Preplanned Studies

Two-Year Surveillance of Dengue, Zika, and Chikungunya Viruses Among Chinese Blood Donors — Guangxi and Yunnan PLADs, China, 2022–2023

Ying Yan[1,&]; Xinru Liu[2,&]; Shaofang Lu[3,&]; Le Chang[1,2]; Jing Dong[3]; Huimin Ji[1]; Huizhen Sun[1]; Lunan Wang[1,2,#]

Summary

What is already known about this topic?

The transmission of emerging and re-emerging arboviruses represent a critical challenge to blood transfusion safety worldwide.

What is added by this report?

This study established a comprehensive quality assurance system for Dengue virus (DENV), Zika virus (ZIKV), and Chikungunya virus (CHIKV) nucleic acid testing (NAT) blood screening. The system included external quality assessment (EQA) implementation across all participating central blood stations and performance evaluation of six domestic blood screening reagents. Surveillance conducted in Yunnan Province and Guangxi Zhuang Autonomous Region revealed no positive cases among 45,383 blood samples screened in 2022. In 2023, screening of 44,972 blood donors identified 9 NAT-reactive samples at the Xishuangbanna central blood station, with 6 confirmed as DENV-1 positive.

What are the implications for public health practice?

Blood stations in border regions must implement comprehensive surveillance systems with enhanced detection sensitivity and robust early warning mechanisms to effectively address emerging disease threats and ensure transfusion safety.

ABSTRACT

Introduction: Emerging and re-emerging transfusion-transmitted arboviruses remain a persistent public health challenge to global blood safety. This study aims to establish a comprehensive nucleic acid testing (NAT) quality control system for Dengue virus (DENV), Zika virus (ZIKV), and Chikungunya virus (CHIKV) screening in blood donations to evaluate the performance of domestic screening reagents, and to assess the prevalence of these arboviruses in border regions.

Methods: Pseudovirus quality control materials based on the Moloney murine leukemia virus (MMLV) vector was constructed to evaluate the limit of detection (LoD) and precision of six blood screening reagents. An external quality assessment (EQA) was conducted across eight central blood stations in Guangxi Zhuang Aotonomous Region and Yunnan Provinces. These blood stations employed either reagent A or E for DENV/ZIKV/CHIKV triplex-assay screening of blood donor samples collected during epidemic seasons (June–August) in 2022 and 2023.

Results: The six reagents exhibited varied LoD. All evaluated reagents exhibited excellent precision with coefficient of variation (CV) values <5%. All eight central blood stations achieved EQA scores above 80. In 2022, a total of 45,383 blood samples were screened, with no positive cases detected. In 2023, 44,972 blood donors were screened, and nine samples tested positive at the Xishuangbanna central blood station. Confirmatory testing verified six Dengue virus serotype 1 (DENV-1) infections among these cases.

Conclusions: This study successfully established a robust quality assurance system for NAT-based arbovirus screening in China. The detection of DENV-positive samples underscore the persisting risk of transfusion-transmitted infections in endemic regions. Continued surveillance and enhanced screening strategies are essential to safeguard blood safety, particularly in arboviral hotspot regions with tropical/subtropical climates prone to recurrent outbreaks.

Emerging and re-emerging transfusion-transmitted diseases represent a critical global threat to blood safety. In China, localized Chikungunya fever outbreaks occurred in Guangdong (2010), Zhejiang (2017), and Yunnan (2019) Provincial-level

administrative division (PLAD), with each occurrence demonstrating progressively longer durations and increasing proportion of imported cases (*1*). Since 2016, sporadic imported Zika cases have been documented. Between 2010 and 2021, Dengue fever exhibited a biennial outbreak pattern in Yunnan Province, characterized by frequent imported cases and a significant increase in local transmission (*2*). Given that over 75% of Dengue virus (DENV) infections are asymptomatic, potentially infected blood donors during epidemic periods pose a substantial risk to transfusion safety (*3*).

