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

Jean Stanley1 | Viroje Chongkolwatana2 | Pham Tuan Duong3 |
Pimpun Kitpoka4 | Susan L. Stramer5 | Nguyen Thi Thanh Dung3 |
Kacie E. Grimm5 | Anyarin Pojanasingchod2 | Panitita Suksomboonvong4 |
Susan A. Galel1

1Medical and Scientific Affairs, Roche Molecular Diagnostics, Pleasanton, California
2Faculty of Medicine, Siriraj Hospital, Mahidol University, Bangkok, Thailand
3Blood Screening, National Institute of Hematology and Blood Transfusion, Hanoi, Vietnam
4Faculty of Medicine, Ramathibodi Hospital, Mahidol University, Bangkok, Thailand
5Scientific Affairs, American Red Cross, Gaithersburg, Maryland

Correspondence
Jean Stanley, Medical and Scientific Affairs, Roche Molecular Diagnostics, 4800 Hacienda Drive, Pleasanton, CA 94588.
Email: jean.stanley@contractors.roche.com

Funding information
Roche Molecular Diagnostics, Grant/Award Number: None

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 donors.
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) transmissible to humans primarily by *Aedes aegypti* and to a lesser extent *Aedes albopictus*.1,2 DENV and ZIKV are members of the Flaviviridae family of viruses, which include West Nile virus and yellow fever virus1,3; 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. 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 Americas12 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 DENV19,25–27 and ZIKV28,29 with documented reports of transfusion transmission of DENV26,30 and ZIKV31,32 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 US Food 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 2.041 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 al44 (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,
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.
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).

**FIGURE 2**  Testing algorithm for cobas Zika

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

| Cobas CHIKV/DENV | Initial reactive (sample ID) | Repeat test × 1 | 1:6 dilution | Altona RealStar dengue 3.0 | Number of replicates | Interpretation |
|-------------------|-----------------------------|----------------|--------------|----------------------------|----------------------|----------------|
|                   | DENV reactive               |                |              | Nonreactive | Reactive | Heminested PCR |                  |
| 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.
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 on the cobas 8800 system was 7 hours and 53 minutes. The 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.
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.\textsuperscript{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,\textsuperscript{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.\textsuperscript{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%\textsuperscript{,51} and in the 2015 outbreak in Taiwan as 0.013%.\textsuperscript{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,\textsuperscript{16,19} although the clinical importance of preventing TT-DENV in the context of mosquito-borne outbreaks is unclear.\textsuperscript{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.\textsuperscript{53} Case reports and phylogenetic analysis suggest that ZIKV has been circulating in Thailand since 2002.\textsuperscript{54} Continued activity in 2019 was demonstrated by infections acquired by individuals who traveled to Thailand.\textsuperscript{55} ZIKV outbreaks in 2018 in India suggest the possibility of persistent activity in that country as well.\textsuperscript{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.\textsuperscript{57,58} In Thailand, there have been periodic CHIKV outbreaks particularly in the southern part of the country, including outbreaks in 2009 and 2019,\textsuperscript{22,59} with 11,484 cases reported in 2019, during the time of the study.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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

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

Abbreviations: QNS, quantity not sufficient.

**Tabla 3** Test results of cobas Zika-reactive sample from Thailand
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.

**ACKNOWLEDGMENTS**

The authors thank Prof. Dr. Udo Reischl at the Institute for Medical Microbiology and Hygiene, University of Regensburg, Germany, for performing the confirmatory testing with the RealStar assays for CHIKV and DENV; and Dr. Michael Busch and Dr. Sonia Bakkour at Vitalant Research Institute, San Francisco, California, for performing the alternate NAT and anti-Zika IgM testing for ZIKV. We also thank those responsible for testing, statistical analysis, and study management, including the staff at the National Institute of Hematology and Blood Transfusion; Faculty of Medicine, Siriraj Hospital, Mahidol University; Ramathibodi Hospital; Sakina Smith at the ARC Scientific Support Office, Bethesda, Maryland; Sabine Locher at Roche Diagnostics International Ltd., Rotkreuz; and Kristin Bick, Kevin Luk, Shardule Shah, and Shikha Chugh at Roche Molecular Diagnostics, Pleasanton, California.

**CONFLICT OF INTEREST**

The studies described in this manuscript were sponsored by Roche Molecular Diagnostics. J.S. and S.A.G. are employees of Roche Molecular Systems, Inc. V.C., P.T.D., P.K., and S.L.S. were principal investigators for the studies. N.T.T.D., K.E.G., A.P., and P.S. managed the studies at their respective sites.

