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DONOR INFECTIOUS DISEASE TESTING

TRANSFUSION

Detection of dengue, chikungunya, and Zika RNA in blood donors from Southeast Asia

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Abstract

Background: Chikungunya (CHIKV), dengue (DENV), and Zika (ZIKV) viruses are of concern due to the potential of transfusion transmission in blood, especially in regions such as Southeast Asia where the viruses are endemic. The recent availability of nucleic acid testing (NAT) to screen blood donations on an automated platform provides the opportunity to detect potentially infectious units in asymptomatic donors.

Study Design and Methods: Three thousand blood donations from Vietnam and 6000 from Thailand were screened with a real-time polymerase chain reaction (PCR) test (cobas CHIKV/DENV, Roche Diagnostics, Indianapolis, IN) and equal numbers on cobas Zika (Roche Diagnostics). Reactive samples were tested by alternative NAT with resolution of discordant results by heminested PCR. Throughput of simultaneous testing of the two assays on the cobas 8800 system (Roche Diagnostics) was evaluated.

Results: In Vietnam, 9 of 3045 samples were reactive for DENV and all were confirmed, for a prevalence (with 95% confidence interval [CI]) of 0.296% (0.135-0.560). In Thailand, 2 of 6000 samples were reactive for CHIKV, 4 of 6000 for DENV, and 1 of 6005 for ZIKV, and all confirmed. The prevalence of CHIKV is 0.033% (0.004-0.120), DENV 0.067% (0.018-0.171), and ZIKV 0.017% (0.000-0.093). The overall specificity for the cobas CHIKV/DENV and cobas Zika tests was 100% (99.959-100).

For the simultaneous assay testing, 960 test results were available in 7 hours and 53 minutes.

Conclusion: Detection of CHIKV, DENV, and ZIKV RNA in donor samples in Vietnam and Thailand indicate the presence of the virus in asymptomatic

Abbreviations: CHIKV, chikungunya virus; DENV, dengue virus; HnPCR, heminested polymerase chain reaction; IDT, individual donation testing; NAT, nucleic acid testing; PCR, polymerase chain reaction; QNS, quantity not sufficient; TT, transfusion-transmitted; ZIKV, Zika virus

blood donors. The cobas 6800/8800 systems (Roche Molecular Systems, Pleasanton, CA) enable screening blood donations in endemic areas for these viruses together or separately.

KEYWORDS

chikungunya, dengue, donor blood screening, nucleic acid testing, simultaneous testing, Zika

1 | INTRODUCTION

The global increase of reemerging pathogens, such as dengue virus (DENV), Zika virus (ZIKV), and chikungunya virus (CHIKV) pose a threat to public health safety and potentially to the safety of the blood supply. All three pathogens are arthropod-borne viruses (arboviruses) transmittable to humans primarily by *Aedes aegypti* and to a lesser extent *Aedes albopictus*.[1,2] DENV and ZIKV are members of the Flaviviradae family of viruses, which include West Nile virus and yellow fever virus[1,3]; and CHIKV is an alpha virus of the family Togaviridae made up of three distinct genotypes, the West African, East Central South African, and Asian genotype (a variant of East Central South African).[4,5]

Four distinct serotypes, DENV-1, DENV-2, DENV-3, and DENV-4 cause dengue disease, with infection by one serotype providing lifelong immunity and only partial or temporary immunity against the other serotypes.[4,6] Approximately 80% of individuals infected with DENV are asymptomatic. The majority of those individuals with symptoms are usually mild, although acute flulike symptoms may occur, occasionally progressing to severe dengue, which can be life threatening.[4,6] According to the World Health Organization the global incidence of dengue has grown dramatically, with the largest number of cases reported in 2019.[6] The World Health Organization states that half of the world population is at risk of dengue, with an estimated 100 million to 400 million infections each year, with Asia representing approximately 70% of the global burden of the disease.[6]

The explosive outbreak of the ZIKV in the Americas during 2015 is another example of a reemerging pathogen. Originally identified in Uganda in 1947, the first reported outbreak was in 2007 from Yap Island in the Federated States of Micronesia, followed by another in 2013 in French Polynesia and again in 2015 in the Americas.[7,8] Similar to dengue, most infections are asymptomatic or with mild symptoms; however, infection during pregnancy can cause microcephaly and congenital Zika syndrome in developing fetuses and newborns.[9] Infection can also cause Guillain-Barré syndrome and other neurological problems in adults and children.[7]