RNA detection serves as the earliest marker of acute infection, making nucleic acid testing (NAT) the primary diagnostic tool for accurate arbovirus identification (*4*). Arbovirus blood screening has been implemented in several countries, with the Asia Pacific Blood Network (APBN) recommending targeted testing in regions experiencing regular seasonal outbreaks (*5*). Since 2022, the National Health Commission of the People's Republic of China (NHC) has implemented NAT screening for Dengue, Zika, and Chikungunya viruses at eight central blood stations in Yunnan and Guangxi PLAD. This initiative aims to adopt screening strategies based on epidemic trends and enhance blood station screening capabilities, enabling rapid epidemic response. To ensure screening quality, the National Center for Clinical Laboratories (NCCL) has developed quality control materials, evaluated all domestic blood screening reagents, conducting external quality assessments (EQA) for participating blood stations, and provided confirmation testing for screening-reactive samples.

Quality control materials were prepared using Moloney Murine Leukemia Virus (MMLV)-based pseudoviruses containing amplified regions corresponding to all domestic NAT screening reagents used in China. For reagent performance evaluation and EQA, pseudoviruses were quantified via digital PCR and diluted to predetermined concentrations in negative plasma. The limit of detection (LoD) and precision evaluations were conducted in accordance with CLSI EP17-A2, EP15-A3, and CNAS-GL039 guidelines. Annual EQA was performed at eight border central blood stations. (Supplementary Materials, available at https://weekly.chinacdc.cn/).

Blood screening for DENV, Zika virus (ZIKV), and Chikungunya virus (CHIKV) was conducted at 8 border central blood stations across 11 cities in Guangxi and Yunnan provinces from June to August in both 2022 and 2023, using NAT reagents from either Manufacturer A or Manufacturer E. Reactive samples were transported to NCCL for confirmatory testing using five NAT reagents (A, B, C, D, and E), with positive confirmation requiring detection by at least two reagents. Verified positive samples underwent DENV (serotype 1–4) determination using two Real-Time RT-PCR reagents (Shanghai Biogerm Medical Technology Co., LTD, Shanghai, China; Daan Gene Co., LTD, Guangzhou, China) (Figure 1A). Genotype analysis was performed using the NCBI database, and a phylogenetic tree was constructed using the neighbor-joining method in Mega (version 7.0; Mega Development Team). All screened reactive samples underwent serological testing for IgG/IgM (Wondfo Biotech Co., LTD, Guangzhou, China) and Non-structural protein 1 (NS1) antigen (Beijing Wantai Biological Pharmacy Enterprise Co., LTD, Beijing, China).

To ensure screening accuracy, this study validated the limit of detection (LoD) and precision of domestic blood screening reagents. Among the 6 DENV/ZIKV/CHIKV NAT reagents evaluated, reagent C demonstrated superior analytical sensitivity in individual donor NAT (ID-NAT) testing, achieving the lowest LoDs of 12.05 copies/mL for DENV, 13.41 copies/mL for ZIKV, and 14.86 copies/mL for CHIKV. In mini-pool NAT(MP-NAT) testing, reagent C maintained optimal sensitivity for CHIKV (33.24 copies/mL) and DENV (103.94 copies/mL), while reagent A exhibited the lowest LoD for ZIKV at 60.94 copies/mL (Table 1). All kits demonstrated excellent precision, with within-run and within-laboratory coefficient of variation (CV) values below 5% at concentrations of 2 LoD and above (Table 2). The 8 participating blood stations consistently achieved EQA scores exceeding 80 over the 2-year period.

In 2022, the screening of 45,383 blood samples yielded no reactive samples. In 2023, among 44,972 samples screened, 9 DENV-reactive samples (designated 2301–2309) were identified at the Xishuangbanna central blood station using reagent A. Six of these samples were subsequently confirmed as DENV-positive (Figure 1B). Further serotype differentiation revealed all six samples were positive for DENV type 1 (Figure 1C).

