	Reagent Ingredients	Quantity Per Kit
		480 Tests
Proteinase Solution (PASE)	Tris buffer, < 0.05% EDTA, calcium chloride, calcium acetate, 8% (w/v) proteinase EUH210: Safety data sheet available on request. EUH208: Contains subtilisin. May produce an allergic reaction.	38 mL
Internal Control (IC)	Tris buffer, < 0.05% EDTA, < 0.001% internal control armored RNA construct (non-infectious RNA encapsulated in MS2 bacteriophage), < 0.002% Poly rA RNA (synthetic), < 0.1% sodium azide	38 mL
Elution Buffer (EB)	Tris buffer, 0.2% methyl-4 hydroxybenzoate	38 mL
Master Mix Reagent 1 (MMX-R1)	Manganese acetate, potassium hydroxide, < 0.1% sodium azide	14.5 mL
Zika Master Mix Reagent 2 (ZIKA MMX-R2)	Tricine buffer, potassium acetate, glycerol, 18% dimethyl sulfoxide, <0.1% Tween 20, EDTA, < 0.14% dATP, dGTP, dCTP, dUTPs, < 0.01% upstream and downstream ZIKA primers, <0.01% internal control forward and reverse primers, < 0.01% fluorescent-labeled ZIKA and internal control probes, < 0.01% oligonucleotide aptamer, < 0.01% Z05D DNA polymerase, < 0.01% AmpErase (uracil-N-glycosylase) enzyme (microbial), < 0.1% sodium azide	17.5 mL

Table 2. cobas® Zika Control Kit

Kit Component	Reagent Ingredients	Quantity Per Kit
Zika Positive Control (Zika (+) C)	< 0.001% Synthetic (armored) Zika RNA encapsulated in MS2 bacteriophage coat protein, Normal human plasma, non-reactive by licensed tests for antibody to HCV, antibody to HIV-1/2 HBsAg, antibody to HBc; HIV-1 RNA, HIV-2 RNA, HCV RNA, HBV DNA, HEV RNA, WNV RNA, CMV DNA, Zika RNA, CHIKV RNA and DENV RNA not detectable by PCR methods. 0.1% ProClin® 300 preservative	16 mL (16 x 1 mL)

Table 3. cobas® NHP Negative Control Kit

Kit Component	Reagent Ingredients	Quantity Per Kit
Normal Human Plasma Negative Control (NHP-NC)	Normal human plasma, non-reactive by licensed tests for antibody to HCV, antibody to HIV-1/2, HBsAg, antibody to HBc; HIV-1 RNA, HIV-2 RNA, HCV RNA, HBV DNA, HEV RNA, WNV RNA, CMV DNA, Zika RNA, CHIKV RNA and DENV RNA not detectable by PCR methods. < 0.1% ProClin® 300 preservative	16 mL (16 x 1 mL)

Table 4. cobas omni Reagents for Sample Preparation

Kit Component	Reagent Ingredients	Quantity Per Kit
cobas omni MGP Reagent (MGP)	Magnetic glass particles, Tris buffer, 0.1% methyl-4 hydroxybenzoate, < 0.1% sodium azide	480 tests
cobas omni Specimen Diluent (SPEC DIL)	Tris buffer, 0.1% methyl-4 hydroxybenzoate, < 0.1% sodium azide	4 x 875 mL
cobas omni Lysis Reagent (LYS)	42.56% (w/w) guanidine thiocyanate, 5% (w/v) polydocanol, 2% (w/v) dithiothreitol, dihydro sodium citrate	4 x 875 mL
cobas omni Wash Reagent (WASH)	Sodium citrate dihydrate, 0.1% methyl-4 hydroxybenzoate	4.2 L

Review Summary

Background

The BLA was submitted as an Electronic Biologic License Application (e-BLA) for **cobas**[®] Zika, Nucleic acid test for use on the **cobas**[®] 6800/8800 Systems, along with the required electronic files (pdf) per the eCopy Program for Medical Device Submissions guidance.

Items Reviewed:

1. Cover Letter/Table of Contents
2. Summary Basis of Approval
3. Chemistry, Manufacturing, and Controls (CMC)
4. Establishment Description
 - o Bioburden
 - o Cleaning Procedures
 - o Environmental Monitoring
5. Process Validation

The manufacturing of all **cobas**[®] 6800/8800 in vitro products takes place at the aforementioned location. The manufacturing suite where each in vitro product is manufactured is listed below in Tables 5-7.

Table 5: cobas[®] Zika Test Kit Manufacturing Information

Kit	Manufacturing Site
cobas Zika test kit (480T)	(b) (4)
cobas Zika Control Kit	

Table 6: cobas[®] Zika Test Kit Component Manufacturing Information

Kit	Component	Manufacturing Site
cobas Zika test kit	Master Mix R2 Master Mix R2 (Bulk)	(b) (4)
	RNA Internal Control RNA Internal Control (Bulk)	
cobas Zika Control Kit	Zika (+) C (Vial) Zika (+) C (Bulk)	

Table 7: cobas omni Reagent and Common Component Manufacturing Information

Kit	Component	Manufacturing Site
Assay Specific Test Kits	Protease Protease (Bulk)	(b) (4)
	Elution Buffer Elution Buffer (Bulk)	
	Master Mix R Master Mix R1 (Bulk)	
Wash Reagent	Wash Reagent (Bottle) Wash Reagent (Bulk)	
Specimen Diluent	Specimen Diluent (Bottle) Specimen Diluent (Bulk)	
Lysis Buffer	Lysis (Bottle) Lysis (Bulk)	
MGP Reagent	Magnetic Glass Particles (Bulk) Magnetic Glass Particles (Buffer)	
Negative Control	Negative RMC (Vial) Negative RMC (Bulk)	

Inspection History

The (b) (4) facility was last inspected (b) (4) and classified VAI. The current CBER Core Team Biologics led Establishment Inspection (EI) was conducted as a CP 7342.008, QSIT Level II Routine inspection for general production system coverage. The complete EIR is in progress. The previous inspection was conducted (b) (4) and classified NAI. The CDRH led EI was conducted as a CP 7382.845, QSIT Level I Postmarket inspection for (b) (4) Test. CAPA and P&PC subsystems were covered. Limited coverage was given to the Design Control subsystem.