**ORCID**

Jean Stanley https://orcid.org/0000-0002-9182-9281  
Susan L. Stramer https://orcid.org/0000-0002-5865-6366

**REFERENCES**

1. Stramer SL. Current perspectives in transfusion-transmitted infectious diseases: emerging and re-emerging infections. ISBT Sci Ser. 2014;9:30–36.
2. Petersen LR, Busch MP. Transfusion-transmitted arboviruses. Vox Sang. 2010;98:495–503.
3. Kuno G, Chang G-JJ, Tsuchiya KR, Karabatsos N, Cropp CB. Phylogeny of the genus flavivirus. J Virol. 1998;72:73–83.
4. Stramer SL, Hollinger FB, et al. Emerging infectious disease agents and their potential threat to transfusion safety (Appendix 2). Transfusion. 2009, updated February 2014;49:45S–233S.
5. Burt FJ, Chen W, Miner JJ, et al. Chikungunya virus: an update on the biology and pathogenesis of this emerging pathogen. Lancet Infect Dis. 2017;17:e107–e117.
6. World Health Organization (WHO). Dengue and severe dengue. 2020. https://www.who.int/news-room/fact-sheets/detail/dengue-and-severe-dengue. Accessed March 13, 2020.
7. Baud D, Gubler DJ, Schaub B, Lanteri MC, Musso D. An update on Zika virus infection. Lancet. 2017;390:2099–2109.
8. Duffy MR, Chen T-H, Hancock WT, et al. Zika virus outbreak on Yap Island, Federated States of Micronesia. N Engl J Med. 2009;360:2536–2543.
9. Rasmussen SA, Jamieson DJ, Honein MA, Petersen LR. Zika virus and birth defects — reviewing the evidence for causality. N Engl J Med. 2016;374:1981–1987.
10. Petersen LR, Stramer SL, Powers AM. Chikungunya virus: possible impact on transfusion medicine. Transfus Med Rev. 2010;24:15–21.
11. World Health Organization (WHO). Chikungunya 2017. Available from: https://www.who.int/news-room/fact-sheets/detail/chikungunya. Accessed March 19, 2020.
12. Lindh E, Argentini C, Remoli ME, et al. The Italian 2017 outbreak chikungunya virus belongs to an emerging aedes albopictus-adapted virus cluster introduced from the Indian subcontinent. Open Forum Infect Dis. 2018;6:1–8.
13. Grandadam M, Caro V, Plumet S, et al. Chikungunya virus, southeastern France. Emerg Infect Dis. 2011;17:910–913.
14. European Centre for Disease Prevention and Control. Cluster of autochthonous chikungunya cases in France – 23 August 2017. Stockholm: ECDC; 2017. https://www.ecdc.europa.eu/sites/portal/files/documents/RRA-Chikungunya-France-revised-Aug-2017.pdf. Accessed April 23, 2020.
15. European Centre for Disease Prevention and Control. Cluster of autochthonous chikungunya cases in Italy. First update. October 9, 2017. Stockholm: ECDC. 2017. https://www.ecdc.europa.eu/sites/portal/files/documents/RRA-chikungunya-Italy-update-9-Oct-2017.pdf. Accessed April 23, 2020.
16. Levi JE, Nishiya A, Felix AC, et al. Real-time symptomatic case of transfusion-transmitted dengue. Transfusion. 2015;55:961–964.
17. Lanteri MC, Busch MP. Dengue in the context of “safe blood” and global epidemiology: to screen or not to screen? Transfusion. 2012;52:1634–1639.
18. Beau F, Mallet HP, Lastère S, Broult J, Laperche S. Transfusion risk associated with recent arbovirus outbreaks in French Polynesia. Vox Sang. 2020;115:124–132.
19. Stramer SL, Linnen JM, Carrick JM, et al. Dengue viremia in blood donors identified by RNA and detection of dengue transmission during the 2007 dengue outbreak in Puerto Rico. Transfusion. 2012;52:1657–1666.
20. Santos FLS, Slavov SN, Bezerra RS, et al. Vaso-occlusive crisis in a sickle cell patient after transfusion-transmitted dengue infection. Transfusion. 2020;60:2139–2143.
21. Brouard C, Bernillon P, Quatrerous S, et al. Estimated risk of Chikungunya viremic blood donation during an epidemic on Reunion Island in the Indian Ocean, 2005 to 2007. Transfusion. 2008;48:1333–1341.
22. Appassakij H, Promwong C, Rujirjindakul P, Wutthanarungsan R, Silpapojakul K. The risk of blood transfusion-associated
Chikungunya fever during the 2009 epidemic in Songkla Province, Thailand. Transfusion. 2014;54:1945–1952.
23. Simmons G, Bres V, Lu K, et al. High incidence of Chikungunya virus and frequency of viremic donations during epidemic, Puerto Rico, USA, 2014. Emerg Infect Dis. 2016;22:1221–1228.
