



Statistical Review and Evaluation

BLA (Final)

Submission Number: STN 125653/0

Device Name: cobas® Zika

Intended Use: Cobas® Zika is a qualitative in vitro nucleic acid screening test for the direct detection of Zika virus RNA in plasma specimens from individual human donors.

Applicant: Roche Molecular Systems, Inc.

CBER Receipt Date(s): April 7, 2017 (original submission)
August 4, 2017 (Amendment 5)
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1 EXECUTIVE SUMMARY

The cobas[®] Zika test for use on the cobas[®] 6800 and cobas[®] 8800 Systems is a qualitative in vitro nucleic acid screening test, intended to screen donor samples for Zika virus RNA in plasma samples from individual human donors.

The clinical specificity of cobas Zika was 99.997% (358,015/358,024; 95% CI: 99.995% to 99.999%) when used to test plasma from donations collected in US states.

Clinical sensitivity was assessed based on data collected during the clinical specificity study. The reactivity of each of 2 cobas Zika repeat tests were compared to 206 alternative nucleic acid test (NAT) positive samples that were initially reactive on cobas Zika. For Repeat Test 1, the clinical sensitivity was determined to be 96.6% (199/206, 95% CI: 93.1 to 98.6%), and this was claimed as the final clinical sensitivity as it was worse than Repeat Test 2. During the mid-cycle meeting, we pointed it out to the product office (DETTD) that this statistics is an assessment of reproducibility, rather than sensitivity. DETTD agreed to address this issue by appropriate terminology in the Package Insert. We defer it to DETTD.

In the reproducibility study, the total CV% was $\leq 2.3\%$ for all positive panel members. Although CV% should be calculated including those samples with non-reactive results by imputation, this request was not made because 1) negative samples were also excluded in the calculations in prior approved submissions; and 2) it should not have a big impact on the assessment based on the small %CV observed.

2 INTRODUCTION

The cobas[®] Zika test for use on the cobas[®] 6800 and cobas[®] 8800 Systems is a qualitative in vitro nucleic acid screening test, 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 (i) as an aid in diagnosis of Zika virus infection, (ii) on samples of other body fluids or cord blood.

This review focuses on the two clinical reports: clinical specificity study (cX8-ZIKA-412) and reproducibility study (cX8-ZIKA-427).

In response to RMS question 10 (BQ160101, November 17, 2016), FDA (i) recommended using data obtained from the specificity study to determine the clinical sensitivity and (ii) requested the statistical analysis plan and the pre-specified acceptance criteria. RMS submitted the requested information in the supplement to BQ160101 (BQ160101/Supplement 1; CBER receipt date: December 21, 2016) on which CBER had no comments.

The mid-cycle meeting was held on July 6, 2017. Subsequently, several Information Requests (IRs) were sent.

3 STATISTICAL EVALUATION

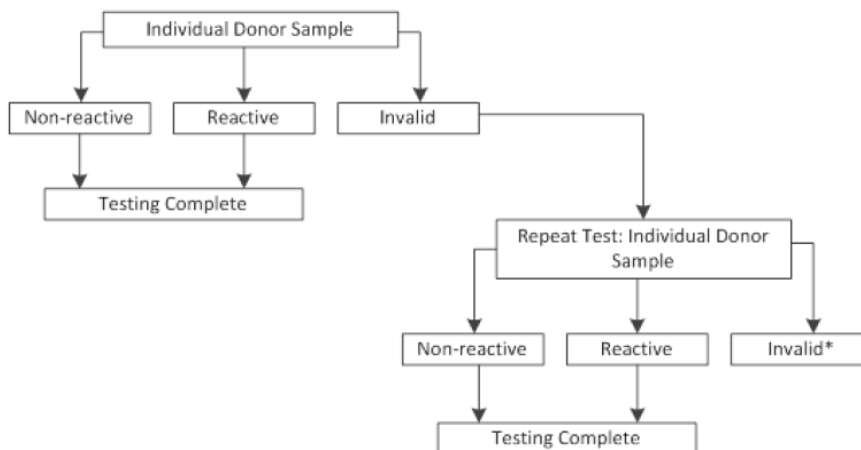
3.1 Clinical Specificity Study

3.1.1 Study Design and Testing Algorithm

Samples from 358,266 donations collected in US states were tested as individual donations using cobas Zika. Samples were tested using 5 reagent lots at 5 US test sites.