RMS is seeking approval for the manufacture of the **cobas**[®] Zika, Nucleic acid test for use on the **cobas**[®] 6800/8800 Systems in the (b) (4) facility. 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.”

Environmental Assessment

Environmental Assessment (EA) and claim for categorical exclusion was submitted to the file. Roche Molecular Systems, Inc. claims a Categorical Exclusion from the submission of an Environmental Impact Statement with the **cobas**[®] Zika test Biologics License Application pursuant to 21 CFR 25.31(c). Based on the materials, concentration, volumes used in the product and the method(s) of product disposal performed in compliance with federal, state, and local environmental regulations, it is

unlikely that the release of any of the substances of the product at the expected level of exposure will be harmful to the environment or toxic to organisms in the environment.

Questions for Information Request: The response was received August 4, 2017 and September 27, 2017. My review of the firm's responses to the following Information Requests dated June 6, 2017, July 25, 2017, September 25, 2017 and September 26, 2017 follows:

IR Responses dated August 4, 2017 (STN 125653/0/5)

1. Please verify that there was no impact to the facility and/or equipment (i.e. facility changes, new equipment, etc.) to establish the Zika Test Kit manufacturing at the (b) (4) facility. CBER Response: the response is adequate. RMS asserts that there is no impact to the facility and equipment to establish the **cobas**® Zika kit manufacturing at the (b) (4) facility.
2. Please provide a description and the results of the process validation studies to include manufacturing processes specific to the **cobas**® Zika Test Kit and **cobas**® Zika Test Kit Component Manufacturing (i.e. automated sample preparation, PCR amplification and detection which identify the critical parameters to be used as in-process control to ensure the success of routine production. CBER Response: The response is adequate. The final reports were provided and reviewed for the validation of the manufacturing process (formulation, bulk hold time, and filling) of **cobas**® 6800/8800 Zika MMX-R2 IVD, bulk (b) (4) and functional performance of the KIT COBAS 6800/8800 Zika 480T IVD M/N 07972466190, **cobas**® 6800/8800 Zika Positive Control (PC), bulk (b) (4) and vial M/N 08129738001 at Roche Molecular Systems (RMS), PCR Manufacturing Center (PMC), (b) (4) per protocol 201701-0042-BB-BLK-VP. The description and the results of the process validation studies are discussed under the Process Validation section of this memo.
3. Please provide microbial testing results to include bioburden, (b) (4) specific to the **cobas**® Zika Test Kit (480T) (7972466190) and Master Mix R2 (7972555001) to ensure lack of sample inhibition. CBER Response: The response is adequate. The **cobas**® Zika Test Kit is not a sterile product and is intended for single use only. The RMS Preservative Effectiveness Program a preservative system included in the formulation of the *in vitro* diagnostic reagents to address the potential introduction of microorganisms in the case that microorganisms are inadvertently introduced during or after the manufacturing process. The RMS Preservative Effectiveness Program is primarily based on (b) (4)
(b) (4)
(b) (4) The studies are intended to test (b) (4) to ensure the product meets its performance claims. The non-commercial **cobas**® Zika MMX-R2 vessel and Zika Positive Control were used in the study.

In accordance with SOP 280.01.08 RMS Preservative Effectiveness Program, testing was only performed for the new components (MMX-R2 and Positive Control) for the **cobas**® Zika Test Kit (480T) and **cobas**® Zika Control Kit (480T). The (b) (4) for pre-existing reagents (RNA IC, Elution Buffer and MMX-R1) was previously submitted and documented in DH-266-136G for the licensed test **cobas**® MPX (BLA STN# 125576). The non-commercial MMX-R2 Vessel, as well as the Zika Positive Control used in the study, were manufactured in the same facility in the final

packaging, and contain the same final formulation produced using the same process steps as in the commercial components of cobas® Zika: MMX-R2 Vessel (07972555001) and Zika Positive Control (08129738001). Therefore, the results of the (b) (4) using the non-commercial components were applied to the commercial components, and respective reagent kits, as shown in Table 8 below for a correlation between non-commercial and commercial M/N for the components and kits.

Table 8: Non-commercial and commercial material numbers for components and kits

(b) (4) Testing – non-commercial components	Corresponding commercial components	Corresponding commercial kit
MMX-R2 Vessel (b) (4)	MMX-R2 Vessel (M/N 07972555001)	cobas® Zika (480T) M/N 07972466190
Zika Positive Control (b) (4)	Zika Positive Control (M/N 08129738001)	cobas® Zika Control Kit (M/N 08129690190)

Bioburden testing will be conducted per SOP 380.03.04 during commercial kit release cobas® Zika Test Kit (480T) and cobas® Zika Control Kit.

(b) (4) Testing of test-specific MMX-R2 and PC control components was conducted to determine the effectiveness of the preservative in the components used in the cobas® Zika Test for use on the cobas® 6800/8800 Systems.

