Duplex nucleic acid test for the detection of chikungunya and dengue RNA viruses in blood donations

Susan L. Stramer,[1] Jean Stanley,[2] Megan L. Nguyen,[1] Rasa Bertuzis,[2] Nancy Huynh,[2] John R. Duncan,[2] Patrick Albrecht,[3] Lisa L. Pate,[2] and Susan A. Galel[2]

BACKGROUND: Chikungunya (CHIKV) and dengue (DENV) viruses are primarily mosquito-borne, but transfusion transmission can occur (DENV) or is likely (CHIKV). In the absence of commercially available blood screening assays, a variety of strategies to ensure recipient safety in the face of expanding CHIKV and/or DENV outbreaks have been used.

STUDY DESIGN AND METHODS: Performance of cobas CHIKV/DENV, a qualitative RNA detection assay for use on the cobas 6800/8800 Systems, was evaluated at two sites (Roche Molecular Systems, Inc. [RMS], and the American Red Cross [ARC]). Analytical sensitivity, genotype inclusion, correlation with other assays, and reproducibility used clinical CHIKV- or DENV-positive samples and secondary standards for DENV Types 1 to 4 and for three CHIKV genotypes (Asian; East Central South African; and West African); each secondary standard was traceable to international reference panels or reagents. Evaluation of analytic specificity assessed other microorganisms for interference and cross-reactivity; clinical specificity was determined by individually testing 10,528 volunteer blood donations from the continental United States.

RESULTS: The 50 and 95% limit of detection (LoD) obtained by RMS for CHIKV, Asian genotype was 1.8 and 6.8 Detectable Units (DU)/mL, respectively, and 0.14 and 0.63 International Units (IU)/mL, respectively for DENV-1. No significant differences in detection occurred by testing at a second site, the ARC (2.4 and 10.5 DU/mL for CHIKV and 0.15 and 0.60 IU/mL for DENV). Clinical specificity was 100% (95% confidence interval, 99.965%-100%) for CHIKV and DENV.

CONCLUSIONS: The high sensitivity and specificity of the cobas CHIKV/DENV test, as demonstrated in these evaluations, indicate its suitability for blood donation screening.

Dengue viruses (DENV) are arthropod-borne RNA viruses (arboviruses) that belong to the *Flaviviridae* family that includes West Nile, yellow fever, Zika, and other viruses.[1] Like other arboviruses, DENV is maintained in an enzootic cycle between blood-feeding mosquitoes (primarily *Aedes aegypti*) and susceptible vertebrate hosts, such as humans.[2,3] DENV is classified into four related, but immunologically distinct serotypes: DENV-1, DENV-2, DENV-3, and DENV-4.

The World Health Organization (WHO) estimates that DENV is endemic in more than 100 countries in the tropics and subtropics, including more than 3.9 billion people at risk.[2,4] In 2017, the number of reported cases of DENV has

ABBREVIATIONS: ARC = American Red Cross; aRNA = armored RNA; CHIKV = chikungunya virus; DU = detectable units; ECSA = East Central South African; LoD = limit of detection; RMS = Roche Molecular Systems, Inc.; WA = West African.

From the [1]Scientific Affairs, American Red Cross, Gaithersburg, Maryland; [2]Medical and Scientific Affairs, Roche Molecular Diagnostics, Pleasanton, California; and the [3]Roche Diagnostics International Ltd, Rotkreuz, Switzerland.

Address reprint requests to: Susan L. Stramer, PhD, American Red Cross, 9315 Gaither Road, Gaithersburg, MD 20877; e-mail: susan.stramer@redcross.org

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increased dramatically in Asian countries such as Vietnam, India, and Sri Lanka.[5] Most clinical DENV infections are classified as "dengue fever," which the WHO defines as fever and at least two other symptoms that include chills, bone pain, myalgia, arthralgia, eye pain, rash, and easy bruising[2]; severe dengue includes hemorrhagic fever and shock.[2]

Chikungunya virus (CHIKV), also an RNA-containing arbovirus in the family *Togaviridae* is maintained in an enzootic cycle between blood-feeding mosquitoes (*A. aegypti* and, since at least 2005, *A. albopictus*) and humans.[2] CHIKV presents with similar symptoms to, and during the same endemic periods as, DENV except CHIKV is characterized by severe joint pains and crippling arthritis that may cause sufferers to be unable to stand up due to intense joint pain.[2,6,7]Although rare, CHIKV fatalities have been reported and are typically the result of encephalitis or encephalopathy, myocarditis, hepatitis, or multiorgan failure.[7,8]