The testing algorithm for individual donor samples is shown in Figure 1. Testing is complete if the test result is reactive or non-reactive. If the result is invalid, repeat testing is performed. Repeat testing is complete if the test result is reactive or nonreactive; if it is still invalid and sufficient volume is unavailable, the result is reported as unresolved.

Figure 1: Testing Algorithm – Individual Donor Samples



*If sufficient sample volume is unavailable for additional testing, the result is reported as unresolved.

Source: Figure 1, Clinical Specificity Study Report (cX8-ZIKA-412, page 21).

Samples reactive on cobas Zika were sent to (b) (4) for the following testing:

- Alternate NAT
- Anti-Zika IgM
- (b) (4)
- Plaque reduction neutralization testing (PRNT) if IgM anti-Zika was positive

Samples reactive on cobas Zika were retested (i) in duplicates and (ii) in a simulated pool of 6 (1: 5 of donor sample and negative human plasma) with cobas Zika at the testing site.

Donors with Zika-reactive donations were followed until seroconversion (a positive test was obtained by serology for Zika virus) for up to a maximum of 8 weeks (and up to a maximum of 2 follow-up visits) after the date of their index donations. The two follow-up visits were to occur within and after 2 weeks of the index donation, respectively. Follow-up collections were tested for:

- Anti-Zika IgM (performed at (b) (4))
- (b) (4)
- PRNT for specimens positive anti-Zika IgM (performed at (b) (4))
- cobas Zika (1 replicate, done at the testing site)

The cobas Zika and anti-Zika IgM results of follow-up samples were also used to determine the Zika status of the index donation.

3.1.2 Statistical Methods

A reactive result on cobas Zika was considered true positive if any of the following were true:

- Alternative NAT was positive
- Index donation was positive for IgM anti-Zika and plaque reduction neutralization testing (PRNT) was consistent with Zika infection
- Donor was positive for IgM anti-Zika and PRNT was consistent with Zika infection on follow-up testing
- Donor was positive for cobas Zika on follow-up testing

Specificity was calculated as the frequency of cobas Zika non-reactive results among status-negative donations which were defined as total donations with complete valid results on cobas Zika minus true positive cobas Zika reactive donations.

As indicated on page 32 of the Clinical Specificity Study Report, on March 1, 2017, FDA agreed that RMS could include as “confirmed positive” donations those that were IgM positive on either index or follow-up without PRNT.

3.1.3 Acceptance Criteria

For a sample size of greater than 50,000 donations, the lower limit of the two-sided 95% exact CI for a clinical specificity of the assay of 99.8% is 99.76%. Therefore, for study sample sizes of greater than 50,000 donations, the acceptance criterion is that the clinical specificity of the assay be at least 99.76%.

3.1.4 Results

This analysis included those donations from US states that were tested individually with plasma samples using cobas Zika. The dispositions of donors included in the study are summarized in Table 1. Of the 358,266 US donations from enrolled donors, 358,038 (99.94%) specimens were evaluable.

Table 1. Summary of Donations Collected From Donors Enrolled

	Number of Donations N (%)
Donations From Enrolled Donors	358,266
Non-Evaluable Donations ^a	228 (0.06)
Evaluable Donations ^b	358,038 (99.94)
Evaluable Donations ^b	358,038
Follow-up Not Required	358,015 (99.99)
Eligible for Follow-up ^c	23 (0.01)
Donors Eligible for Follow-up ^c	23
Enrolled in Follow-up	13 (56.52)
Declined Follow-up ^d	5 (21.74)
Donors Lost to Follow-up ^d	5 (21.74)
Donors Enrolled in Follow-up	13
Donors Completed Follow-up	11 (84.62)
Did not Complete Follow-up ^e	2 (15.38)

^a Donations with an invalid/missing cobas Zika test result were considered non-evaluable.