As required by SOP 090.04.03 “(b) (4) for IVD Reagents” the Testing is to be performed per the procedure described in the current (b) (4), using the procedure for (b) (4) products. Testing was performed on (b) (4) mL sample volumes. The test was performed by an external testing laboratory, (b) (4), following the (b) (4) protocol DH-04482.03-331A. The acceptance criteria required that there was no increase ((b) (4)) from the initial calculated count at (b) (4) of (b) (4). The following microorganisms: (b) (4)

(b) (4). As a result, the Zika MMX-R2 and Zika Positive Control of cobas® Zika passed time point (b) (4) of (b) (4) testing. An unplanned deviation occurred due to time point (b) (4) of the (b) (4) testing not being tested within (b) (4) after the date of manufacturing of the tested components as outlined in the study protocol (DH-04482.03-331A). Since testing at a later time point reflects a more stringent condition, this deviation was deemed acceptable and had no impact on the outcome of the study. The time point (b) (4) for the cobas® Zika Control Kit was not performed after (b) (4), as mentioned in the study protocol (DH-04482.01-331A). Since the current shelf life for control kit was assigned with 6 months (DH-04482.03-330B1), (b) (4) was tested at (b) (4) months, supporting the current shelf life. The Zika MMX-R2 (NC LBLD C68/88 Zika MMX-R2 480T VESSEL; (b) (4)) passed the time points up to 15 months and Zika Positive Control (NC LBLD C68/88 Zika ARNA PC (b) (4) ML IVD; (b) (4)) of the cobas® Zika test passed the time points up to 7 months and satisfy the (b) (4) for preserved samples.

4. You indicated under the addendums titled Test Method Validation Equivalency Final Report Addendum for Additional Materials that identical formulations of test materials can be incorporated into previous test method validations by an addendum without additional validation tests. Please provide a list of identically formulated test materials along with the documented history of revision and history of the performance of the **cobas**[®] Zika Test Kit. CBER response: The response is adequate. RMS asserts that there are two types of test method validation (TMV) reports in the BLA submission:
- Methods that are new and specific to **cobas**[®] Zika, which capture TMV for Zika specific reagents and kits: Zika assay kit, control kit, MMX-R2, Zika specific primers and probe (purification and synthesis) and Zika positive control (PC) stock
 - Existing methods for components used for **cobas**[®] Zika, which capture TMV previously completed for other existing products that were not specific for Zika but are needed for the Zika assay/manufacture, e.g., ZO5D (b) (4) DNA polymerase enzyme, enzyme UNG (b) (4), and Bulk Generic Specimen Diluent

The Zika specific TMV reports were documented as full validation reports except for those for primers/probe and the armored RNA for PC stock which were categorized as “additional materials” per SOP 430.01.09. The reports for these components were documented on “Test Method Validation Equivalency Final Report Addendum for Additional Materials” templates (SOP 430.01.09 EFH). There were no Zika specific components that met the criteria for “identically formulated test materials”. In the TMV reports for non-Zika specific components (type 2 reports), “identically formulated materials” were identified and were documented using the “Test Method Validation Equivalency Addendum for Identical Materials” template (SOP 430.01.09EFB). The test method validation, however, was completed for existing products and was not repeated during the development of **cobas**[®] Zika.

5. Provide documentation to illustrate that a risk analysis was performed for the revised formulation for the **cobas**[®] Zika Positive Control and the **cobas**[®] Zika Negative Control. CBER Response: The response is adequate. RMS would like to clarify that the existing risk analysis adequately addresses the identified risks. There were no new risks identified for the revised formulation of the **cobas**[®] Zika Positive Control and the **cobas**[®] Zika Negative Control, thus no additional impact analysis was conducted for the switch.

The negative control is a commercial control which is used for other licensed blood screening assays (**cobas**[®] MPX, **cobas**[®] WNV) and therefore no additional risk for the negative control was identified.

To demonstrate the usability of the new positive control kit, release data of three batches of the positive control were assessed. This is documented in DH-04482.03-008B (Attachment 7). (b) (4) replicates from three batches of the positive control were tested, all results were valid and within the defined target specific Ct-range.

6. If applicable, in regards to purchasing controls, please describe the controls and measures in place to ensure that the supplier(s) meet the specified requirements. CBER Response: The response is adequate. RMS has multiple levels of control over suppliers of raw materials which include determining material/supplier criticality, supplier audits, evaluation of suppliers, supplier acceptance of RMS specifications, qualification of suppliers, performance monitoring of suppliers and supplier corrective action. A listing of relevant SOPs was included and reviewed.

Specifications (a selection of test attributes and variables with acceptance criteria that are material specific to determine material acceptability) were written for direct materials following SOP 100.03.21, "Development of a Quality Control Raw Material Specification" and SOP 180.06.01, "Raw Material Testing Requirements". Once prospective suppliers of direct materials are identified and have been subject to an initial evaluation, they are provided with a copy of RMS specifications for the requested material and are asked to review the specification to assure that the material that they supply can meet the necessary requirements. This process is described in SOP 440.06.02, "Supplier Review and Acceptance of RMS Raw Material, Packaging and Labeling Specifications." Acceptance of RMS specifications is one input into the qualification process.

RMS controls the acceptance activities of a supplier's products and services through multiple functions including material criticality, testing or inspection of incoming materials against pre-established specifications, the supplier's historical performance of same or similar materials or components, supplier review and acceptance of RMS specifications, and results of supplier audits.

7. You mentioned that manufacturing is campaigned in each production area followed by a cleaning procedure. Please clarify whether manufacturing is campaigned per lot or by product. CBER Response: The response is adequate. RMS asserts that manufacturing is campaigned per lot and after each lot is manufactured, a cleaning takes place prior to subsequent production.

IR Responses dated September 27, 2017 (STN 125653/0/14)

8. Please clarify whether requalification was performed for any of your cleaning processes. If so, specify. Is equipment multi-use? CBER Response: The response is adequate. The Zika reagents were assessed against the most difficult to clean reagents for each piece of multi-use equipment per the validated and approved SOP 430.02.10. RMS stated no need for requalification due to the inclusion of Zika reagent manufacturing because no Zika reagent was considered more difficult to clean than the existing reagents of approved in-vitro diagnostic products.