Three distinct genotypes of CHIKV have been identified: the West African (WA), East Central South African (ECSA), and Asian that is an ECSA variant.[8] Since 2000, CHIKV has reemerged to cause outbreaks of more severe forms of the disease than previously reported.[8] India, Indonesia, Maldives, Myanmar, and Thailand have reported over 1.9 million CHIKV cases since 2005.[9] An explosive CHIKV outbreak occurred on Reunion Island and islands of the southwest Indian Ocean, east of Africa, from late 2005 through 2007, that included approximately 300,000 clinical cases on Reunion Island (41% of the island's population), of which at least 75% were symptomatic.[2,10] An additional 700 cases were imported from this outbreak to France.[11] Due to concerns of CHIKV transfusion transmission on Reunion Island, red blood cell (RBC) collections were suspended with importation of RBCs and plasma from France.[11] Selective screening by nucleic acid testing (NAT) was subsequently implemented for platelets (PLTs) using an assay with a detection limit of 350 copies/mL[9] in addition to the introduction of pathogen inactivation.[11] Of the PLTs that were screened, 0.4% tested CHIKV RNA positive.[10] No cases of transfusion-transmitted CHIKV were documented as a result of these interventions. Pakistan and Kenya are experiencing epidemics that began in 2016,[9] and since 2007, many reports of outbreaks due to CHIKV have been reported in Italy and France; the European outbreaks in 2017 were accompanied by public health recommendations to discontinue blood collections in affected regions and to defer donors who traveled to these regions.[12,13] CHIKV is also a concern in the Americas and the Caribbean. For 2017, through December 22, 2017, the Pan American Health Organization reported 123,087 confirmed (121,734 in Brazil) and 61,613 suspected CHIKV autochthonous transmission cases in South America, the Caribbean, and North America, including 99 deaths in Brazil.[14]

While CHIKV transfusion transmission has not been documented, the potential for transfusion-transmitted CHIKV infection is based on the transfusion transmissibility of other arboviruses, like DENV,[2,15–18] in addition to RNA detection in blood donors using NAT.[10,19] Approximately 53% to 87% of DENV infections and approximately 3% to 25% of CHIKV infections are asymptomatic.[2,7,11,15,20] Because infected donors may not develop clinically significant disease or remain asymptomatic, questioning of blood donors about recent symptoms suggestive of CHIKV or DENV infection has limited efficacy for identifying infected donors.

Blood donations collected in CHIKV/DENV outbreak areas are not currently screened for the presence of RNA of these viruses. However, the adoption of NAT for blood donation screening has contributed to the early detection of viral infections such as human immunodeficiency virus and hepatitis in potentially infected donors, thereby reducing the risk of transfusion transmission. The successful implementation of NAT extends to Zika virus in the United States including Puerto Rico.[21,22] The cobas CHIKV/DENV nucleic acid test for use on the cobas 6800/8800 Systems (Roche Molecular Systems, Inc., RMS) is the first CE-marked qualitative test for the detection and discrimination of CHIKV and DENV RNA viruses in blood from human donors, simultaneously or as a single target in an individual donation or in pooled plasma from individual donations. The evaluation of the assay performance is described in this article.

MATERIALS AND METHODS

Assay and systems

The cobas CHIKV/DENV test for use on the cobas 6800/8800 Systems is a qualitative in vitro duplex test for the direct detection of CHIKV RNA and DENV RNA in human plasma. The test may be used to screen donor samples for CHIKV or DENV RNA alone or simultaneously.

The cobas 6800/8800 Systems provide fully automated sample preparation for nucleic acid extraction and purification with ready to use reagents, followed by polymerase chain reaction (PCR) amplification and detection. Automated data management is performed by the System software assigning test results of nonreactive, reactive, or invalid.

The cobas CHIKV/DENV master mix contains detection probes specific for CHIKV, DENV, and an internal control (IC). The specific CHIKV, DENV, and IC detection probes are each labeled with one of three unique fluorescent dyes that act as a reporter. The three reporter dyes are measured at defined wavelengths, thus permitting simultaneous detection and discrimination of the amplified CHIKV and DENV targets and the IC.[23,24]

Technical performance verification

Studies performed at RMS included evaluation of analytic sensitivity, analytic specificity, genotype inclusion, correlation with other assays, and reproducibility.

