



DEPARTMENT OF HEALTH & HUMAN SERVICES

Food and Drug Administration
Center for Biologics Evaluation and Research
Office of Blood Research & Review
Division of Hematology Research & Review

Internal Memorandum

September 18, 2017

STN: BL125653

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Reviewer: Krishna Mohan V. Ketha, Ph.D., PRB/DETTD/OBRR

Through: David A. Leiby, Ph.D, Chief, PRB/DETTD/OBRR
Pradip N. Akolkar, Ph.D. Team Lead, PRB/DETTD/OBRR

To: Vasantha Kumar, PhD, RPM, OBRR

Product: cobas ZIKA NAT

Specific Scientific Disciplines Reviewed: Non-clinical

Recommendation: No additional comments

Documents reviewed: All Documents in Non-Clinical Pharmacology & Toxicology section

Summary:

This is a BLA submission from Roche Molecular Systems for the cobas ZIKA NAT and has been assigned a 6-month priority review. I have been assigned to review the **Non-Clinical** section (included in section Non-clinical Pharmacology and Toxicology) of the submission. This is a mid-cycle memo requesting additional information from the sponsor.

INTENDED 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.

Documents Reviewed: Non-Clinical Section

A. PDF Documents

1. **DH-266-062F**- *Summary report Cross Contamination*
2. DH-04482.01-029- *Investigational Assay Analytical Studies Report*
3. DH-04482.03-107F- *Supplemental Cross-reactivity / Analytical Specificity*
4. DH-04482.03-109F- *Summary Report: Exogenous Interferences*
5. DH-04482.03-115F- *Summary Report : Clinical Specimen Stability*
6. DH-04482.03-122F- *Summary Report: On Board and Open Kit Stability*

B. Excel Spreadsheets-Line Listing

1. 04482.03-107D_LL
2. 04482.03-109D_LL
3. 04482.03-115D_LL
4. 04482.03-122D_LL_480T
5. 04482.03-122D_LL_RMC
6. DH-04482.01-029_3_LoD_LL
7. **DH-266-062D**_Cross Contamination_19Jan2015
8. DH-04482.01-029_4_Specificity_LL
9. DH-04482.01-029_5.2.3.titer_assignment_LL
10. DH-04482.01-029_5.3.1_confirmed_specimens_LL
11. DH-04482.01-029_5.3.1_additional_specimens_LL
12. DH-04482.01-029_5.3.3_contrived_specimens_LL
13. DH-04482.01-029_6_Detection_at_LoD_LL
14. DH-04482.01-029_7_Endogenous_Interference_LL
15. DH-04482.01-029_8_Cross_Reactivity_LL
16. DH-04482.01-029_9_Matrix_Equivalency_LL

1. Limit of Detection (LoD)

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. The stock titer was provided by the vendor (BNI, Bernhard Nocht Institute, Hamburg, Germany), and it was assigned using a serial dilution of Zika RNA transcript for which concentration was determined by photometric absorbance. A total of three (3) independent panels of 5 concentration levels plus blank were prepared by three different operators. Each panel (dilution series) consists of 5 concentration levels (16, 12, 8, 4, 2 cp/mL, and a blank-0 cp/mL).