^b Donations with valid cobas Zika test results were considered evaluable.

^c Eligibility for enrollment in the Follow-up Study was based on a reactive cobas Zika result for the donation.

^d Donors who did not enroll in the Follow-up Study, either declined to enroll in the Follow-up Study or contact with the donor for enrollment in the Follow-up Study was never established.

^e Two donors enrolled in the Follow-up Study but were lost to Follow-up before their second Follow-up visit.

Source: Table 6, Clinical Specificity Study Report (cX8-ZIKA-412, page 35).

Note: There were actually 358,817 (99.91%) evaluable donations. However, a total of 779 evaluable donations were tested twice (contributing non-reactive results both times) leaving 358,038 evaluable donations for analysis.

Among the 358,038 evaluable donations, only 23 (0.01%) donations had reactive cobas Zika results. Seven of them were first confirmed true positive by alternative NAT on their index donation (Reactivity Category 1, Table 2). An additional 7 reactive index donations were confirmed by positive anti-Zika IgM results (Reactivity Category 2); 2 of these 7 index donations were repeat reactive on cobas Zika. The remaining 9 of the 23 donations were classified as false reactive (Reactivity Category 3). No donor status was changed by the follow-up results.

Table 2. Testing Reactivity Patterns and Donation Status Summary

Reactivity Category ^a	Index Donation Testing				Repeat/ Simulated Pool cobas Zika Testing Results Reactive? ^b	Follow-up Study cobas Zika Results Reactive? ^c	Follow-up Study IgM Positive? ^c	Donation Status ^d	Number of Donations (N = 358,038)
	Donation ID	cobas Zika Result	Alter- native NAT	IgM Positive?					
1	W036816575175	Reactive	+	-	(+)/++	-/-	+/+	Positive	1
	W036816673743	Reactive	+	+	(+)/++	-/-	E/+	Positive	1
	W036816766809	Reactive	+	-	(+)	ND	ND	Positive	1
	W036816873167	Reactive	+	E	(+)/++	+	+	Positive	1
	W223216601314	Reactive	+	+	(-)/-/+	ND	ND	Positive	1
	W223216602201	Reactive	+	-	(+)/++	ND	ND	Positive	1
	W223216604713	Reactive	+	-	(+)/++	ND	ND	Positive	1
2	W036816264951	Reactive	-	+	(-)/-/-	ND	ND	Positive	1
	W036816538546	Reactive	-	+	(-)	-/-	+/+	Positive	1
	W036816563795	Reactive	-	+	(-)/-/-	-/-	+/+	Positive	1
	W036816726179	Reactive	-	+	(-)/-/-	-/-	E/?	Positive	1
	W036816770797	Reactive	-	+	(-)/-/-	ND	ND	Positive	1
	W036816783592	Reactive	-	+	(-)/+/-	-/-	+/+	Positive	1
	W223216603883	Reactive	-	+	(-)/+/-	ND	ND	Positive	1
3	W036816568301 W036816584805 W036816656625 W036816722019 W044616353023 W115116264389 W138716146823 W140916016741 W223216603505	Reactive	-	- or ?	(-)/-/- or (-)/-/-	-/- or - or ND	-/- or - or ?/? or ND	Negative	9
4	N/A	Non-Reactive	N/A	N/A	N/A	N/A	N/A	Negative	358,015

Note: Only evaluable donations are included in this summary table.

a Reactivity Categories were defined as follows:

Category 1 – cobas Zika reactive index donations with positive alternative NAT results.

Category 2 – cobas Zika reactive index donations with non-reactive alternative NAT and positive anti-Zika IgM results.

Category 3 – cobas Zika reactive index donations with no further reactivity on additional index testing or follow-up testing (ie, false reactive cobas Zika results).

Category 4 – Donations with non-reactive cobas Zika results.

b Additional cobas Zika results from simulated pool testing (1:6 dilution), if performed, are displayed within parentheses. cobas Zika results from additional testing of neat replicates, if performed, are displayed with no parentheses.

c Follow-up study results from up to 2 follow-up visits are displayed separated by a ‘/’.

d Donation Status was assigned based on the testing reactivity pattern observed on the index donation (initial and additional index testing) and/or based on followup study results.