Limits may be set which examine the potential adverse effects for product function in the event of cross contamination and contamination with cleaning agent residue. RMS's in-vitro diagnostic products are not directly used in or on the human body. Their rationale for their

approach to cleaning validation is based on the intended use of the products, the composition of the product, and the technology upon which the products are designed. RMS uses (b) (4) to show removal of (b) (4) and (b) (4) show removal of product residuals. Other tests may be applied based on the specific product requirements. The bioburden of PCRW is (b) (4) and the bioburden specification for some RMS products is (b) (4); therefore, the bioburden specification for a RMS cleaning validation is (b) (4) for rinse water samples of clean equipment.

RMS validates the cleaning processes with the reagents that are considered the most difficult to clean product (highest risk soil), degradation product, or contaminant (SOP 430.02.10, Conducting Equipment Cleaning Validation). Representative contaminants from each contaminant family (chemical (cleaning agents/buffer preparation soils), downstream formulation, fill soils, biological, microbiological) must be identified for testing. Then, the most concentrated contaminants in each family must be determined. The worst-case contaminant is determined through quantitative experimentation and visual inspection according to SOP 430.02.11, Evaluation of the Most Difficult to Clean Product/Material that Contacts Manufacturing Surfaces. The most difficult to clean product/material will be used for the cleaning validation. If the new soils are easier to clean than the most difficult soil already being cleaned by a validated procedure, introduction of the new material using existing cleaning procedures can be made with confidence (PDA Technical Report #49). The clean-ability of the cleaning process was evaluated through the (b) (4) Methods and (b) (4) in areas where (b) (4) is not removed by the (b) (4) represent the most difficult to clean areas of the equipment.

9. Please submit a revised claim for a Categorical Exclusion to include both the CFR citation and confirmation that you are not aware of any extraordinary circumstances that require the preparation of an environmental assessment. CBER Response: The response is adequate. RMS revised the statement to include no awareness of any extraordinary circumstances that would require the preparation of an environmental assessment.

Bioburden Testing

The **cobas**[®] Zika Test Kit is not a sterile product and is intended for single use only. The RMS Preservative Effectiveness Program is a preservative system included in the formulation of the *in vitro* diagnostic reagents to address the potential introduction of microorganisms in the case that microorganisms are inadvertently introduced during or after the manufacturing process. The RMS Preservative Effectiveness Program is primarily based on (b) (4) and if the (b) (4) results do not meet the acceptance criteria, the testing can be extended by an additional (b) (4) product studies. The studies are intended to test (b) (4) to ensure the product meets its performance claims.

In accordance with SOP 280.01.08, RMS Preservative Effectiveness Program, testing was only

performed for the non-commercial new components (MMX-R2 and Positive Control) for the **cobas**[®] Zika Test Kit (480T) and **cobas**[®] Zika Control Kit (480T). The (b) (4) for pre-existing reagents (RNA IC, Elution Buffer and MMX-R1) was previously submitted and documented in DH-266-136G for the licensed test **cobas**[®] MPX (BLA STN# 125576). The non-commercial MMX-R2 Vessel, as well as the Zika Positive Control used in the study, were manufactured in the same facility in the final packaging, and contain the same final formulation produced using the same process steps as in the commercial components of **cobas**[®] Zika: MMX-R2 Vessel (07972555001) and Zika Positive Control (08129738001). Therefore, the results of the (b) (4) using the non-commercial components were applied to the commercial components and their respective reagent kits.

RMS' procedures for the quantification of bioburden levels in (b) (4) vial components was provided and reviewed to include details concerning sampling, testing, evaluation of results and retesting. (b) (4)

Vial components may be tested in place of (b) (4) if (b) (4) is not available. Bulk Normal Human Plasma (NHP) is purchased from (b) (4) by RMS for use in RMS products if the purchased materials meet RMS quality requirements. Product Quality Specifications (PQS) documents for NHP include bioburden specifications, require that bioburden testing of NHP be performed by the vendor, and require that vendor certificates of analysis include bioburden results. Filled components for bioburden testing are collected by the Operations department after the completion of the filling process. The number of vials to be removed for bioburden testing depends on the fill volume of the vial component as specified in the filling batch records. The diluent used for bioburden testing of a product is determined based on results from inhibition testing and three bioburden test runs for that product. The product tables will be updated as required based on the testing outcomes. (b) (4) of bioburden samples must be performed within the confines of a certified bio-safety cabinet. Prior to (b) (4) of vial components, the contents of the vials must be (b) (4) into sterile containers.

RMS conducts bioburden testing in accordance with (b) (4) through monitoring of the test environment ((b) (4)), diluent controls, media controls and microbial identification. Exposure of all (b) (4) occurs during the sample preparation and testing by (b) (4). Upon completion of the (b) (4) and placement of the (b) (4)

The results are expressed in (b) (4)

(b) (4)

Bioburden testing will be conducted per SOP 380.03.04 during commercial kit release **cobas**[®] Zika Test Kit (480T) and **cobas**[®] Zika Control Kit.

Bulk and Fill and Closure Systems

The CCIT methods are not applicable to microbiologically controlled *in vitro* liquid components. A preservative system is included in the formulation of *in vitro* diagnostic reagents to address the potential introduction of microorganisms if microorganisms are introduced inadvertently during or after the manufacturing process. (b) (4) acceptance criteria were established to meet the current (b) (4) requirements for (b) (4) products.

RMS provided a report to include the Container and Closure Systems used for **cobas**[®] Zika, **cobas**[®] MPX, **cobas**[®] WNV, Omni Reagents and Common Components. Specifically, the report included the bulk and fill containers and closures container composition, closure type, bulk/use, supplier and address, chemical resistance, chemical treatment and indication details as noted below in Tables 9-15.