Figure 1: Study Design Limit of Detection

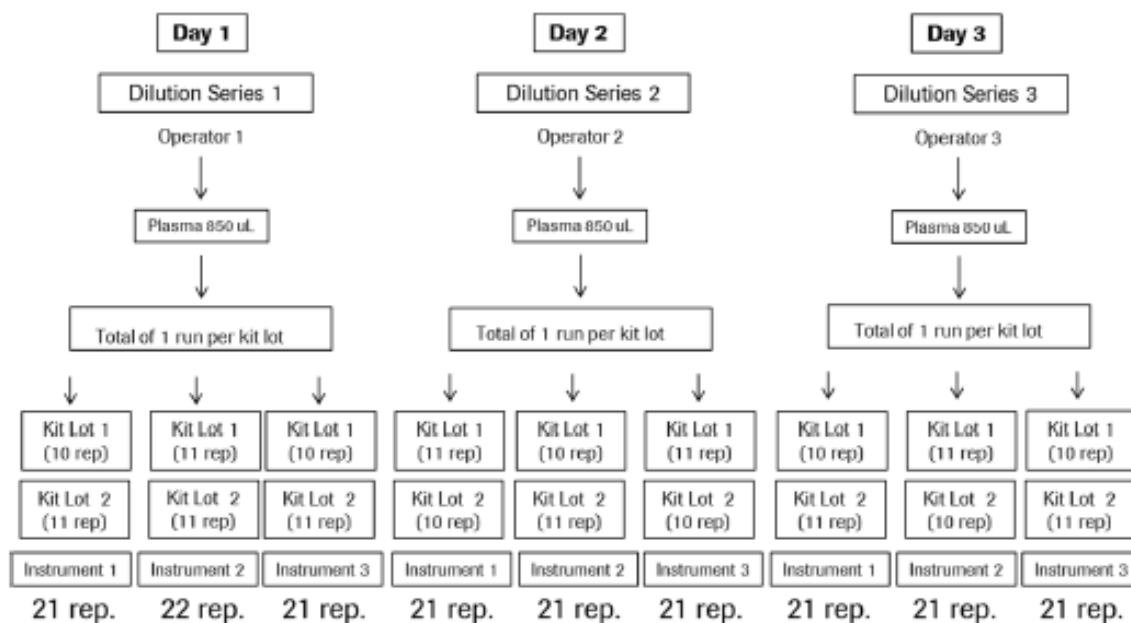


Table 4: Summary LoD for all lots combined

Zika RNA concentration (cp/mL)	Number of reactives	Number of valid replicates	% reactive	95% lower confidence 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%
LoD by PROBIT analysis (95% Reactive Rate)	8.1 cp/mL 95% confidence range: 6.1 – 13.6 cp/mL			

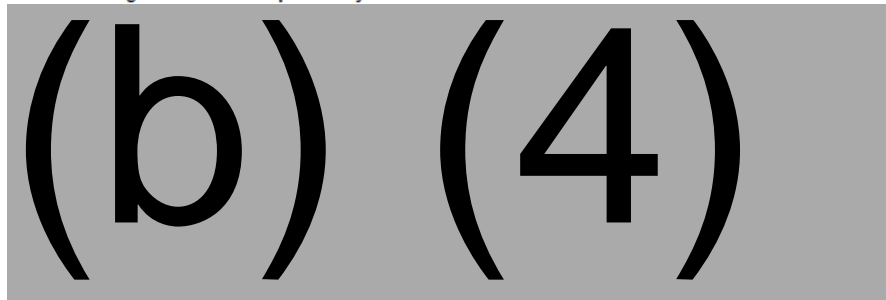
2. Repeatability

Results from valid runs were used to determine the Limit of Detection / Analytical Sensitivity. 19 runs were performed in this study, of which 18 runs were valid. One run was invalid due to a run abort (no data available).

Since at least 20 of 21 replicates for each concentration level tested on each day were valid, the invalid sample was not repeated. Invalid, results were excluded from data analysis.

a. Lot-to-Lot

Table 8: Reagent Lot-to-Lot Repeatability Results



b. Day-to-day

Table 9: Day-to-Day Repeatability Results

c. Instrument-to-Instrument

Table 10: Instrument-to-Instrument Repeatability Results

The **cobas®** Zika IA Limit of Detection for Zika in EDTA plasma based on 95% Probit analysis for all lots combined is **8.1 cp/mL** (CI 6.1-13.6 cp/mL).

Additional analysis of four Zika concentrations (16 cp/mL, 12 cp/mL, 8 cp/mL and 4 cp/mL) across three separate days/operators, two reagent lots and three instruments, showed overlapping 95% confidence intervals for the reactive rates. These results indicate that the **cobas®** Zika IA test is reproducible over multiple days/operators, reagent lots and instrument.

Reviewer Comments:

- a. Both the LoD and Repeatability Studies were performed using only 2 lots. RMS had proposed using three lots for these studies in the Pre-submission.
The above comment was resolved during the Internal Meeting (07/06/17) and the data for two lots was found acceptable by the DETTD management.
- b. RMS states using three reagent lots in Section 3.3.2. - "Combined LoD Results for Zika in EDTA plasma over 3 reagent lots", while providing data for only two lots.
- c. Software alert: All these studies were performed using the Software version 01.01.09; ASAP version 9.0.0.