E = equivocal; N/A = not applicable; ND = not done; - = negative/non-reactive; + = positive/reactive; ? = Inconclusive; I = invalid.

Source: Table 7, Clinical Specificity Study Report (cX8-ZIKA-412, page 37).

Among the 23 donations with reactive cobas Zika results, 14 were classified as donation status positive because of a positive alternative NAT result and/or a positive anti-Zika IgM result. The remaining 9 donations were considered status negative. The clinical specificity of cobas Zika for donations tested individually was 99.997% (358,015/358,024; 95% CI: 99.995% to 99.999%) when used to test plasma from 358,024 donations collected in US states. Specificity results were similar across the 5 test sites

3.1.5 Invalid and Invalid Batches and Results

A summary of valid and invalid cobas Zika batches and results is tabulated in Table 3 by this reviewer. This summary included only cobas Zika batches and results from initial testing on index donations from US states.

Table 3. Summary of Invalid and Invalid Batches and Results

Batch	Number of batch (%)	Test results	Number of donations	Number of evaluable donations	
Valid	4,796 (97.5%)	Valid	358,817 (99.91%)	Tested once	357,259
				Tested twice	779*
				Total	358,038
		Invalid	306 (0.09%)		
		Total	359,123		
Invalid	123 (2.5%)	Invalid	Not reported		
Total	4,919				

* A total 779 evaluable donations were tested twice, each contributing 2 valid non-reactive results and the first valid cobas Zika result was retained in statistical analyses.

In total, 4,919 batches were performed, of which 4,796 (97.5%) were valid and 123 (2.5%) were invalid. Majority of the invalid batches (103) were due to positive and negative control failure.

3.2 Clinical Sensitivity

3.2.1 Study Design and Statistical Methods

The clinical sensitivity was assessed using 218 index donations (211 from Puerto Rico and 7 from US states) collected in the specificity study which had both a reactive cobas Zika result and a positive alternative NAT result. Repeat testing (2 replicates) was performed on these alternative NAT-positive samples. The clinical sensitivity was calculated as the percentage of alternative NAT-positive samples with reactive results from repeat testing.

Note: the clinical specificity study in Section 3.1 was only based on donations collected from US states; donations collected from Puerto Rico were not included.

3.2.2 Acceptance Criteria

For known positive donations, the clinical sensitivity of cobas Zika should be greater than or equal to 95% with a lower bound of the two-sided 95% Clopper-Pearson exact confidence interval for the estimate of clinical sensitivity greater than or equal to 90%.

3.2.3 Results

Among the 218 evaluable alternative NAT-positive specimens, 12 were excluded from the analysis as they did not have repeat testing performed, therefore, clinical sensitivity was calculated based on 206 specimens (218-12).

Of the 206 specimens, 198 pairs of repeat results were concordant reactive; 2 pairs of repeat results were concordant non-reactive; 6 pairs of repeat results were discordant. For Repeat Test

1, the clinical sensitivity was determined to be 96.6% (199/206; 95% CI: 93.1 to 98.6%). For Repeat Test 2, the clinical sensitivity was determined to be 98.5% (203/206; 95% CI: 95.8 to 99.7%). See Table 4. The applicant claimed the sensitivity using Repeat Test 1 as this represented the worst outcome.

Table 4. Clinical Sensitivity of cobas Zika

		cobas Zika Repeat Test Result			
cobas Zika Repeat Testing	Total Alternative NAT-Positive Samples*	Reactive	Non-Reactive	Sensitivity Estimate	95% Exact CI
Repeat Test 1	206	199	7	96.6%	(93.1%, 98.6%)
Repeat Test 2	206	203	3	98.5%	(95.8%, 99.7%)

* Alternative NAT-positive specimens are defined as index donations with reactive cobas Zika results and positive alternative NAT results.

Source: Table 12, Clinical Specificity Study Report (cX8-ZIKA-412, page 44).