All nine reactive samples tested negative for DENV IgG/IgM antibodies. Two samples (2308 and 2309) showed positivity for NS1 antigen. Follow-up testing of sample 2308 at three months post-initial screening

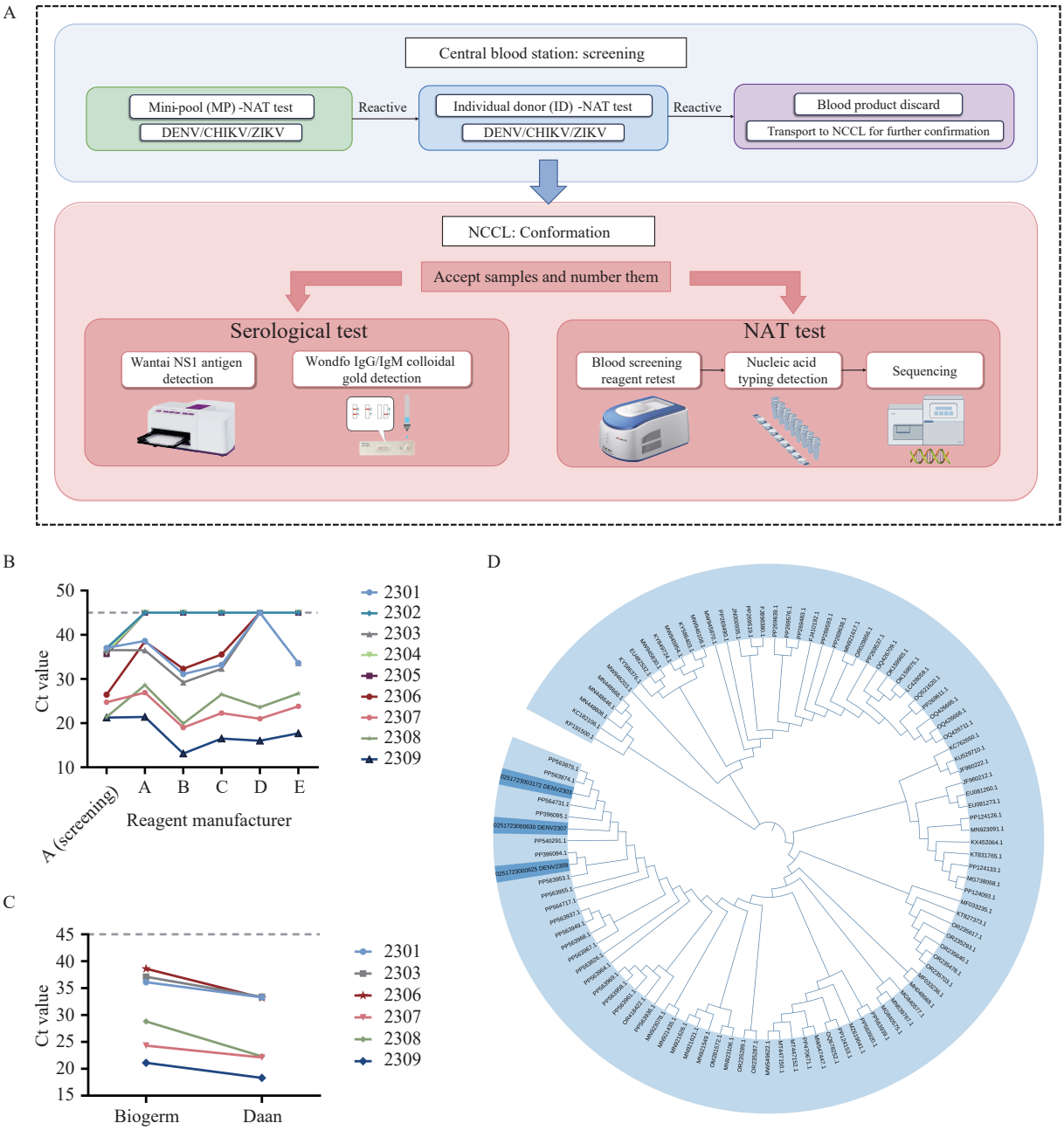

FIGURE 1. Screening and confirmation testing for Dengue, Chikungunya, and Zika viruses. (A) Strategy for screening and confirmation. (B) Results of individual donor testing in screening and retesting of reactive samples using blood screening reagents. (C) Results of DENV serotype differentiation. (D) Phylogenetic tree of E gene of DENV-1 genotype I in Xishuangbanna blood donors.
Note: Samples 2301, 2303, 2306, 2307, 2308, and 2309 demonstrated reactivity in two or more reagents upon retesting and were confirmed as DENV-positive. In pannel C, all six samples tested positive for DENV type 1 using DENV typing reagents.
Abbreviation: CHIKV=Chikungunya virus; DENV=Dengue virus; ZIKV=Zika virus; NCCL=national center for clinical laboratories; NS1=non-structural protein 1; NAT=nucleic acid testing.

revealed seroconversion with positive DENV IgG/IgM results, while both NS1 antigen and NAT testing were negative, providing definitive evidence of prior DENV infection.

Successful sequencing of samples 2303, 2307, and 2309 identified them as DENV 1 genotype I (Asian type). These strains showed close phylogenetic relationships with contemporary local epidemic strains PP563874/Guangzhou/2023, PP396094/Yunnan/2023, and MW549622/Hainan/2023. Furthermore,