3. Specificity

 negative EDTA-plasma samples tested: 0 positives –  (99.3 – 100%)

Table 12: Summary Specificity

No. of Valid Replicates	Percent of Non-reactive results Zika	Acceptance Criteria
(b) (4) negative individual EDTA-plasma specimens	100% (99.3 – 100%)	Specificity (b) (4)

Reviewer Comments: Data acceptable

4. Analytical Sensitivity

Multiple samples from recent Zika outbreaks were tested. NAT-positive Zika specimens were identified in an in-house multi-step process, first by screening (b) (4)

(b) (4)

(b) (4)

(b) (4)

All 25 positive by both cobas ZIKA and CDC

(b) (4)

(b) (4)

Contrived specimen

(b) (4)

(b) (4)

(b) (4)

Reviewer Comments: No additional comments

5. Detection of ZIKV isolates at LoD

(b) (4) runs were performed in this study, of which (b) (4) runs were valid. The (b) (4) valid runs contained (b) (4) valid results ((b) (4)).

Table 23. Zika Results for samples diluted to near the Limit of Detection

Specimen ID	cobas® Zika (Ch 3) Ct		cobas® Zika (Ch 3) RFI		Overall Result
	mean	SD	mean	SD	
(b) (4)					21/21, 100% Reactive
					21/21, 100% Reactive
					21/21, 100% Reactive
					21/21, 100% Reactive
					21/21, 100% Reactive

All replicates (21/21, 100%) of each of the five confirmed Zika positive specimens were detected at ~13.6 copies/mL. This study demonstrated that the **cobas®** Zika test for use on the **cobas®** 6800/8800 Systems is capable of detecting isolates near the Limit of Detection of the test.

Reviewer Comments: No additional comments

6. Endogenous Interference

The effect of endogenous potentially interfering substances on the sensitivity and the specificity of the **cobas®** Zika IA test was determined by testing (b) (4) normal negative EDTA-plasma specimens that were spiked with one of the potential interferents albumin, bilirubin, hemoglobin, triglycerides and human DNA, as well as the respective interferent-free normal negative EDTA-plasma specimens used as controls for the study). The interferent spiked samples and the non-spiked samples were tested with ~3xLoD of Zika target (sensitivity) and without Zika target (specificity).

Levels of albumin, bilirubin, hemoglobin, and triglycerides for the spiked samples tested were those recommended by the (b) (4). Since human DNA levels are not listed in the (b) (4) guidelines, 2.0 mg/L was tested, which is significantly above the average naturally occurring DNA level (~0.04 mg/L) and the expected maximum naturally occurring DNA level (~0.24 mg/L).

Table 27: Sensitivity of the cobas[®] Zika test

Interferent	Source of Interferent ^a	Channel	Reactivity		CT values		RFI Values	
			#	%	Mean	SD	Mean	SD
Albumin	Spiked	3 (Zika)	(b) (4)	100	(b) (4)			
		5 (RNA IC)		100				
	N/A (PSC)	3 (Zika)		100				
		5 (RNA IC)		100				
Bilirubin	Spiked	3 (Zika)		100				
		5 (RNA IC)		100				
	N/A (PSC)	3 (Zika)		100				
		5 (RNA IC)		100				
Hemoglobin	Spiked	3 (Zika)		100				
		5 (RNA IC)		100				
	N/A (PSC)	3 (Zika)		100				
		5 (RNA IC)		100				
Human DNA	Spiked	3 (Zika)		100				
		5 (RNA IC)		100				
	N/A (PSC)	3 (Zika)		100				
		5 (RNA IC)		100				
Triglycerides	Spiked	3 (Zika)		100				
		5 (RNA IC)		100				
	N/A (PSC)	3 (Zika)		100				
		5 (RNA IC)		100				

N/A (PSC): Positive Spike Control: EDTA-plasma samples spiked with ~3x LoD Zika but not spiked with the potential interferent