(b) (4)

(b) (4)

(b) (4)

(b) (4)

Table 13. Fill Containers and Closures

Container/Material Number	Closure/Material Number	Where Used (Component)	Supplier
VESSEL COBAS 68/8800 34ML PP BLACK 7002670001	PLUG COBAS 68/8800 RGT VES PP NATURAL 7002645001 ROLL STOCK VESSEL INDUCTION SEAL PP 1" 6949355001	LBDL COBAS 6800/8800 PASE 96T VESSEL LBDL COBAS 6800/8800 EB 96T VESSEL LBDL COBAS 6800/8800 MMX-R1 480T VESSEL LBDL COBAS 68/8800 MPX MMXR2 480T VESSEL LBDL COBAS 68/8800 WNV MMXR2 480T VESSEL LBDL C68/88 ZIKA MMX-R2 480T VESSEL LBDL COBAS 6800/8800 RNA IC 96T VESSEL	(b) (4)
VESSEL COBAS 68/8800 54ML PP BLACK 7002653001	PLUG COBAS 68/8800 RGT VES PP NATURAL 7002645001 ROLL STOCK VESSEL INDUCTION SEAL PP 1" 6949355001	LBDL COBAS 6800/8800 PASE 480T VESSEL LBDL COBAS 6800/8800 EB 480T VESSEL LBDL COBAS 6800/8800 RNA IC 480T VESSEL	(b) (4)

Table 14. Fill Containers and Closures cont'd

Container/Material Number	Closure/Material Number	Where Used (Component)	Supplier
VESSEL COBAS 68/8800 14ML PP BLACK 7002661001	PLUG COBAS 68/8800 RGT VES PP NATURAL 7002645001 ROLL STOCK VESSEL INDUCTION SEAL PP 1" 6949355001	LBD COBAS 6800/8800 MMX-R1 96T VESSEL LBD COBAS 68/8800 MPX MMX-R2 96T VESSEL LBD COBAS 68/8800 WNV MMX-R2 96T VESSEL	(b) (4)
VESSEL/CSTE COBAS 68/8800 200ML PP BLACK 7002629001	ROLL STOCK MGP INDUCTION SEAL PP 1.44" 6949347001	KIT COBAS 6800/8800 MGP IVD	
BOTTLE 5L SQUARE NATURAL 7002572001	SCREW CAP SEALING LIP WHITE KS40 4647254001	KIT COBAS 6800/8800 WASH IVD	
BOTTLE 1L RECT RIGHT HAND HDPE NATURAL 7002602001	SCREW CAP SEALING LIP WHITE KS40 4647254001	KIT COBAS 6800/8800 SPEC DIL REAGENT IVD	

Table 15. Fill Containers and Closures cont'd

Container/Material Number	Closure/Material Number	Where Used (Component)	Supplier
BOTTLE 1L RECT LEFT HAND HDPE BLACK 7002599001	SCREW CAP PP SEALING LIP BLACK KS40 6464815001	KIT COBAS 6800/8800 LYS REAGENT IVD	(b) (4)
Tube 2.0 mL PP Natural 5163668001	CLOSURE NAT w/FOIL 13mm PPCO 6906648001	LBD COBAS 6800/8800 NHP NEG CTL 1.0 ML	
Tube 2.0 mL PP Natural 5163668001	CLOSURE RED w/FOIL 13mm PPCO 6906644001	LBD COBAS 6800/8800 MPC POS CTL 1.0 mL	
Tube 2.0 mL PP Natural 5163668001	CLOSURE BLUE w/FOIL 13mm PPCO 6906672001	LBD COBAS 68/8800 HIV-1-O POS CTL 1.0ML LBD C68/88 ZIKA ARNA PC 1.0 ML	
Tube 2.0 mL PP Natural 5163668001	CLOSURE WHITE w/FOIL 13mm PPCO 6906656001	LBD COBAS 6800/8800 HIV2 POS CTL 1.0 ML	
Tube 2.0 mL PP Natural 5163668001	CLOSURE ORANGE w/FOIL 13mm PPCO 6906737001	LBD COBAS 6800/8800 WNV POS CTL 1.0 ML	

Environmental Control

RMS monitors three primary potential sources of contamination to include environmental sources, contamination from personnel, and manufacturing procedures and materials, including cleaning and preparation of equipment and containers. Specifically, two types of contamination control are practiced: (1) control of potential microbiological contamination, and (2) control of potential nucleic acid contamination. The methods used to control contamination include: segregated manufacturing areas designed to maintain the proper environment of each area and to prevent cross contamination; use of

HEPA filtered environmentally controlled areas for manufacture, as appropriate; controlled air pressure differentials in the manufacturing and filling rooms; use of laminar air flow hoods for defined manufacturing activities; restricted personnel access to manufacturing areas; use of closed manufacturing systems whenever possible; use of 0.2 µm filtration on appropriate reagents as the product is not sterile; requirements for clean room attire, including full or partial gowning as applicable, use of disinfecting and sanitizing agents with periodic rotation to control microbiological contamination; and during the use of (b) (4) cleaning solutions to control oligonucleotide and nucleic acid contamination. Lastly, restrictions in the movement of personnel between areas where amplified DNA is present or generated, i.e., PCR amplification testing laboratories, high concentrations of nucleic acid presence, i.e. oligo manufacturing rooms, areas where DNA/RNA controls and standards are manufactured, and controlled manufacturing areas that are free of both target and amplified DNA (Bulk Manufacturing and Filling Areas).

Cleaning Procedures

Manufacturing is campaigned per lot in each production area followed by a cleaning procedure which mitigates cross contamination prior to subsequent production. Cleaning and sanitizing procedures and schedules have been established for the manufacturing areas in the (b) (4) facilities to reduce the level of microorganisms and nucleic acids that could potentially affect the performance of the products. These procedures control microbiological and nucleic acid contamination in the manufacturing areas.