TABLE 1. Limit of detection of six domestic NAT kits for research.

Virus	Test types (copies/mL)	Reagent manufacturer					
		A	B	C	D	E	F
CHIKV	MP-NAT (95% *CI*)	426.02 (247.86, 978.56)	3,490.82 (2,119.48, 7,909.66)	33.24 (24.72, 72.53)	1,598.18 (1,049.40, 4,868.64)	103.02 (72.04, 195.57)	498.15 (287.16, 1,332.18)
	ID-NAT (95% *CI*)	36.03 (24.31, 67.31)	428.05 (279.21, 833.91)	14.86 (11.30, 24.79)	375.00	20.04 (13.73, 57.71)	97.14 (57.30, 274.93)
DENV	MP-NAT (95% *CI*)	605.53 (388.85, 1,163.10)	702.56 (438.75, 1,695.06)	103.94 (72.99, 195.01)	9,809.46 (7,722.29, 16,303.33)	8,178.76 (4,552.37, 19,956.30)	9,209.55 (5,930.77, 19,098.41)
	ID-NAT (95% *CI*)	199.46 (122.48, 432.15)	218.79 (122.89, 575.78)	12.05 (9.49, 19.21)	8,421.45	907.22 (566.85, 1,867.53)	5,252.56 (3,473.96, 10,411.01)
ZIKV	MP-NAT (95% *CI*)	60.94 (39.11, 140.65)	379.65 (248.93, 772.45)	168.29 (111.68, 339.06)	2,105.09 (1,433.55, 4,772.67)	129.83 (88.89, 238.80)	217.74 (141.18, 491.16)
	ID-NAT (95% *CI*)	16.66 (10.15, 44.01)	34.85 (23.70, 77.47)	13.41 (10.32, 22.06)	2,655.70 (1,002.01, 1,881,589.12)	22.70 (15.03, 69.14)	51.51 (36.07, 99.15)

Note: The limit of detection, at which measurement results yielded a positive classification with a 95% probability, was calculated using a probit regression model and is shown with a 95% confidence interval.
Abbreviation: CHIKV=Chikungunya virus; DENV=Dengue virus; ZIKV=Zika virus; NAT=nucleic acid testing; MP-NAT=mini-pool NAT; ID-NAT=individual donor NAT; *CI*=confidence interval.

TABLE 2. Precision of six domestic NAT kits for research.