Unlike DENV or ZIKV infection, the majority of CHIKV infections are symptomatic and may cause acute, subacute, or chronic disease, although rare fatalities have been reported.[5,10] The most notable symptom from the disease is severe joint pain and crippling arthritis that can be very debilitating and may remain months or years after resolution of acute disease.[5,11] A resurgence of CHIKV since 2000 has led to multiple outbreaks in Africa, Asia, and the Americas[12] and recently in Europe with reports of both imported and autochthonous cases of CHIKV identified in France and Italy.[5,13–15]

Interestingly, based on the number and magnitude of outbreaks for these viruses in endemic areas, one would expect a larger number of reported transfusion-transmitted (TT) cases. Reasons suggested for the relatively low number of documented TT-DENV infections include a high level of IgG seroprevalence in endemic areas, enhanced pathogenicity due to mosquito saliva factors compared to an intra-venous transfusion from an infected unit, and potentially less severe clinical outcomes, which may not be recognized in infected recipients.[16–18] Nevertheless, clinically significant illnesses have been documented in TT-DENV cases.[16,19,20]

Reports of global outbreaks due to these viruses still raise concerns about the impact on blood safety. Viral RNA has been detected in blood donors in prior outbreaks of CHIKV,[21–25] DENV[19,25–27] and ZIKV[28,29] with documented reports of transfusion transmission of DENV[16,30] and ZIKV[31,32] from asymptomatic blood donors. Although CHIKV is endemic in many of the same countries as DENV and ZIKV, there are no reports of TT.[1,4] Regardless, preventive actions to stop blood donations during CHIKV outbreaks have taken place. Between 2005 and 2007, a massive outbreak due to CHIKV on Reunion Island in the Indian Ocean caused a halt to blood donations as a precaution,[21] as did a 2007 CHIKV outbreak in northern Italy.[33] The European Center for Disease Control also recommended cessation of blood donations during the 2017 CHIKV outbreaks in France and Italy.[14,15] In 2016, during the ZIKV-outbreak in the Americas, the USFood and Drug Administration required all blood components collected in Zika active areas of the United States and its territories to be screened with ZIKV nucleic acid testing (NAT) or to treat the blood components with a Food and Drug Administration–approved pathogen reduction technology.[34]

Although outbreaks of these viruses continue in various parts of the world, blood donations are not routinely

screened for CHIKV or DENV and screening for ZIKV RNA is limited to the United States and Singapore. In most countries, other methods of prevention have been implemented including vector control and personal protection against mosquito bites. Screening of blood may include serology testing for the detection of IgM and IgG antibodies; however, the presence of these antibodies occurs several days postinfection. The efficacy of donor blood screening with NAT for other reemerging pathogens has been demonstrated with West Nile Virus and ZIKV.[29,35] One of the arguments against NAT is the cost and need for highly skilled personnel; however, the availability of automated platforms makes it possible to test a large volume of donations with minimal hands-on time and reduces the potential for human error.

The cobas CHIKV/DENV test and cobas Zika test detect viral RNA in human plasma and can be run on an automated NAT using real-time polymerase chain reaction (PCR) technology (cobas 6800/8800 Systems; Roche Molecular Systems, Pleasanton, CA). The cobas CHIKV/DENV test (Roche Diagnostics, Indianapolis, IN) is a duplex test that directly detects and discriminates CHIKV and DENV RNA and cobas Zika (Roche Diagnostics) directly detects ZIKV RNA. Studies were conducted to determine the prevalence in the donor population in Southeast Asian countries, a region known to be endemic for these viruses.[6,11,36] We also evaluated the ability to test samples on the cobas 8800 system for all three targets simultaneously, measuring throughput in an 8-hour work shift.