Cleaning is conducted in manufacturing areas using methods appropriate for the processes performed in each area. Area-specific SOPs describe the cleaning of the equipment and work surfaces (for example, benchtops) in the area. Qualified cleaning agents are (b) (4) and qualified sanitizers are used as needed to eliminate any spore forming microorganisms.

Only personnel who have been trained in procedures used for cleaning are authorized to clean in the production areas.

Validated cleaning agents, including disinfectants and sanitizers (sporicides), are specified by SOP 110.02.07. Disinfectants are (b) (4) and sanitizing agents are used as needed or in response to elevated environmental monitoring results. Cleaning of walls, floors, doors, ceilings, vents, windows, pass-thru areas, (b) (4) mats (or large tacky mats), cabinetry, and bench tops are scheduled and performed per SOP 110.02.08.

Records are kept to ensure that rooms are cleaned in accordance with the existing SOPs. Each cleaning activity and the cleaning agent used are recorded on the Room Activity Logbook.

Processing equipment is cleaned using methods defined in written batch records, standard operating procedures, and/or maintenance procedures. Procedures are specific for the equipment being cleaned and are performed following a set schedule. The cleaning of major equipment is documented in the equipment log.

Environmental Monitoring

The environmental monitoring program has been established for all environmentally controlled manufacturing areas in the (b) (4) facilities. Environmental monitoring is performed in accordance with the established Environmental Monitoring Master Plan for the PCR Manufacturing Center (0706-742-BB-EM-MP), existing SOPs which specify the rooms to be monitored, the procedures to be used for sampling and measuring, and the frequency of the monitoring, as well as the use of SAP and the forms used for recording the results of the monitoring procedures and for describing the corrective actions to be taken if alert and/or action levels are exceeded. These procedures are described in more detail below and are summarized below in Tables 16, 17 and 18.

The environmental monitoring program is intended to demonstrate the stability of the controlled manufacturing environments and the effectiveness of the environmental control procedures based on information gathered during facility and process validations activities to include monitoring for airborne viable particles, airborne non-viable particle levels, and surface microbiological quality. The parameters are monitored in the environmentally controlled areas after cleaning is performed and are used to demonstrate the efficacy of the cleaning agents and effectiveness of the cleaning procedures, stability of the environment, and proper operation of the air handling units. Since product manufacture is conducted under conditions which are categorized as microbiologically controlled, and not aseptic or sterile, the use of static (before operations) sampling has been incorporated into the applicable SOPs. RMS does not utilize environmental monitoring data for product release decisions, though, bioburden testing is performed on components of blood screening test kits and specifications have been established.

Room-to-room air pressure differentials (Delta-Ps) are monitored continuously by the automated Building Management System (BMS). The BMS operates continuously and personnel monitor the alarms continuously. When a Delta-P excursion is detected, the BMS activates visual alarms within the affected suite, and issues alarms to active BMS work stations. When the condition is cleared, the visual alarm inside the suite ceases and the BMS workstation alarm changes status and displays it as an inactive alarm.

If a Delta-P condition persists in any suite, the visual and BMS alarms remain active. If such alarms occur and persist during working hours, suite occupants follow their internal protocol for stopping or containing production, and they notify the Facilities Department, which investigates to identify and remediate the cause. If the conditions occur during nights or weekends, the Facilities Department identifies them in the daily morning alarm review, initiates remediation, and notifies the affected department prior to commencement of manufacturing, if not remedied.

Monitoring of surfaces and air for microbes and non-viable particles are performed using surface contact plates, a centrifugal air sampling device, and a laser particle counter, respectively, on an established schedule. Samples are collected in the specified rooms and support areas after the room has been cleaned and prior to (before) manufacturing operations. Dynamic samples are taken from the areas where critical manufacturing such as, Bulk formulation and Filling processes occur. Alert and action levels have been established for microbiological levels in air and on surfaces and for non-viable particle levels in air.

An Environmental Nonconformance (ENC) Investigation procedure per SOP 220.03.03 is followed to

evaluate those results that exceed the alert or action level and to initiate corrective actions should they be necessary. SOPs define the schedule and specific areas and sites to be sampled and test results are recorded in the SAP database System. If action levels are exceeded, corrective action is taken as defined in written SOPs. The effectiveness of corrective actions is confirmed by follow-up environmental monitoring.

Table 16. Building ^{(b) (4)} PMC Environmental Monitoring Procedures and Criteria

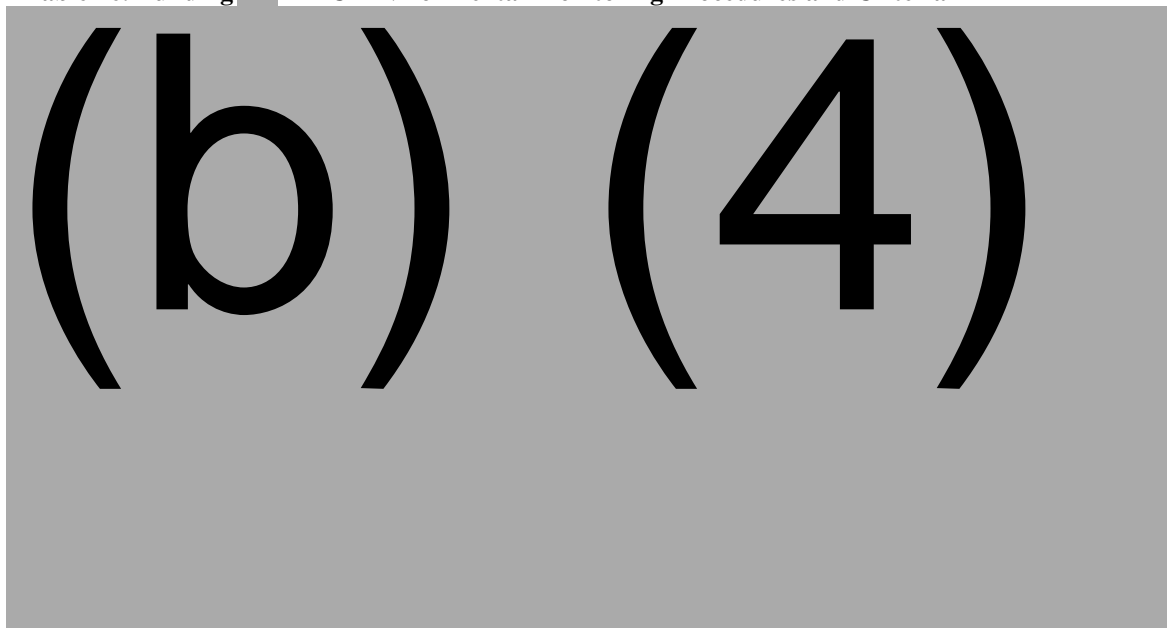
A large gray rectangular area covering the entire content of Table 16, indicating that the table's data has been redacted. The text "(b) (4)" is printed in large, bold, black font across the top of this area.