Table 28: Specificity of the cobas[®] Zika IA test

Interferent	Source of Interferent ^a	Channel	Negativity		CT values		RFI Values	
			#	%	Mean	SD	Mean	SD
Albumin	Spiked	3 (Zika)	(b) (4)	0	(b) (4)			
		5 (RNA IC)		0				
	N/A (NSC)	3 (Zika)		0				
		5 (RNA IC)		0				
Bilirubin	Spiked	3 (Zika)		0				
		5 (RNA IC)		0				
	N/A (NSC)	3 (Zika)		0				
		5 (RNA IC)		0				
Hemoglobin	Spiked	3 (Zika)		0				
		5 (RNA IC)		0				
	N/A (NSC)	3 (Zika)		0				
		5 (RNA IC)		0				
Human DNA	Spiked	3 (Zika)		0				
		5 (RNA IC)		0				
	N/A (NSC)	3 (Zika)		0				
		5 (RNA IC)		0				
Triglycerides	Spiked	3 (Zika)		0				
		5 (RNA IC)		0				
	N/A (NSC)	3 (Zika)		0				
		5 (RNA IC)		0				

N/A (NSC): Negative Spike Control: EDTA-plasma samples that were not spiked with the potential interferent

^a One specimen was tested with three replicates

Plasma samples with high levels of triglycerides (up to 33.2 g/L), hemoglobin (up to 2.9 g/L), unconjugated bilirubin (up to 0.28 g/L), albumin (up to 61.4 g/L), and human DNA (up to 2 mg/L) were tested with and without Zika virus added to a concentration of approximately 3 x LoD of the **cobas[®] Zika IA test**. Samples containing these substances did not interfere with the sensitivity or specificity of the **cobas[®] Zika IA test**.

These results meet the product requirement.

Reviewer Comments: No additional comments

7. Cross Reactivity

The analytical specificity of the **cobas®** Zika IA test was evaluated by testing cross reactivity with (b) (4) clinical specimens including HIV, HBV, HCV positive samples and 6 cultured microorganisms at 1E+06 copies/mL including Chikungunya virus, Dengue virus serotype 1-4 and West Nile virus.

Specimens reflecting *Treponema pallidum* (syphilis) were not available at the time of testing and were therefore not included in testing. FDA has agreed that cross- reactivity testing of syphilis is not required for the Investigational Assay, but **this testing will be required as part of a BLA filing.**

The clinical specimens were tested with and without Zika virus added to a concentration of approximately 3x LoD of the Zika IA test. The cultured microorganisms were added to normal, virus-negative, human pooled plasma and tested with and without Zika virus added to a concentration of approximately 3x LoD of the **cobas®** Zika IA test.

Table 3: Sensitivity of the cobas® Zika in the Presence of Microorganisms

Microorganism	Zika (Channel 3) Results						IC (Channel 5) Results					
	Reactivity		Ct Values		RFI Values		Reactivity		Ct Values		RFI Values	
	#	%	Mean	SD	Mean	SD	#	%	Mean	SD	Mean	SD
Japanese Encephalitis virus (JEV)	3/3	100	(b) (4)				3/3	100	(b) (4)			
Murray Valley Encephalitis virus (MVEV)	3/3	100					3/3	100				
St. Louis Encephalitis Virus (SLEV)	3/3	100					3/3	100				
Usutu Virus	3/3	100					3/3	100				
Yellow Fever Virus	3/3	100					3/3	100				
<i>Treponema pallidum</i> (syphilis)	3/3	100					3/3	100				
Positive Spike Control	3/3	100					3/3	100				

Table 4: Specificity of the cobas® Zika in the Presence of Microorganisms

Microorganism	Zika (Channel 3) Results		IC (Channel 5) Results			
	Reactivity		Reactivity		Ct Values	
	#	%	#	%	Mean	SD
Japanese Encephalitis virus (JEV)	0/3	0	3/3	100	(b) (4)	
Murray Valley Encephalitis virus	0/3	0	3/3	100		
St. Louis Encephalitis Virus	0/3	0	3/3	100		
Usutu Virus	0/3	0	3/3	100		
Yellow Fever Virus	0/3	0	3/3	100		
<i>Treponema pallidum</i> (syphilis)	0/3	0	3/3	100		
Negative Spike Control	0/3	0	3/3	100		

Reviewer Comments: Not clear why only 3 replicates were tested for each interferent.

The above comment was clarified during the Internal Meeting (07/06/17). The three replicate requirement was conveyed to the sponsor during the pre-IND stage and was found acceptable by the management.