2 | MATERIALS AND METHODS

2.1 | Assays and systems

The cobas CHIKV/DENV test is a qualitative in vitro test for the detection of CHIKV RNA and DENV RNA, serotypes 1 through 4 in human plasma. The cobas CHIKV/DENV is designed to test for CHIKV and DENV RNA either alone or simultaneously. The cobas Zika test is a qualitative in vitro test for the detection of ZIKV RNA. Both tests allow plasma from donations of whole blood and blood components to be tested individually or in pools composed of individual samples.[37,38]

The cobas CHIKV/DENV and cobas Zika tests are based on fully automated sample preparation (nucleic acid extraction and purification) followed by PCR amplification and detection on the cobas 6800/8800 systems. The cobas 6800/8800 systems are highly automated testing platforms consisting of sample supply, transfer, processing, and analytic modules. Reagents are ready to use, requiring no preparation, and can be stored on the instrument. Minimal operator hands-on time is required once reagents, consumables, and samples are loaded onto the instrument. The cobas 6800/8800 software performs automated data management that assigns test results that can be reviewed directly on the system screen and printed as a report or transmitted to a laboratory information system.[39] Up to 384 test results can be generated on the cobas 6800 and up to 960 test results on the cobas 8800 within an 8-hour work shift.[40]

2.2 | Clinical prevalence and specificity testing

Deidentified ethylenediaminetetraacetic acid plasma samples were collected from routine blood donations in Vietnam between October and December 2018, and stored at −30°C for a maximum of 4 months before testing at the National Institute of Hematology and Blood Transfusion in Hanoi, Vietnam. Ethylenediaminetetraacetic acid plasma samples were collected in Bangkok and eastern and southern Thailand, between May and November 2019, and stored at −40°C or −60°C for a maximum of 40 days before testing at the Faculty of Medicine, Siriraj Hospital, Mahidol University, or at the Faculty of Medicine, Ramathibodi Hospital, Mahidol University in Bangkok. Each of the laboratory sites tested a minimum of 3000 samples by individual donation testing (IDT) using cobas CHIKV/DENV and a minimum of 3000 samples by IDT using cobas Zika. The ethics committee or institutional review board for each site approved the study protocols and materials.

Each site tested the samples once by IDT with the respective cobas test and samples nonreactive were considered RNA negative for the targeted viruses. Samples reactive with cobas CHIKV/DENV were further tested to confirm reactivity (Figure 1). Additional testing included repeat testing by cobas CHIKV/DENV neat and in a 1:6 dilution format, and testing by an alternative NAT using an in vitro diagnostic test (RealStar Chikungunya RT-PCR Kit 2.0[41] or RealStar Dengue RT-PCR Kit 2.042; Altona Diagnostics,Plain City,OH) based on the reactive target reported in the cobas CHIKV/DENV test. An enhanced sample input volume was used for the RealStar assays based on prior studies comparing the sensitivity of the RealStar assays to cobas CHIKV/DENV, and each concentration was tested in multiple replicates.[43] Samples with one or more reactive replicates on the target-specific Altona test were considered confirmed for the target. DENV serotypes and CHIKV genotypes were not determined. Samples reactive by cobas Zika were further tested, including a repeat test by cobas Zika neat and in a 1:6 dilution, by an alternative NAT in duplicate, and anti-Zika IgM test as described in Galel et al[44] (Figure 2). Samples reactive by alternative NAT or anti-Zika IgM confirmed the presence of ZIKV. Discordant results between initial and additional testing for CHIKV, DENV,

