

MEMORANDUM

Department of Health and Human Services
Public Health Service
Food and Drug Administration

Date: September 21, 2017
To: Caren Chancey, Ph.D.
Through: David A. Leiby, Ph.D.
From: Julia Tait Lathrop, Ph.D., OBRR/DETTD/PBR. Clinical reviewer
Subject: BL125653/0 Roche Molecular **cobas**[®] Zika Test Clinical review memo
Scope of review: Clinical studies, clinical reproducibility (b) (4) data

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.

Background

BL125653/0 was submitted to DETTD on April 7, 2017 for licensure as an ID-NAT donor screening test for detection of Zika Virus in donated plasma. The purpose of this study was to evaluate the clinical specificity of the **cobas** Zika for testing blood donor samples. In addition, samples identified as positive were re-tested as an approximation of the ability of the test to detect virus in samples upon re-testing. Clinical reproducibility was evaluated in a separate study.

Study Design and Results

Clinical specificity: In § 20.0, Clinical specificity (page 49 of the clinical study report cX8-ZIKA-412), the Sponsor provided the following results. A total of 358,266 donations were collected in U.S. states from donors enrolled in the study, of which 358,038 donations were evaluable, and 228 donations were not evaluable. A total of 4,919 batches containing donations collected in U.S. states were tested, of which 4,796 (97.5%) were valid and 123 (2.5%) were invalid. Of the 358,038 evaluable donations, 358,015 (99.99%) were non-reactive on **cobas** Zika,

and 23 (0.01%) of donations were reactive on cobas Zika. Of the 23 cobas Zika reactive U.S. donations, 14 were confirmed as reactive (“true positive”) on either index or a follow-up donation with alternative NAT results, anti-Zika IgM results, or both. Nine of the 23 cobas Zika reactives were not confirmed and were classified as false positives. The clinical specificity of cobas Zika is 99.997% (385,015/358,024; 95% CI: 99.995%–99.999%) (Table 1). Clinical specificity was similar at all five testing sites, ranging from 99.994% to 100%. The pre-specified acceptance criterion stated that, for a sample size of greater than 50,000 donations, the clinical specificity must be at least 99.76%, with a lower bound of the two-sided 95% exact CI of 99.8%. Based on 358,024 donations collected in the continental U.S., the clinical specificity of cobas Zika is 99.997% (95% CI: 99.995%–99.999%), which exceeds 99.76%.

Table 1. Clinical Specificity

cobas [®] Zika Result	Zika Donation Status*		Total
	Positive	Negative	
Reactive	14	9	23
Non-Reactive	0	358,015	358,015
Total	14	358,024	358,038
Clinical Specificity (95% CI)	-	99.997% (99.995–99.999%)	-

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

Note: Seven index donations with reactive cobas Zika results were confirmed by positive anti-Zika IgM results, two of these seven index donations were repeat reactive on cobas Zika.

* Donation Status was assigned based on the testing reactivity pattern observed on the index donation (initial and additional index testing) and/or based on Follow-up study results.

cobas Zika = cobas Zika for use with the cobas 6800/8800 Systems; index donation = donation reactive on cobas Zika for use with the cobas 6800/8800 Systems.

Clinical sensitivity: In §20.2 (page 51 of the clinical study report cX8-ZIKA-412), the Sponsor provided the following results. Clinical sensitivity was assessed using available data collected in the specificity study including both in the continental U.S. and Puerto Rico rather than using well-characterized known positive samples. The clinical sensitivity assessment used a total of 218 index donations collected under the cobas Zika specificity protocol in Puerto Rico (n = 211 donations) and the continental U.S. (n = 7 donations) to estimate clinical sensitivity. Each of these 218 index donations had both a reactive cobas Zika result and a positive alternative NAT result; each donation was tested between April 4, 2016, and October 9, 2016. Clinical sensitivity of the cobas Zika test was calculated as the percentage of alternative NAT samples with reactive cobas Zika results on cobas Zika replicate testing (performed at the testing laboratory, per the specificity protocol). Using this approach, the clinical sensitivity was estimated to be 96.6% based on Repeat Test 1 (95% CI 93.1–98.6% 95% CI) and 98.5% based on Repeat Test 2 results (95% CI 95.8–99.7%). The lower bound (LB) for replicate 1, which the single test that would be performed when the test is used in the field, does not meet the original acceptance criterion of 95%.