Analytic sensitivity

The limit of detection (LoD) of cobas CHIKV/DENV was determined using Roche DENV secondary standards traceable to the First International Reference Panel for Dengue virus Types 1 to 4 for NATs.[25] Although no international standards are currently available for CHIKV genotypes, the CHIKV Roche standards (CHIKV-Asian, CHIKV-ECSA), and armored RNA (aRNA; CHIKV-WA) are traceable to the CBER CHIKV RNA reference reagents.[26] The secondary standards for all except CHIKV-WA were heat-inactivated virus culture supernatants (characteristics of the materials are shown in Table S1 in the supplemental information, available as supporting information in the online version of this paper).

For DENV-1 and CHIKV-Asian, three independent coformulated dilution series of both viral standards were prepared with human EDTA-plasma that was prescreened for CHIKV/DENV nucleic acid. Each dilution series was tested using three different lots of cobas CHIKV/DENV test kits with 63 replicates per lot, for a total of 189 replicates per concentration.

Three independent series of each viral standard were coformulated for DENV-2 and CHIKV-ECSA and individually formulated for DENV-3, DENV-4, and CHIKV-WA using prescreened CHIKV/DENV nucleic acid–negative human EDTA-plasma, as above. Each dilution series was tested 42 times across three different cobas CHIKV/DENV reagent lots for a total of 126 replicates per concentration. Probit analysis on the combined data from all replicates tested for each virus was used to estimate the LoD and two-sided 95% confidence intervals (CIs).

Analytic specificity

Human immunodeficiency virus–, hepatitis B virus–, and hepatitis C virus–seronegative EDTA-plasma samples collected from February 2011 to April 2012 in CHIKV/DENV-nonendemic regions (continental United States) were tested individually with cobas CHIKV/DENV using two different lots of reagents (500 specimens per lot) on two cobas 8800 Systems.

Cross-reactivity and interference was evaluated by testing a panel of 31 cultured microorganisms (24 viral isolates—including seven flaviviruses, six bacterial strains, one yeast isolate) at 10^5 to 10^6 copies, genome equivalents, international units (IU), or colony-forming units per milliliter (CFU/mL); (Table S2 in supplemental information). The virus isolates included West Nile, Yellow fever, St. Louis encephalitis, Japanese encephalitis, Murray Valley encephalitis, Usutu, and Zika. All samples were tested in triplicate with and without spiking of approximately 3× LoD of CHIKV-Asian and DENV-1. Results were analyzed with and without target.

Genotype inclusion

To ensure consistent detection of all three CHIKV genotypes and four DENV serotypes, multiple specimens of each claimed genotype and serotype (as available) were tested in EDTA-plasma using one reagent lot. Genotypes and serotypes were determined by sequencing at the Bernhard Nocht Institute. Positive clinical specimens, cultured viral isolates, and aRNA particles were tested neat and at approximately 4× LoD with one replicate per concentration.

Comparison with RealStar assays

The sensitivity of cobas CHIKV/DENV was compared to that of Altona RealStar assays in two phases. An initial exploratory study was performed using serially diluted culture supernatants. The viral concentrations of the undiluted culture supernatants were 5×10^4 copies/mL for the DENV materials and 1×10^4 copies/mL for the CHIKV materials. This study utilized an enhanced sample input volume for the RealStar assays,[27,28] and each concentration was tested in multiple replicates on each assay. All testing was performed at RMS. The second study compared detection of clinical specimens, utilizing standard sample input volume for the RealStar assays[28,29] and a single replicate for each sample. In this second study, the RealStar testing was performed at the Institute for Medical Microbiology and Hygiene of the University of Regensburg. For the RealStar testing, an IC was included throughout extraction and amplification.

Comparison using culture supernatants. Culture supernatants of CHIKV-Asian and DENV 1 to 4 were serially diluted 10-fold in EDTA-plasma. Each concentration was tested in three replicates on cobas CHIKV/DENV. Each concentration was also tested in six replicates by the first generation RealStar Chikungunya RT-PCR kit[27] or second generation RealStar Dengue RT-PCR kit [28] (Altona Diagnostics).

For the RealStar assays, three extractions were performed on each viral concentration using the QIAamp Viral RNA Mini Kit (QIAGEN), with sample input volume doubled from 140 to 280 μL to increase sensitivity. From each extraction 60 μL was eluted and two replicates of RealStar RT-PCR testing were performed from each eluate using 10 μL of eluate per PCR, for a total of six PCR results for each viral concentration.