24. Gallian P, de Lamballerie X, Salez N, et al. Prospective detection of chikungunya virus in blood donors, Caribbean 2014. Blood. 2014;123:3679–3681.
25. Dias LL, Amarilla AA, Poloni TR, Covas DT, Aquino VH, Figueiredo LT. Detection of dengue virus in sera of Brazilian blood donors. Transfusion. 2012;52:1667–1671.
26. Linnen JM, Vinelli E, Sabino EC, et al. Dengue viremia in blood donors from Honduras, Brazil, and Australia. Transfusion. 2008;48:1355–1362.
27. Mohammed H, Linnen JM, Munoz-Jordan JL, et al. Dengue virus in blood donations, Puerto Rico, 2005. Transfusion. 2008;48:1348–1354.
28. Musso D, Nilles EJ, Cao-Lormeau V-M. Rapid spread of emergence of Zika virus in the Pacific area. Clin Microbiol Infect. 2014;20:O595–O596.
29. Kuehnert MJ, Basavaraju SV, Moseley RR, et al. Screening of blood donations for Zika virus infection - Puerto Rico, April 3–June 11, 2016. MMWR Morb Mortal Wkly Rep. 2016;65:627–628.
30. Sabino EC, Loureiro P, Lopes ME, et al. Transfusion-transmitted dengue and associated clinical symptoms during the 2012 epidemic in Brazil. J Infect Dis. 2016;213:694–702.
31. Barjas-Castro ML, Angerani RM, Cunha MS, et al. Probable transfusion-transmitted Zika virus in Brazil. Transfusion. 2016;56:1684–1688.
32. Motta IJF, Spencer BR, Cordeiro da Silva SG, et al. Evidence for transmission of Zika virus by platelet transfusion. N Engl J Med. 2016;375:1101–1103.
33. Liumbruno GM, Calteri D, Petropulacos K, et al. The chikungunya epidemic in Italy and its repercussion on the blood system. Blood Transfus. 2008;6:199–210.
34. U.S. Food and Drug Administration. Guidance for Industry: Revised Recommendations for Reducing the Risk of Zika Virus Transmission by Blood and Blood Components. 2018. [cited April 24, 2020]. Available from: https://www.fda.gov/regulatory-information/search-fda-guidance-documents/revised-recommendations-reducing-risk-zika-virus-transmission-blood-and-blood-components.
35. Petersen LR. Epidemiology of West Nile virus in the United States: implications for arbovirology and public health. J Med Entomol. 2019;56:1456–1462.
36. European Centre for Disease Prevention and Control. Zika virus transmission worldwide – April 9, 2019. Stockholm: ECDC. 2019. https://www.ecdc.europa.eu/sites/portal/files/documents/zika-risk-assessment-9-april-2019.pdf. Accessed September 2, 2020.
37. Roche Molecular Systems, Inc. (2018). cobas® CHIKV/DENV for use on the cobas® 6800/8800 Systems. 08077851001-02EN (CE-IVD).
38. Roche Molecular Systems, Inc. cobas® Zika test for use on the cobas® 6800 and cobas® 8800 Systems. (2019). 08082871001-04EN.
39. Roche Diagnostics GmbH. cobas® 6800/8800 Systems operator’s manual, version 2.0. 2015.
58. European Centre for Disease Prevention and Control. Communicable disease threats report: week 8, 16-22 February 2020. Stockholm: ECDC. 2020. https://www.ecdc.europa.eu/en/publications-data/communicable-disease-threats-report-16-22-february-2020-week-8. Accessed June 1, 2020.

59. Appassakij H, Silpapojakul K, Promwong C, Rujirojindakul P. The potential impact of chikungunya virus outbreaks on blood transfusion. Transfus Med Rev. 2020;34:23–28.

60. U.S. Food and Drug Administration. Use of nucleic acid tests to reduce the risk of transmission of West Nile virus from donors of whole blood and blood components intended for transfusion. 2009. [cited 2020 April 28]. Available from: https://www.fda.gov/regulatory-information/search-fda-guidance-documents/use-nucleic-acid-tests-reduce-risk-transmission-west-nile-virus-donors-whole-blood-and-blood.

61. U.S. Food and Drug Administration. Revised recommendations for reducing the risk of Zika virus transmission by blood and blood components. 2018 [cited 2020 April 28]. Available from: https://www.fda.gov/regulatory-information/search-fda-guidance-documents/revised-recommendations-reducing-risk-zika-virus-transmission-blood-and-blood-components.

How to cite this article: Stanley J, Chongkolwatana V, Duong PT, et al. Detection of dengue, chikungunya, and Zika RNA in blood donors from Southeast Asia. Transfusion. 2021;61:134–143. https://doi.org/10.1111/trf.16110