3.3 Reproducibility Study

3.3.1 Study Design

The reproducibility of cobas[®] Zika for use on the cobas[®] 6800/8800 Systems was established by testing a twelve member panel composed of three negative plasma samples and three samples positive for Zika virus at three different concentrations (approximately 0.5x, 1-2x, and 3x the LoD of cobas[®] Zika).

Reproducibility was conducted at three sites with 1 cobas[®] 6800 System or cobas[®] 8800 System at each site. An operator at each site performed five days of testing with each of three lots of cobas[®] Zika reagents and two valid panel runs (i.e., two batches, each batch composed of one panel and two independent controls) per day. For each panel member there were up to 270 tests: 3 lots × 3 sites × 5 days × 2 batches × 3 replicates.

3.3.2 Statistical Methods

Only valid test results were included in statistical analyses. Data were summarized by the percent agreement and associated Clopper-Pearson exact 95% confidence interval (CI) at each expected viral concentration and were presented by site/instrument, lot, day, and batch.

Analysis of variance was done on the cycle threshold (Ct) values for each positive panel member with valid reactive results using a mixed model with random effects lot; site; day nested within lot and site; batch nested within lot, site, and day; and within-batch. It was done using the PROC MIXED procedure of SAS/STAT[®] software with the restricted maximum likelihood estimates (REMLs) option. Results from the model fitted to the Ct value were presented as the percentage of variance and percentage coefficient of variation (CV%) for each effect (eg, lot) by positive panel member concentration.

3.3.3 Acceptance Criteria

For panel members with concentrations at or above the LOD (eg, 1 to $2 \times \text{LOD}$) of the test, the lower limit of the 2-sided 95% Clopper-Pearson exact confidence interval (exact CI) of the percent agreement with the viral target was to be equal to or greater than 91.9%. This allowed a hit rate of at least 95.2% with at most 13 misses out of 270 tests.

3.3.4 Results

There were 5 invalid results out of 1080 (0.46%). The percent agreement was 100% for all concentrations except for $\sim 0.5 \times \text{LOD}$ (see Table 5). The percent agreements for positive panel members by site, lot, day, and batch separately are omitted in this review.

Table 5. Percent Agreement by Panel Member

Viral Target	Expected Viral Concentration	Tests N	Results in Agreement With Viral Target n	Percent Agreement $n/N \times 100$	Exact 95% Confidence Interval
Negative	0	268	268	100.0	(98.6, 100.0)
Zika	$\sim 0.5 \times \text{LOD}$	268	204	76.1	(70.6, 81.1)
	1-2 $\times \text{LOD}$	269	269	100.0	(98.6, 100.0)
	$\sim 3 \times \text{LOD}$	270	270	100.0	(98.6, 100.0)

Source: Table 3, Reproducibility Study Report (cX8-ZIKA-427, page 24).

In the analysis of variance, the total CV% was $\leq 2.3\%$ for all positive panel members. Within each component, the CV% was $\leq 2.1\%$ across positive panel members.

4 REVIEWER'S COMMENTS

- 1) RMS claimed that the clinical sensitivity of the cobas Zika assay to be 96.6% (page 43 of the Specificity Clinical Study Report: cX8-ZIKA-412) based on the repeat testing of alternative NAT-positive specimens. This approach is problematic because the analysis was based on the repeat test on specimens initially reactive on cobas Zika; therefore, the so-called clinical sensitivity was in fact a measure of reproducibility, rather than sensitivity. DETTD agreed to address this issue by appropriate terminology in the Package Insert. We defer it to DETTD.
- 2) In the reproducibility study, the total CV% was $\leq 2.3\%$ for all positive panel members. Within each component, the CV% was $\leq 2.1\%$ across positive panel members. Technically CV% should be calculated including those samples with non-reactive results by imputing the Ct values with 50. However, this request was not made because 1) negative samples were also excluded in the calculations in prior approval submissions; and 2) it should not have a big impact on the assessment based on the small %CV observed.
- 3) Several IR items were sent on July 25, 2017 to request for clarification regarding the number of invalid results/batches. The responses are satisfactory.