Comparison using clinical specimens. Reactivity of the cobas CHIKV/DENV test was compared with the second-generation RealStar Chikungunya RT-PCR kit,[29] which has increased sensitivity compared to the first-generation RealStar Chikungunya RT-PCR kit,[27] and the second-generation RealStar Dengue RT-PCR kit.[28] Samples from endemic areas were characterized as CHIKV or DENV NAT–positive based on reactivity when tested with cobas CHIKV/DENV. Samples reactive for either CHIKV or DENV were then retested by cobas CHIKV/DENV and with the RealStar assay for that target. One-hundred DENV NAT–positive serum or EDTA-plasma samples and 100 CHIKV NAT–positive EDTA-plasma samples were tested individually. For CHIKV, the testing included 67 individual

clinical specimens and 33 diluted specimens with final concentrations ranging from approximately 10 Detectable Units (DU)/mL to 2.9×10^5 DU/mL. For DENV, sufficient volumes of 100 clinical samples were available to test the samples undiluted.

For the RealStar assays, nucleic acid was extracted from each sample using the viral RNA mini kit with a sample input of 140 μL, an eluate of 50 μL, and 10 μL of eluate per PCR procedure. Results of cobas CHIKV/DENV and RealStar assays were compared by McNemar's test with two-sided p-values calculated.[30]

Reproducibility

Reproducibility of cobas CHIKV/DENV was determined using the Roche secondary standard for DENV-1 and the Roche secondary standard for CHIKV-Asian. Three panels of coformulated CHIKV and DENV members at concentrations of approximately 0.5×, 1×, and 2× the LoD of cobas CHIKV/DENV for each virus were tested. Testing was performed for day-to-day variability over 3 days, lot-to-lot variability using three different reagent lots of cobas CHIKV/DENV, and instrument-to-instrument variability using three different cobas 8800 Systems. Each of the three panels was tested in 21 replicates for a total of 63 replicates for each reagent lot. All valid reproducibility data were evaluated by calculating the percentage of reactive test results for each concentration level across all variables. The limits of two-sided 95% CIs were calculated for each of the three levels of CHIKV and DENV tested across 3 days, three reagent lots, and three cobas 8800 Systems.

Clinical performance validation

The American Red Cross (ARC) Scientific Support Office in Gaithersburg, Maryland, conducted specificity and sensitivity studies under an Investigational New Drug application approved by the Food and Drug Administration.

Analytic sensitivity

Coded randomized panels were prepared from serial dilutions of CHIKV-Asian and DENV-1 secondary standards. Multiple dilution series of co-formulated CHIKV and DENV at five concentrations, ranging from approximately 0.125 to 2× the LoD, as well as CHIKV/DENV-negative panel members were tested. Each viral concentration was tested in 24 replicates using three different reagent lots of cobas CHIKV/DENV. The acceptance criteria for each viral target was a comparison of the estimated LoD from all lots combined from testing at the ARC compared to the LoD claimed for the cobas CHIKV/DENV by RMS. The difference of the determined log LoDs (estimated – claimed) was not to exceed ±0.3 for each viral target.[31]

Clinical specificity

Samples from ARC donations collected October 6, 2015 to November 19, 2015, from the continental United States, were deidentified and shipped to the ARC after completion of routine testing. Each donor identification number was linked to a new study sample identification number, the tubes from that donor identification number were relabeled with the study identification number, and the samples were tested individually on cobas CHIKV/DENV.

Samples testing nonreactive were considered negative for CHIKV/DENV RNA. Reactive samples were to undergo further testing by alternative NAT with possible additional testing of the amplification and detection plates by hemi-nested PCR or probe fragment analysis.

The prevalence and specificity of the cobas CHIKV/DENV test was calculated as the percentage of CHIKV/DENV RNA confirmed-positive samples of the total tested (prevalence) or the percentage of negative samples that were nonreactive on cobas CHIKV/DENV. Two-sided 95% Clopper-Pearson[32] exact CIs were calculated for point estimates.

RESULTS

Technical performance verification

Analytic sensitivity

Table 1 and Tables S3 to S9 in the supplemental information summarize the overall results of the Probit analysis of the CHIKV and DENV LoDs combining results across three cobas CHIKV/DENV reagent lots, three instruments, and three dilutions series. The 50 and 95% lower LoDs obtained by RMS for CHIKV, Asian genotypes were 1.8 and 6.8 DU/mL, respectively, and 0.14 and 0.63 International Units (IU)/mL, respectively, for DENV-1.