Table 17. Other Environmentally Controlled Manufacturing Areas

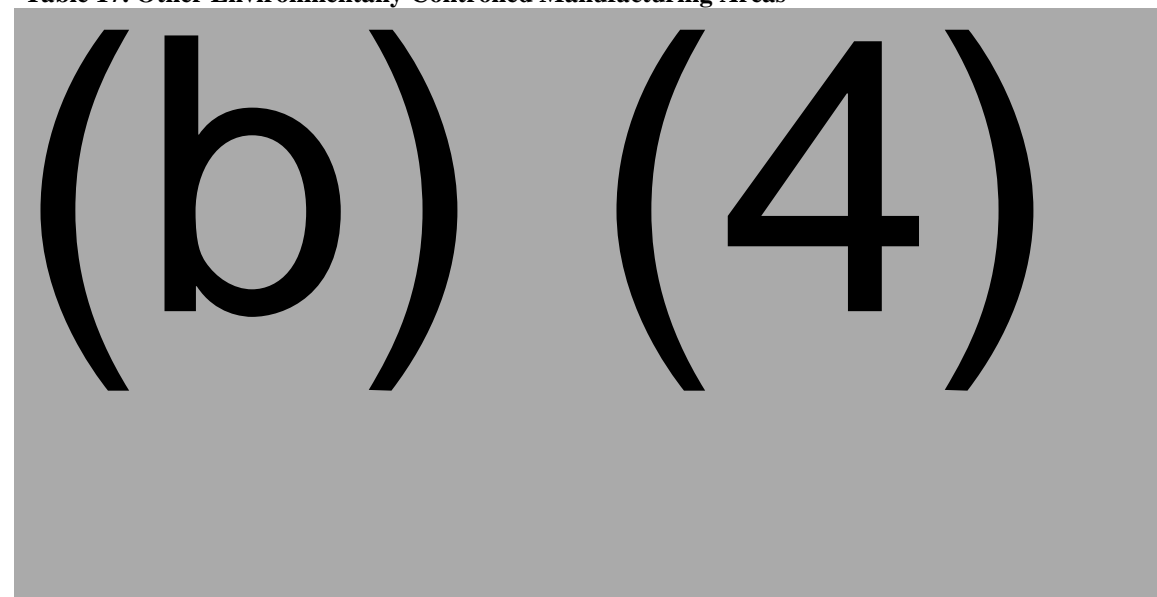
A large gray rectangular area covering the entire content of Table 17, indicating that the table's data has been redacted. The text "(b) (4)" is printed in large, bold, black font across the top of this area.

Table 18. Building ^{(b) (4)} Manufacturing Areas-Monitoring Procedures and Criteria

(b) (4)

Process Validation

Process Validation studies were only performed for the new components (MMX-R2 and Positive Control) for the **cobas**[®] Zika Test Kit (480T) and **cobas**[®] Zika Control Kit (480T) as the pre-existing reagents (RNA IC, Elution Buffer and MMX-R1) were previously submitted and documented in DH-266-136G for the licensed test **cobas**[®] MPX (BLA STN# 125576). The manufacturing processes of **cobas**[®] 6800/8800 Zika MMX-R2 and **cobas**[®] 6800/8800 Zika Positive Control were evaluated to verify that the processes consistently delivered products (Zika MMX-R2, Test Kit and Positive Controls) that met the established acceptance criteria. The final reports were provided and reviewed for the validation of the manufacturing process (formulation, bulk hold time, and filling) of **cobas**[®] 6800/8800 Zika MMX-R2 IVD, bulk (b) (4) and functional performance of the KIT COBAS 6800/8800 Zika 480T IVD M/N 07972466190, **cobas**[®] 6800/8800 Zika Positive Control (PC), bulk M/N 0(b) (4) and vial M/N 08129738001 at Roche Molecular Systems (RMS), PCR Manufacturing Center (PMC), (b) (4) according to protocols 201701-0042-BB-BLK-VP and 201701-0043-BB-CON-VP.