As communicated within the statistical analysis plan (BQ160101_s01_01, RMS Response to Question 10 of FDA Feedback of November 17, 2016), the worst performing repeat testing of 96.6% (Repeat Test 1) was claimed as the sensitivity of the cobas Zika assay. The lower bound

of the two-sided 95% exact CI of 93.1% for the sensitivity estimate of 96.6% (Repeat Test 1) was greater than or equal to the protocol specified acceptance criteria of a lower bound of greater than or equal to 90%. This plan establishes sensitivity as a worst-case scenario.

However, the Sponsor also proposed combining the two replicates by an algorithm wherein if either replicate 1 or replicate 2 are positive, the donation is considered positive. The clinical sensitivity based on at least 1 of the 2 repeats being reactive was 99.0% (N = 204/206, 95% CI 96.5 to 99.9%). The Sponsor further suggested (b) (4).

Comments:

1. Sensitivity analysis. The cobas Zika test will be used for donor screening as a single replicate test. Negative results are not re-tested. Thus, the test's sensitivity should be based on the results of the first of two replicates. The Sponsor's new recommendation that sensitivity should be based on the proposed interpretation of the results presented above, in which at least one of two replicates are positive, is not appropriate. The Sponsor proposed this interpretation when the test failed to reach a 95% CI LB of 95% and post-hoc analysis is not appropriate; therefore, I recommend that we do not accept the "one out of two" algorithm as a demonstration of the clinical sensitivity of the test. As the pre-specified "worst performing repeat" was the first one, that only the first repeat is considered does not require re-analysis of the results. Furthermore, because there is no other test, I do recommend that DETTD determine that the clinical sensitivity as evidenced by the performance of replicate 1, 96.6% (95% CI 93.1–98.6%) is acceptable. I noted that this is the lowest sensitivity of the individual tests that were presented, and is therefore less likely to be artificially high.

(b) (4) results is also not appropriate because it does not reflect how the test would be used in actual practice and therefore this analysis is not relevant to results expected during actual screening.

2. Description of the clinical "sensitivity" study in the package insert and SBRA. It is incontrovertible that the design of the Clinical Sensitivity study—using as clinical truth samples that were so designated by the investigational device—is not an appropriate design for a true sensitivity study. An appropriate sensitivity study designs uses samples designated as clinical truth by an independent, licensed, and preferably orthogonal method(s). However, there is no other licensed test with which to determine clinical truth nor are there signs and symptoms of disease in the apparently healthy blood donor population. Therefore, given these limitations, the ability of the device to detect positive samples was approximated by using samples identified as positive in the Specificity study and confirmed positive through Alt Nat. These were blinded along with negative samples and sent to the original testing labs for testing as unknowns. These samples were primarily taken from unit (i.e., (b) (4)) plasma, not from the EDTA tubes from which the initial reactives were identified. Although the study is, strictly speaking, testing the ability of the device to reproducibly detect positive samples in a different matrix, it was not powered or designed to serve as a reproducibility study, and should not be characterized in the package insert in this way. Therefore, my recommendation is that the clinical performance sections of the PI and the SBRA describe how the study was performed and include the results from only the first re-test as described above. Suggested wording might be along the lines of:

“Because no licensed test was available to select Zika-positive samples for testing sensitivity, samples that were positive in the Specificity study and that were confirmed by Alt Nat were tested again in a blinded fashion by three independent sites to determine the ability of the test to detect Zika positive donations”.

- PRNT testing. In the IND the Sponsor proposed that one method of confirming positive results was a positive result in a plaque reduction neutralization test (PRNT) (§ 19.1). In § 15.1 (page 31) The Sponsor stated that, because of cross-reactivity between Dengue virus and Zika, and based on CDC recommendations, PRNT confirmation is no longer recommended. The FDA agreed on March 1, 2017, that the Sponsor did not have to confirm positives using PRNT. Thus, no PRNT testing was performed.