Analytic specificity

There were no reactive results in 1000 EDTA-plasma samples from nonendemic regions. Thus, the specificity for cobas CHIKV/DENV was 100% (95% CI, 99.6%-100%).

No interference with CHIKV/DENV detection at 3× the assay LoD nor cross-reactivity in the absence of spiked CHIKV/DENV was observed in samples containing 31 different microorganisms, including seven other flaviviruses (Japanese encephalitis, Murray Valley encephalitis, St. Louis encephalitis, Usutu, West Nile, Yellow fever, and Zika viruses).

Genotype inclusion

All clinical samples, cultured viral isolates, and aRNA particles of CHIKV genotypes and DENV types were detected undiluted and at 4× the cobas CHIKV/DENV LoD with the test, including 12 CHIKV RNA–positive samples and 34 DENV RNA–positive samples (Table 2).

Correlation with RealStar assays

Results of the comparison of cobas CHIKV/DENV with Altona RealStar assays are shown in Tables 3 and 4. In the testing of culture supernatants, cobas CHIKV/DENV showed detection out to a higher dilution than the RealStar assays,

TABLE 1. PROBIT analysis defining the cobas CHIKV/DENV LoD obtained with CHIKV/DENV standards in EDTA-plasma

Analyte	Measuring units*	Probit (% LoD)	LoD	Lower 95% confidence limit	Upper 95% confidence limit
DENV-1	IU/mL	95	0.63	0.54	0.78
		50	0.14	0.12	0.16
DENV-2	IU/mL	95	1.02	0.84	1.31
		50	0.24	0.20	0.28
DENV-3	IU/mL	95	1.05	0.86	1.34
		50	0.23	0.20	0.25
DENV-4	IU/mL	95	0.37	0.31	0.49
		50	0.09	0.06	0.12
CHIKV-Asian	DU/mL	95	6.8	5.9	8.1
		50	1.8	1.7	2.0
CHIKV-ECSA	DU/mL	95	9.3	7.9	11.5
		50	2.6	2.4	2.9
CHIKV-WA	DU/mL	95	7.1	6.1	8.7
		50	2.2	2.0	2.4

* IU: International Units; DU: Detectable Units

TABLE 2. Reactivity of CHIKV/DENV clinical samples, cultured isolates, and armored RNA

Target	Genotype/serotype	Samples	% Reactive (reactive/samples tested neat)	% Reactive (reactive/samples tested) diluted to 4× LoD
CHIKV	Asian	10 clinical samples	100 (10/10)	100 (10/10)
	ECSA	1 cultured isolate	100 (1/1)	100 (1/1)
	WA	1 armored RNA	100 (1/1)	100 (1/1)
DENV	1	10 clinical samples	100 (10/10)	100 (10/10)
	2	10 clinical samples	100 (10/10)	100 (10/10)
	3	3 clinical samples, 1 cultured isolate	100 (4/4)	100 (4/4)
	4	10 clinical samples	100 (10/10)	100 (10/10)

despite doubling the volume of sample input for extraction. Regarding the 100 clinical specimens for each virus, 85 CHIKV samples and 81 DENV samples were reactive by both cobas CHIKV/DENV and the relevant RealStar assay. Fourteen CHIKV specimens and 19 DENV specimens were reactive only on cobas CHIKV/DENV and were nonreactive on the RealStar assays. There were no specimens reactive only on the RealStar assay. The remaining CHIKV sample that was negative by both assays had been reactive for CHIKV on initial testing by cobas CHIKV/DENV but with a high Ct value, suggesting a low viral load.

Reproducibility

Table 5 provides the cobas CHIKV/DENV lot-to-lot reproducibility after testing at 2× to 0.5× the assay's LoD, demonstrating cobas CHIKV/DENV reproducibility at the stated analytic sensitivity level for CHIKV-Asian and DENV-1.