Three (3) Process Operational Qualification (pOQ) bulk batches (A - C), three (3) Process Performance Qualification (pPQ) 480T vessel batches (1 - 3) of **cobas**[®] 6800/8800 ZIKA MMX-R2 IVD were manufactured and met all specifications and acceptance criteria per Production Batch Record (PBR) 7972440001, Filling Labeling Record (FLR) 7972555001 and protocol 201701-0042-BB-BLK-VP. Additionally, three unique combinations of components were tested using the kit method and met the acceptance criteria of the protocol demonstrating that the new master mix reagent functions in the kit together with the existing reagents. Each batch (Bulk batches A-C) was sampled and tested for kit function (SOP 335.01.53) on DOC and at the end of bulk hold time ((b) (4) from DOC). Additionally, each bulk batch and vessel batch were tested for Bioburden (SOP 380.03.04) on DOC, the end of bulk hold time and final fill day. The batches were subsequently filled (Vessel batches 1 – 3) utilizing the (b) (4) Filling and Capping Machine per FLR7972555001. Vessel batches 1 - 3 were tested at 100% of filling for function using the kit test method per SOP 335.01.53 and met the acceptance criteria as documented in the SAP Business System for (b) (4). All test results met the

acceptance criteria of the protocol. As noted in the Tables 19A, 19B, 19C, 20A, 20B and 21 below.

One deviation, Deviation: 327847, occurred during the execution of this protocol. Bioburden testing was performed on DOC, DOF and 100% of the filling process utilizing red-lined SOP 380.03.04A, prior to the document becoming approved and effective. Batches (b) (4) [REDACTED] were impacted. The cause of this nonconformance was due to ineffective communication of the validation documentation requirements during training. The redlined SOP 380.03.04A used during testing of the sample materials was compared against the effective version of the SOP and it was determined that there was no difference. Therefore, the diluents and dilution factors used during testing of the sample materials were correct. As such, there is no impact to product. This nonconformance had no negative impact to the process validation. A communication was initiated to communicate the findings of this nonconformance to Process Validation personnel responsible for performing training on a validation protocol to ensure that all validation protocol requirements are covered during training. No CAPA was required and no revision to pFMEA was required.

2 pages have been determined to be not releasable: (b)(4)

(b) (4)

For the **cobas**[®] 6800/8800 Zika PC, three (3) Process Operational Qualification (pOQ) validation bulk batches (A - C) and three (3) Process Performance Qualification (pPQ) validation vial batches (1 - 3) of **cobas**[®] 6800/8800 Zika PC were manufactured and filled and met all specifications and acceptance criteria Production Batch Record (PBR8129681001), Filling Labeling Record (FLR08129738001) and protocol 201701-0043-BB-CON-VP. Each batch (Bulk batches A-C) was sampled and tested for kit function (SOP 335.01.54) on DOC and at the end of bulk hold time (b) (4) from DOC). Additionally, each bulk batch was tested for Bioburden (SOP 380.03.04) on DOC, the end of bulk hold time and final fill day. The batches were subsequently filled (Vial batches 1 – 3) utilizing the (b) (4) Filling and Capping Machine in Building (b) (4), Room (b) (4) per FLR8129738001. Vial batches 1 - 3 were tested at 100% of filling for kit function (SOP 335.01.54) and for Bioburden (SOP 380.03.04). All test results met the acceptance criteria of the protocol.

Two (2) deviations, Deviations: 327847 and 32860, occurred during the execution of this protocol. Deviation 327847: Bioburden testing was performed on DOC utilizing red-lined SOP 380.03.04A, prior to the document becoming approved and effective. Batches (b) (4) were impacted. The cause of this nonconformance was due to ineffective communication of the validation documentation requirements during training. The redlined SOP 380.03.04A used during testing of the sample materials was compared against the effective version of the SOP and it was determined that there was no difference. Therefore, the diluents and dilution factors used during testing of the sample materials were correct. As such, there is no impact to product. This nonconformance had no negative impact to the process validation. A communication was initiated to communicate the findings of this nonconformance to Process Validation personnel responsible for performing training on a validation protocol to ensure that all validation protocol requirements are covered during training. No CAPA was required and no revision to pFMEA is required.

Deviation 328601: An unplanned deviation occurred during the filling process of the **cobas**[®] Zika Positive Control vial batch (b) (4) when the filling team noticed the caps/closures were leaking. This issue is due to the identification of a nonconforming batch of 6800/8800 closures with foil during filling of the validation batch. As the closure batch was manufactured by the vendor prior to the implementation of additional QC testing corrective action, the nonconforming closures were not identified during vendor release, and consequently were received by RMS and used in filling. The filling team stopped using the caps/closures once they noticed the leaks and switched over to a different (non-leaking batch) of caps. Since the validation batch was completed using an alternate batch of caps, there was no impact to the validation.

1 page has been determined to be not releasable: (b)(4)

The data demonstrates the process for the manufacturing (formulation, hold time, and filling) of **cobas**[®] 6800/8800 ZIKA MMX-R2 and ZIKA Test Kit is reproducible and consistently meets established acceptance criteria. Furthermore, the data demonstrates that the new ZIKA Master Mix functions in the kit together with the existing MMX-R1, RNA IC, EB and PASE components. The validation of the manufacturing process for the **cobas**[®] 6800/8800 ZIKA MMX-R2 and ZIKA Test Kit is considered complete.

cobas[®] 6800/880 Zika Positive Control validation bulk batches A - C and vial batches 1 - 3 were formulated, held and filled and met all specifications and acceptance criteria of the material as documented in the SAP business system and validation protocol 201701-0043-BB-CON-VP. The test results provide sufficient evidence to demonstrate that the manufacturing process of the **cobas**[®] 6800/8800 Zika Positive Control result in positive controls that meets established acceptance criteria.

References:

- CBER SOPP 8401.4: Review Responsibilities for the CMC Section of Biologic Applications and Supplements, April 2005
- CBER SOPP 8410 “Determining When Pre-Licensing/Pre-Approval Inspections are Necessary
- 21 CFR Part 820 – Quality System Regulation
- Guidance for Industry: Content and Format of Chemistry, Manufacturing and Controls Information and Establishment Description Information for a Biological *In Vitro* Diagnostic Product, March 1999
- Guidance for Industry and FDA Staff: Quality System Information for Certain Premarket Application Reviews, August 1999

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