Clinical reproducibility

The clinical reproducibility of the device is presented in the Clinical Reproducibility Study Report cX8-ZIKA-427, § 14.3. Reproducibility was evaluated by testing a twelve member panel composed of three negative plasma samples and three samples positive for Zika virus at three different concentrations (approximately 0.5 x, 1–2 x, and 3x the LoD of cobas[®] Zika).

Operators at each of three sites performed five days of testing with each of three lots of reagents and two valid panel runs (i.e., two batches, each batch composed of one panel and two independent controls) per day were completed to yield up to 270 tests per panel member virus type at each of the three concentrations.

All valid batches and test results were analyzed by calculating the percentage of reactive test results for each panel member. For overall reproducibility, the acceptance criterion for panel members with concentrations at or above LOD (i.e., 1–2 × LOD and ~3 × LOD) was that the lower limit of the 2-sided 95% exact CI of the percentage agreement with the viral target (Zika RNA) was ≥ 91.9%. The lower limit of the 2-sided 95% exact CI of the percentage agreement with the viral target for both panel members was 98.6%; therefore, the study acceptance criterion for overall reproducibility was met.

Table 2. Overall reproducibility

				Standard Deviation [SD] and Percent Coefficient of Variation [CV%]											
				Within-Batch		Between-Batch		Between-Day		Between-Site/ Instrument		Between-Lot		Total	
Viral Target	Expected Viral Concentration	n*/ N	Mean Ct	SD	CV %	SD	CV %	SD	CV%	SD	CV	SD	CV %	SD	CV%
Zika	~0.5 x LOD	204/268	38.59	0.83	2.1	0.00	0.0	0.15	0.4	0.17	0.4	0.07	0.2	0.86	2.2
	1-2 x LOD	269/269	37.31	0.80	2.1	0.00	0.0	0.13	0.4	0.13	0.4	0.20	0.5	0.85	2.3
	~3 x LOD	270/270	36.53	0.70	1.9	0.00	0.0	0.09	0.3	0.06	0.2	0.18	0.5	0.73	2.0

*n is the number of reactive results which contribute Ct values to the analysis. N is the total number of valid tests for the panel member.

Note: Ct = cycle threshold; CV = coefficient of variation; LOD = limit of detection; SD = standard deviation. Data Source: Appendix 5, Table 4.

Evaluation of individual parameters Across all components, 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.

Table 3. Individual component reproducibility

Viral Concentration	Between Site		Between Lot		Between Day		Between Batch	
	ID	% Reactive Results	ID	% Reactive Results	ID	% Reactive Results	ID	% Reactive Results
~0.5 x LoD	1	82.2% (74/90)	1	74.4% (67/90)	1	74.1% (40/54)	1	78.5% (106/135)
	2	71.9% (64/89)	2	78.7% (70/89)	2	79.6% (43/54)	2	73.7% (98/133)
	3	74.2% (66/89)	3	75.3% (67/89)	3	79.6% (43/54)	-	-
	-	-	-	-	4	73.1% (38/52)	-	-
	-	-	-	-	5	74.1% (40/54)	-	-
1-2 x LoD	1	100.0% (89/89)	1	100.0% (90/90)	1	100.0% (54/54)	1	100.0% (135/135)
	2	100.0% (90/90)	2	100.0% (89/89)	2	100.0% (53/53)	2	100.0% (134/134)
	3	100.0% (90/90)	3	100.0% (90/90)	3	100.0% (54/54)	-	-
	-	-	-	-	4	100.0% (54/54)	-	-
	-	-	-	-	5	100.0% (54/54)	-	-
~3 x LoD	1	100.0% (90/90)	1	100.0% (90/90)	1	100.0% (54/54)	1	100.0% (135/135)
	2	100.0% (90/90)	2	100.0% (90/90)	2	100.0% (54/54)	2	100.0% (135/135)
	3	100.0% (90/90)	3	100.0% (90/90)	3	100.0% (54/54)	-	-
	-	-	-	-	4	100.0% (54/54)	-	-

Viral Concentration	Between Site		Between Lot		Between Day		Between Batch	
	ID	% Reactive Results	ID	% Reactive Results	ID	% Reactive Results	ID	% Reactive Results
	-	-	-	-	5	100.0% (54/54)	-	-

(b) (4) [Redacted]

[Large redacted area]