Clinical performance validation

Analytical sensitivity

Table 6 provides a comparison of the estimated versus claimed 50 and 95% LoDs for each agent using cobas CHIKV/DENV. ARC testing yielded point estimates (and

TABLE 3. Sensitivity comparison of cobas CHIKV/DENV with RealStar RT-PCR assays using serially diluted culture supernatants*

	cobas CHIKV/DENV results (No. reactive/No. tested)					RealStar RT-PCR results (No. reactive/No. tested)				
Dilution	CHIKV	D1†	D2	D3	D4	CHIKV	D1	D2	D3	D4
Undiluted	NA	NA				6/6	6/6	6/6	6/6	6/6
10(−1)	3/3	3/3	3/3	3/3	3/3	6/6	6/6	6/6	4/4	6/6
10(−2)	3/3	3/3	3/3	3/3	3/3	4/6	4/6	6/6	6/6	4/6
10(−3)	0/3	3/3	3/3	3/3	3/3	0/6	0/6	2/6	2/6	0/6
10(−4)	0/3	3/3	3/3	2/3	1/3	0/6	0/6	0/6	0/6	0/6
10(−5)	0/3	1/3	0/3	1/3	0/3	0/6	0/6	0/6	0/6	0/6

* Sample input volume for the Altona assays, 280 μL.
† D1-D4 = Dengue Serotypes 1 to 4.
NA = not available.

TABLE 4. Comparison of reactivity by cobas CHIKV/DENV and RealStar assays using clinical CHIKV- or DENV-positive samples*

Result		Number of samples	
Second-generation RealStar CHIKV RT-PCR kit or RealStar DENV RT-PCR kit	cobas CHIKV/DENV	CHIKV	DENV
Nonreactive	Nonreactive	1	0
Reactive	Nonreactive	0	0
Nonreactive	Reactive	14	19
Reactive	Reactive	85	81
Total		100	100
McNemar's Test, p value (two-sided, $\alpha = 0.05$)		0.0001	0.0000

* Sample input volume for the Altona assays, 140 μL.

95% CIs) at the 50% CHIKV LoD of 2.4 (2.1 to 2.8 DU/mL), 95% CHIKV LoD of 10.5 (8.2 to 14.6 DU/mL), 50% DENV LoD of 0.15 (0.12 to 0.17 IU/mL), and 95% DENV LoD of 0.60 (0.47 to 0.86 IU/mL); each met the target of less than a 0.3 log difference versus that of the RMS-claimed LoDs. Tables S10 and S11 in the supplemental information summarize the LoD estimation for CHIKV-Asian and DENV-1.

Clinical specificity

There were 10,571 donations from the continental United States tested on cobas CHIKV/DENV, of which 10,528 donations (99.6%) had valid results. None was reactive. The prevalence of CHIKV or DENV RNA in US donations was 0% (0/10,528; 95% exact CI, 0%-0.035%). The specificity of the cobas CHIKV/DENV test was 100% (95% exact CI, 99.965%-100.000%) for both the CHIKV and the DENV targets.

DISCUSSION

The performance characteristics of the cobas CHIKV/DENV test for use on the cobas 6800/8800 Systems were evaluated at two sites (RMS and the ARC). Analytic sensitivity was determined for each of three CHIKV genotypes and four DENV serotypes. Evaluation of analytic specificity used challenging microorganisms for interference and cross-reactivity studies; clinical specificity was determined by individually testing 10,528 volunteer blood donations from the continental United States.

The assay demonstrated high analytic sensitivity for both CHIKV and DENV in the RMS studies that were confirmed by ARC studies; each study used secondary

TABLE 5. Cobas CHIKV/DENV test reagent lot-to-lot reproducibility

Analyte	Concentration (LoD)	Reagent lot	% Reactive(reactive/valid replicates)	Lower limit of 95% CI	Upper limit of 95% CI
DENV-1	2× LoD	1	100 (63/63)	94.3	100
		2	100 (63/63)	94.3	100
		3	100 (63/63)	94.3	100
	1× LoD	1	100 (63/63)	94.3	100
		2	100 (63/63)	94.3	100
		3	96.8 (61/63)	89.0	99.6
	0.5× LoD	1	92.1 (58/63)	82.4	97.4
		2	84.1 (53/63)	72.7	92.1
		3	82.5 (52/63)	70.9	90.9
CHIKV-Asian	2× LoD	1	100 (63/63)	94.3	100
		2	100 (63/63)	94.3	100
		3	100 (63/63)	94.3	100
	1× LoD	1	100 (63/63)	94.3	100
		2	100 (63/63)	94.3	100
		3	98.4 (62/63)	91.5	100
	0.5× LoD	1	77.8 (49/63)	65.5	87.3
		2	87.3 (55/63)	76.5	94.4
		3	73.0 (46/63)	60.3	83.4