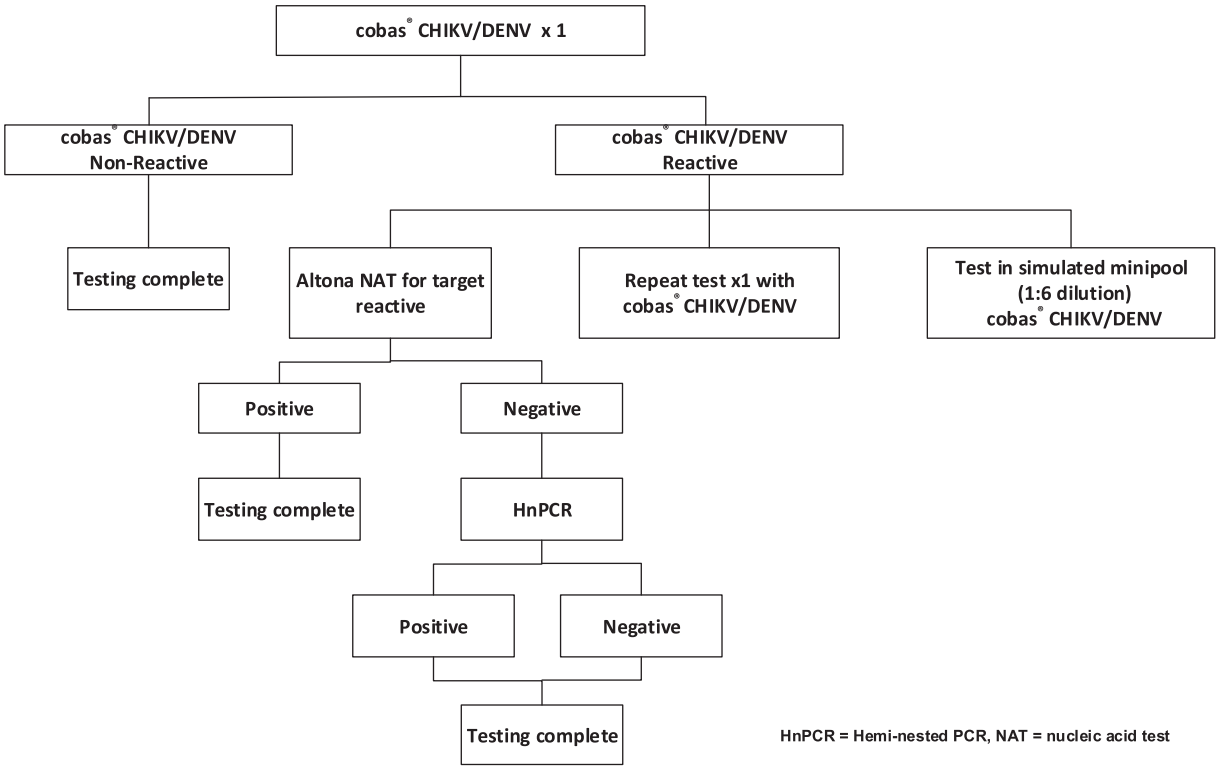

FIGURE 1 Testing algorithm for cobas CHIKV/DENV

or ZIKV were tested by heminested PCR (HnPCR) performed on the amplification product of the initial testing to resolve the status of the sample.

The prevalence of each virus was calculated as the percentage of samples confirmed to contain target-specific RNA among samples with valid cobas CHIKV/DENV or cobas Zika results. The specificity of each test was calculated as the percentage of RNA negative samples that were nonreactive on cobas CHIKV/DENV or cobas Zika.

2.3 | Multiassay testing

Approximately 1500 deidentified ethylenediaminetetraacetic acid plasma samples were collected from infectious disease screened (including Zika), nonreactive volunteer donations from the continental United States. Samples were unscreened for CHIKV or DENV. No known Zika-, CHIKV-, or DENV-positive samples were included because the purpose of the study was solely to evaluate testing throughput on the cobas 8800 system. Samples were tested by IDT with cobas CHIKV/DENV and cobas Zika for use with the cobas 6800/8800 systems according to the manufacturer's instructions.[37,38]

After centrifugation, a maximum of 460 samples were continuously loaded onto a cobas 8800 system. Each sample was pipetted by the instrument onto a processing plate for concurrent testing with cobas CHIKV/DENV and cobas Zika. Each processing plate contained up to 46 samples plus 2 controls per assay for a total of 96 tests. Time was captured at the beginning of processing of the first samples (start time), time to first available test result, and time to last available test result. Total processing time was evaluated to measure the maximum number of test results available in an 8-hour work shift.

3 | RESULTS

3.1 | Prevalence and specificity

3.1.1 | Vietnam

No samples were reactive by cobas Zika, and no samples were reactive for CHIKV by cobas CHIKV/DENV. Nine of 3045 (1:338) samples were reactive for DENV (Table 1**)**. Six of the nine samples were confirmed by the Altona RealStar Dengue RT-PCR Kit 2.0 test; and HnPCR confirmed the three Altona nonreactive samples. In addition, eight of the nine reactives were reactive upon repeat testing by cobas CHIKV/DENV neat, including the three that were nonreactive on the Altona test. None of the reactive samples was tested at 1:6 dilution, as the quantity of remaining plasma was not sufficient for testing