8. Matrix Equivalency

Three runs were performed in this study, of which three runs were valid. These valid runs contained (b) (4) valid results. The valid runs contained 3 invalid results (all of one specimen) due to clot detection during sample aspiration (flag: P02T).

The invalid sample was repeated until a valid result was generated and the repeated valid test results were used for the data analysis.

Table 33: Sensitivity of the cobas® Zika test.

Matrix	Channel	Reactivity		CT values		RFI Values	
		#	%	Mean	SD	Mean	SD
EDTA	3 (Zika)	(b) (4)					
	5 (RNA IC)						
(b) (4)							

Table 34: Specificity of the cobas® Zika test.

Matrix	Channel	Negativity		CT values		RFI Values	
		#	%	Mean	SD	Mean	SD
EDTA	3 (Zika)	(b) (4)					
	5 (RNA IC)						
(b) (4)							

Reviewer Comments: No additional comments

9. Internal Control and RMC Failure Rates

There was no dedicated testing to determine the IC and Control Failure Rate as well as the Sample Reliability of the cobas® Zika test. Results were obtained by analyzing data generated in the following studies:

IC Failure Rate, Control Failure Rate as well as Sample Reliability were calculated as follows:

$$IC \text{ Failure Rate in } \% = \frac{\text{Number of IC Failures}}{\text{Number of RMC and Samples}} \times 100$$

$$Control \text{ Failure Rate in } \% = \frac{\text{Number of Invalid Controls}}{\text{Number of RMC}} \times 100$$

$$Sample \text{ Reliability in } \% = \left(1 - \frac{\text{Number of Invalid Samples and not Processed Samples}}{\text{Number of Samples}}\right) \times 100$$

Table 38: Summary IC Failure Rate, Control Failure Rate and Sample Reliability

Study Name	IC Failure Rate			Control Failure Rate	Sample Reliability
	Positive samples	Negative samples	combined		
Limit of Detection	(b) (4)			(4)	
Specificity					
Clinical Sensitivity					
Detection of Isolates near LoD					
Cross Reactivity					
Matrix Equivalency					
Overall					
Upper one-sided 95% confidence interval					

* Lower one-sided 95% confidence interval

The IC Failure Rate was 0.00% for positive samples, negatives samples and positives and negatives combined. The RMC Control Failure Rate was 0.00% and the Sample Reliability was 99.23%.

Reviewer Comments: *No additional comments*

10. Kit Stability

The study was performed according to the kit release procedures for the test-specific reagent kit and positive control kits described in [DH-04482.01-024](#) and [DH-04482.01-028](#), respectively. The **cobas®** Zika MMX-R2 reagent stability results are documented in [DH-04482.01-037](#). The **cobas®** Zika Positive Control Kit stability results are documented in [DH-04482.01-036](#).

(b) (4)

(b) (4)

the kit remain stable for at least the specified 10 hours at (b) (4) °C (On Board Stability).

Reviewer Comments:

- Documents mentioned in submission but not provided:
 - [DH-04482.01-024](#)
 - [DH-04482.01-028](#)
 - [DH-04482.01-037](#)
 - [DH-04482.01-036](#)
- RMS states "The data supports at least 4 months stability for the Zika MMX-R2 and at least 7 months of stability for the Zika Positive Control". No supporting data provided for this claim.

Letter Ready Comments: Additional Information request

1. Stability Studies: Please provide the following documents for kit release procedures for the test-specific reagent kit and positive control kits:
 - DH-04482.01-024
 - DH-04482.01-028
2. Additionally please provide the following documents for **cobas®** Zika MMX-R2 reagent stability results and the **cobas®** Zika Positive Control Kit stability results:
 - DH-04482.01-037
 - DH-04482.01-036
3. Please note that the On Board and Open kit stability data provided in the submission (DH-04482.03-122F) support a shelf claim of only 30 days for Open Kit stability and 20 hours at (b) (4) °C for On Board stability for the 480 Test specific reagents and 10 hours at (b) (4) °C for On Board stability of the cobas ZIKA control kit. Please provide additional information to support claims for 7-month stability of the Positive controls and 4-month stability of the MMX-R2.

Recommendation: Additional Information request

The SL informed this reviewer that the reviewer's comments have been resolved by DETTD upper management and hence were not communicated to the sponsor. There are no additional comments.

Recommendation: No additional comments.

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