TABLE 6. Summary of cobas CHIKV/DENV claimed versus estimated LoD

Viral target	LoD estimate (%)	Claimed cobas CHIKV/ DENV LoD in units/mL (log units/mL)	95% CI of claimed LoD	ARC Estimated LoD in units/mL (log units/mL)	95% CI of estimated ARC LoD	Difference of log LoDs (estimated – claimed)	Difference of log LoDs within ±0.3 log units/mL
CHIKV*	50	1.8 (0.255)	(1.7 to 2.0)	2.4 (0.382)	(2.1 to 2.8)	0.127	Yes
	95	6.8 (0.833)	(5.9 to 8.1)	10.5 (1.021)	(8.2 to 14.6)	0.189	Yes
DENV†	50	0.14 (–0.854)	(0.12 to 0.16)	0.15 (–0.838)	(0.12 to 0.17)	0.016	Yes
	95	0.63 (–0.201)	(0.54 to 0.78)	0.60 (–0.220)	(0.47 to 0.86)	–0.021	Yes

* CHIKV target is measured in DUs/mL.
† DENV target is measured in IU/mL.

standards traceable to internationally recognized reference reagents.[25,26] No significant differences in detection occurred by testing at the ARC.

All three CHIKV genotypes and four DENV serotypes were detected consistently confirming inclusivity for the cobas CHIKV/DENV test. The cobas CHIKV/DENV test demonstrated reproducibility when tested across three days, with three different reagent lots, and on three cobas 8800 Systems. Comparison studies between cobas CHIKV/DENV and the Altona RealStar Chikungunya RT-PCR and RealStar Dengue RT-PCR kits indicated that cobas CHIKV/DENV was more sensitive compared to the Altona RealStar RT-PCR kits for the detection of CHIKV- and DENV-positive samples. Although use of the cobas CHIKV/DENV for identification of the samples for the clinical sample study could have created a selection bias, it was not possible to obtain enough samples from endemic areas that were characterized by alternate molecular methods having high sensitivity, defined in relation to international reference standards. Precharacterization of clinical samples by an established method is not possible when the first commercial tests are developed for new agents.

Studies of cross-reactivity and interference demonstrated that other pathogens did not affect the sensitivity of the cobas CHIKV/DENV test when present in high concentrations and did not cause cross-reactivity in the assay. Clinical specificity from testing donors collected in a low-prevalence area (continental United States) was 100% (95% CI, 99.965%-100%) for both CHIKV and DENV.

The high sensitivity and specificity of the cobas CHIKV/-DENV test, as demonstrated in these evaluations, indicate its suitability for blood donation screening. The fully automated assay, run on the cobas 6800/8800 Systems, detects and simultaneously discriminates CHIKV and DENV RNA.

Currently, blood donations collected in CHIKV and DENV outbreak areas are not screened for the presence of these viruses, for a variety of reasons including the unavailability of a blood donor screening assay. Other considerations include the cost and/or need for such interventions due to the unclear clinical importance of transfusion transmission versus that by mosquitoes.[18] Even so, the clinical impact of transfusion transmission of these agents is likely underrecognized considering the significant adverse outcomes in severely ill patients in which many comorbidities that may relate to DENV and/or CHIKV transfusion transmission are not evaluated.[15–17] In addition, the CHIKV outbreak in Reunion Island and discovery of an ECSA mutation with greater virulence that allows CHIKV replication in *A. albopictus* has expanded the risk areas of CHIKV.[33] *A. albopictus* has subsequently been implicated as the mosquito vector for outbreaks in India, Northern Italy, and the Caribbean.[33] Recent and ongoing CHIKV/DENV outbreaks in the Americas, Europe, and Asia[5,12,14] require further vigilance ensuring the availability and access to safe blood products. Geographic expansion and the explosive nature of arboviral infections suggest that a preparedness strategy for blood product safety should be considered. Until recently, the only preventive step that could be taken for RBC collections was to interrupt blood donations during outbreaks as was done in Reunion Island, France, and Italy for CHIKV.[10,12,13,34] The availability of the cobas CHIKV/DENV test provides a suitable alternative to enhanced blood safety in the face of an increasing worldwide expansion of these agents.