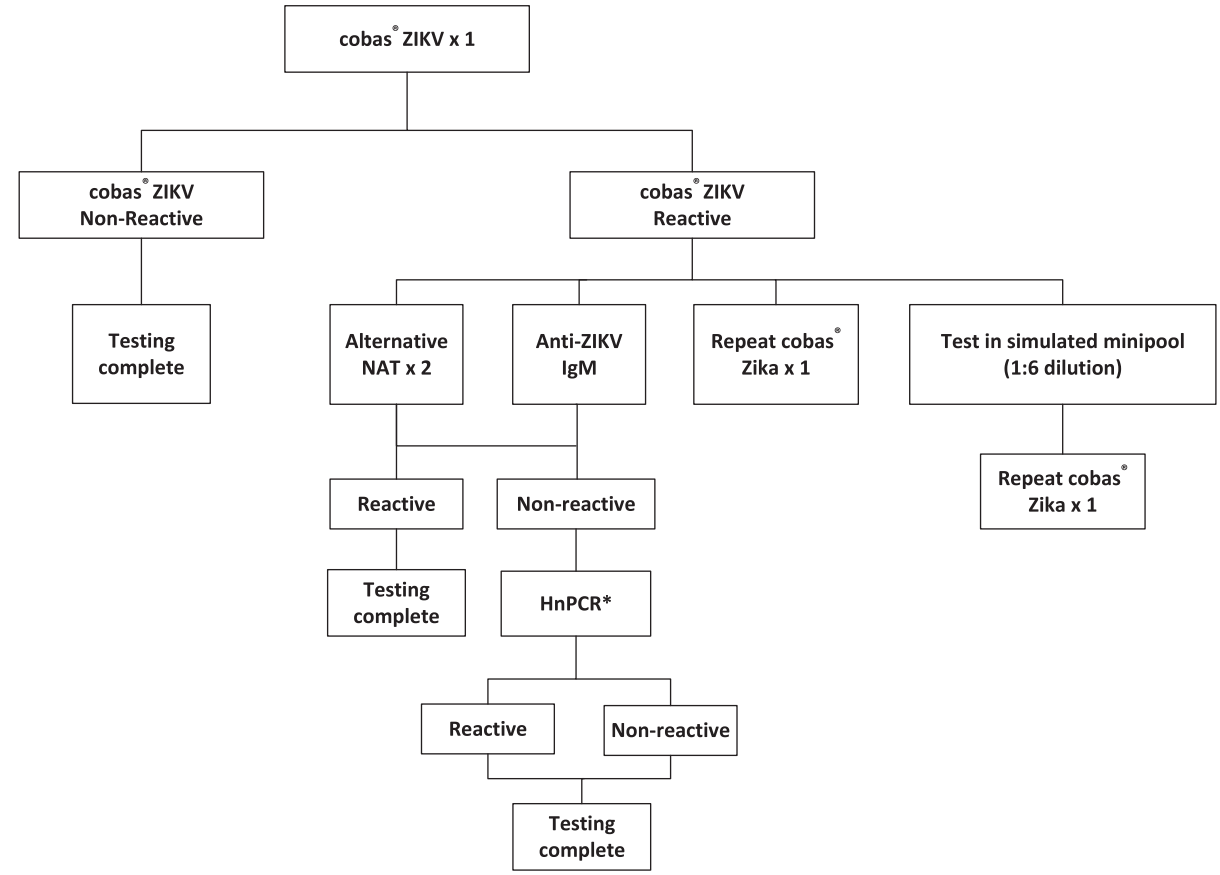

FIGURE 2 Testing algorithm for cobas Zika

TABLE 1 Test results of cobas CHIKV/DENV-reactive samples from Vietnam

Cobas CHIKV/DENV			Altona RealStar dengue 3.0 Number of replicates			
Initial reactive (sample ID) DENV reactive	Repeat test × 1	1:6 dilution	Nonreactive	Reactive	Heminested PCR	Interpretation
NIHCVDV1405D	Reactive	QNS	0	15	N/A	Positive
NIHCVDV2142D	Reactive	QNS	0	15	N/A	Positive
NIHCVDV2526D	Reactive	QNS	0	15	N/A	Positive
NIHCVDV1397D	Reactive	QNS	7	8	N/A	Positive
NIHCVDV1398D	Reactive	QNS	8	7	N/A	Positive
NIHCVDV3315D	Nonreactive	QNS	14	1	N/A	Positive
NIHCVDV1801D	Reactive	QNS	15	0	Reactive	Positive
NIHCVDV2224D	Reactive	QNS	15	0	Reactive	Positive
NIHCVDV2422D	Reactive	QNS	15	0	Reactive	Positive

Abbreviations: QNS, quantity not sufficient; PCR, polymerase chain reaction.