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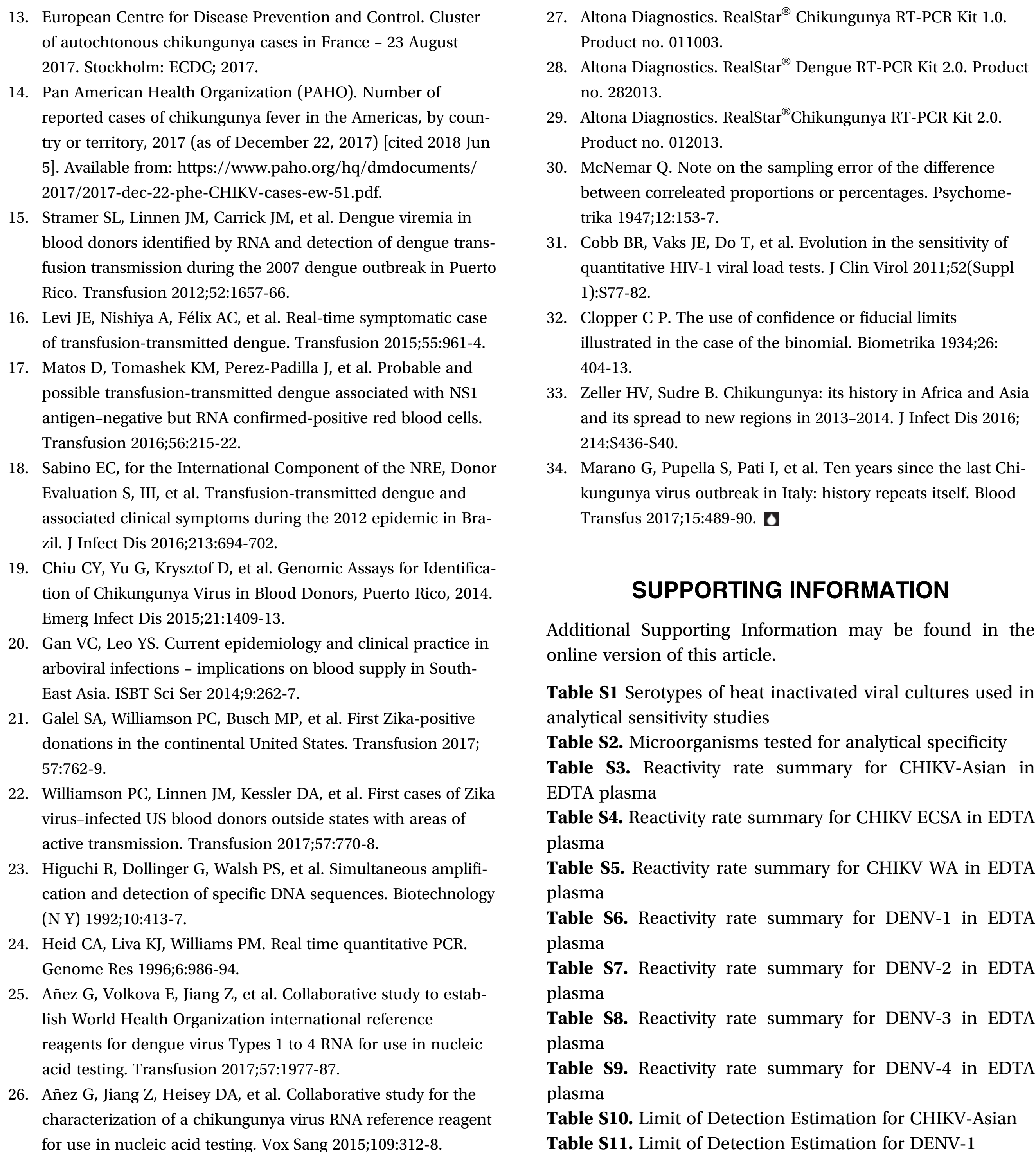

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article.

Table S1 Serotypes of heat inactivated viral cultures used in analytical sensitivity studies
Table S2. Microorganisms tested for analytical specificity
Table S3. Reactivity rate summary for CHIKV-Asian in EDTA plasma
Table S4. Reactivity rate summary for CHIKV ECSA in EDTA plasma
Table S5. Reactivity rate summary for CHIKV WA in EDTA plasma
Table S6. Reactivity rate summary for DENV-1 in EDTA plasma
Table S7. Reactivity rate summary for DENV-2 in EDTA plasma
Table S8. Reactivity rate summary for DENV-3 in EDTA plasma
Table S9. Reactivity rate summary for DENV-4 in EDTA plasma
Table S10. Limit of Detection Estimation for CHIKV-Asian
Table S11. Limit of Detection Estimation for DENV-1