(QNS). In summary, all nine samples initially reactive by cobas CHIKV/DENV were confirmed positive for DENV RNA.

The prevalence of DENV in the Vietnam study is 0.296% (95% confidence interval [CI], 0.135-0.560) and the prevalence of CHIKV is 0.00% (95% CI, 0.00-0.121).

3.1.2 | Thailand

Six of 6000 valid tests were initially reactive by cobas CHIKV/DENV, two reactive for CHIKV (1:3000) and four reactive for DENV (1:1500). One of the two CHIKV-reactive samples and three of the four DENV-reactive samples were confirmed by the Altona tests. The one remaining CHIKV and one DENV cobas reactive, Altona nonreactive samples were confirmed with HnPCR testing (Table 2**)**.

Only one CHIKV and one DENV Altona reactive samples had sufficient remaining volume to repeat testing with the cobas test neat, and both were reactive. Three of the four DENV-initial-reactive samples and one of two CHIKV-initial-reactive samples were reactive when tested at 1:6 dilution with cobas CHIKV/DENV, and the remaining samples were QNS for this testing.

One of 6005 (1:6005) valid tests was initially reactive by cobas Zika and was confirmed reactive in duplicate by the alternate NAT. The sample was nonreactive for Zika anti-IgM and was QNS for repeat testing by neat or 1:6 dilution by cobas Zika (Table 3**)**.

The combined prevalence of DENV in the two Thailand sites is 0.067% (95% CI, 0.018, 0.171) and of CHIKV is 0.033% (95% CI, 0.004-0.120). The combined prevalence of Zika in the Thailand sites is 0.017% (95% CI, 0.00-0.093).

The combined clinical specificity of all three sites for cobas Zika is 100% (95% CI, 99.959-100) and for cobas CHIKV/DENV is 100% (95% CI, 99.959-100).

3.2 | Multiassay testing

All results were valid and nonreactive for CHIKV, DENV, and ZIKV. The time to process and report 960 test results for cobas CHIKV/DENV and cobas Zika on the cobas 8800 system was 7 hours and 53 minutes. The first 96 test results for cobas CHIKV/DENV and cobas Zika were available 2 hours and 59 minutes from sample loading. This time included pipetting and processing of each sample and amplification and detection of potential CHIKV, DENV, and ZIKV targets. Another 96 test results were available approximately every 33 minutes.

4 | DISCUSSION

A major aspect to ensuring a safe blood supply is the ability to detect pathogens that may cause TT infections. Advancements in technology such as NAT have provided an additional layer to blood safety with the ability to detect very low levels of RNA or DNA in human blood.

TABLE 2 Test results of cobas CHIKV/DENV-reactive samples from Thailand

Cobas CHIKV/DENV				Altona RealStar Chikungunya 2.0		Altona RealStar dengue 3.0		Heminested PCR	
Initial reactive (sample ID)				Number of replicates		Number of replicates			
CHIKV reactive	DENV reactive	Repeat test	1 : 6 dilution	Non-reactive	Reactive	Non-reactive	Reactive		Interpretation
RAMCVDV2315C		QNS	QNS	10	0			Reactive	Positive
SIRCVDV2298C		Reactive	Reactive	14	1			N/A	Positive
	RAMCVDV1584D	QNS	Reactive			0	15	N/A	Positive
	RAMCVDV2490D	QNS	QNS			0	10[a]	N/A	Positive
	RAMCVDV1661D	QNS	Reactive			15	0	Reactive	Positive
	SIRCVDV2906D	Reactive	Reactive			0	15	N/A	Positive

[a]Available sample volume only allowed for 10 replicates.
Abbreviations: QNS, quantity not sufficient; N/A, not applicable; PCR, polymerase chain reaction.

TABLE 3 Test results of cobas Zika-reactive sample from Thailand

Cobas Zika			Alternative NAT (Number of replicates)			
Initial reactive (sample ID)	**Repeat test x1**	**1:6 dilution (Roche)**	**Non-reactive**	**Reactive**	**Anti-Zika IgM**	**Interpretation**
RAMZIKA2053	QNS	Nonreactive	0	2	Nonreactive	Positive

Abbreviations: QNS, quantity not sufficient.

This is especially important for identifying a potentially infectious unit of blood for transfusion. The utility of NAT has been shown for human immunodeficiency virus and hepatitis, especially in the detection of early infection in a donor.[45–47]

The results of these studies demonstrate the ability of cobas CHIKV/DENV and cobas Zika to detect CHIKV, DENV, and ZIKV RNA in blood donations from Thailand and Vietnam, two countries located in Southeast Asia endemic for these viruses. Both countries have reported an increase of DENV reactivity in the general population. During the time that the studies were conducted 86 418 cases were reported in Thailand for 2019 and 126 682 cases were reported in Vietnam for 2018,[48] so it is not unexpected that DENV may be present in the blood donor population. The prevalence of DENV RNA in other studies report a range of 0.02% to 0.54% in blood donors during similar outbreaks, although most were conducted in the Americas.[19,25–27,49,50] In Asia, a similar-size study of 3000 blood donors conducted during the 2014 Guangzhou outbreak reported a DENV RNA prevalence of 0.07%,51 and in the 2015 outbreak in Taiwan as 0.013%.[52] DENV is clearly transmissible by transfusion. Clinical DENV and even dengue hemorrhagic fever have been reported in some recipients of DENV-positive blood components,[16,19] although the clinical importance of preventing TT-DENV in the context of mosquito-borne outbreaks is unclear.[30]

The finding of a Zika RNA-positive donation in Thailand is consistent with reports of the long-term presence of the virus in Southeast Asia.[53] Case reports and phylogenic analysis suggest that ZIKV has been circulating in Thailand since 2002.[54] Continued activity in 2019 was demonstrated by infections acquired by individuals who traveled to Thailand.[55] ZIKV outbreaks in 2018 in India suggest the possibility of persistent activity in that country as well.[56] Thus, although the prevalence of ZIKV in this study is low, the detection of a ZIKV-positive donation indicates the presence of ongoing virus activity and need for continued vigilance. Similarly, there has been long-term and ongoing CHIKV activity in multiple countries in Asia.[57,58] In Thailand, there have been periodic CHIKV outbreaks particularly in the southern part of the country, including outbreaks in 2009 and 2019,[22,59] with 11 484 cases reported in 2019, during the time of the study.[48] Appassakij estimated the risk of TT-CHIKV to be approximately 1 in 2000 during the 2009 outbreak. A donor-screening CHIKV assay provides a potential mitigation strategy for preventing TT-CHIKV and may be more practical than cessation of blood collection during large outbreaks.

The discrepant results shown between some of the initial-reactive samples and the Altona testing results indicate that some of the samples may have contained low viral loads for CHIKV or DENV. The Altona RealStar tests are less sensitive than the cobas tests.[37,38,41–43]

Of note is that those samples that had sufficient plasma to be tested in a 1:6 dilution format for CHIKV and DENV were reactive, indicating that mini-pool testing is also suitable for detecting the presence of the viruses. Even though mini-pool testing may be more economical, IDT is more sensitive for the detection of low viral loads. An algorithm for switching from mini-pool testing to IDT based on specific factors similar to the one used by the United States for West Nile Virus and Zika testing may be an option.[60,61]

In addition to increasing the sensitivity of testing to detect potential TT pathogens, blood centers need to be prepared for new emerging or reemerging pathogens that may threaten the blood supply. The ability to implement a new test with minimal disruption to the current workflow provides an advantage to respond to the situation quickly and efficiently. Here, we confirm the manufacturer's claim for the cobas 8800 system's ability to produce 960 test results in less than 8 hours. In addition, the ability to run cobas CHIKV/DENV and cobas Zika simultaneously confirms that the cobas 6800/8800 systems are ideal for screening a large number of donations in a timely fashion. The full automation of the systems reduces staff hands-on time and reduces the opportunity for human error, as well as frees up staff to perform other tasks while the samples are tested on the instruments.

Outbreaks of CHIKV, DENV, and ZIKV will most likely continue to occur in tropical and subtropical areas of the

world where the *Aedes* mosquito breeds. Even with vector control, personal protection, and most recently a dengue vaccine, infections are likely to occur. Asymptomatic blood donors who pass all other donor requirements may still be infected with one of these viruses, and the possibility of transfusion transmission exists. The ability to proactively screen for these viruses in endemic areas provides an option for an additional layer of safety to the blood supply. Decisions regarding blood safety mitigations can be made in the context of local epidemiology and resources.

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CONFLICT OF INTEREST

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