Reagent Manufacturer	Precision	MP-NAT									ID-NAT								
		CHIKV			DENV			ZIKV			CHIKV			DENV			ZIKV		
		12,000	1,500	187.5	12,000	1,500	187.5	12,000	1,500	187.5	12,000	1,500	187.5	12,000	1,500	187.5	12,000	1,500	187.5
A	Average Ct value	32.70	35.78		34.14	37.36		28.61	31.67	34.72	30.78	33.53	37.11	31.60	34.69		26.68	29.66	32.83
	Within-run CV (%)	2.22	1.70		0.76	2.01		0.94	1.23	1.30	2.80	2.49	2.38	1.07	1.19		1.76	1.35	1.29
	Within-lab CV (%)	3.81	3.96		1.60	2.44		3.08	2.68	2.67	2.99	5.70	3.52	2.14	1.97		2.50	3.10	1.95
B	Average Ct value	27.90			28.03	31.74		26.34	29.34		25.31	28.87		25.33	28.85		23.65	26.86	
	Within-run CV (%)	1.25			1.01	2.70		1.35	1.20		0.78	1.54		0.79	1.22		0.98	0.92	
	Within-lab CV (%)	2.38			1.41	2.74		1.40	1.50		0.98	2.82		1.31	2.05		1.47	1.70	
C	Average Ct value	32.06	34.94	38.67	33.42	36.98		33.50	36.64		29.45	32.53	35.27	31.05	33.74	37.55	30.64	33.31	36.48
	Within-run CV (%)	1.45	1.02	1.18	1.73	1.83		1.58	1.34		1.45	1.21	0.84	1.78	1.40	1.12	1.72	1.38	1.70
	Within-lab CV (%)	1.73	1.55	1.62	2.94	2.96		1.97	1.69		2.30	1.83	1.69	1.81	1.75	1.63	2.22	2.84	2.40
D	Average Ct value	26.89			30.15			29.59			24.18	27.31		27.54			27.43		
	Within-run CV (%)	0.92			2.14			1.84			0.85	2.13		2.24			1.73		
	Within-lab CV (%)	1.19			2.10			3.47			0.92	2.29		2.71			2.02		
E	Average Ct value	28.47	31.41		33.16			27.63	30.69		26.18	29.35	32.18	31.34	34.31		25.37	28.58	31.43
	Within-run CV (%)	0.40	0.74		0.91			0.37	0.80		0.27	0.32	0.94	0.90	1.11		0.25	0.43	1.14
	Within-lab CV (%)	0.65	0.81		1.36			0.53	0.91		0.53	0.47	0.97	0.93	1.11		0.49	0.62	1.17
F	Average Ct value	31.37	34.25		37.38			32.03	34.46		29.49	32.49		35.79			30.13	32.96	
	Within-run CV (%)	0.84	1.71		3.50			0.83	1.27		0.76	1.11		2.12			1.49	0.84	
	Within-lab CV (%)	2.95	3.03		3.59			2.31	2.27		1.18	1.80		3.89			2.14	2.15	

Abbreviation: CHIKV=Chikungunya virus; DENV=Dengue virus; ZIKV=Zika virus; NAT=nucleic acid testing; MP-NAT=mini-pool NAT; ID-NAT=individual donor NAT; CV=coefficient of variation.

they clustered with viral strains from Singapore (MF033236/Singapore/2015), Malaysia (MH048669/Malaysia/2014), and Thailand (MZ619041/Thailand/2019) (Figure 1D), suggesting importation from Southeast Asian countries.

DISCUSSION

Blood products play a vital role in healthcare delivery and significantly reduce clinical mortality. However, clinical evidence demonstrates that arboviruses can occasionally cause adverse events through transfusion-transmitted infections (*6*–*7*).

This study worked to establish a comprehensive quality assurance system for arbovirus screening by implementing EQA programs for blood stations and evaluating screening reagent performance. All blood stations successfully passed the EQA requirements. The six evaluated kits demonstrated excellent within-run and within-laboratory precision, with CV values below 5%, indicating robust repeatability and reproducibility. However, LoD values varied significantly among reagents and deviated from manufacturer-declared specifications. This variation likely stems from manufacturers using different in-house quality control samples and non-standardized evaluation methods, which were inadequately detailed in their instructions. Our study employed standardized quantitative quality control samples and a unified evaluation protocol, ensuring direct comparability across manufacturers. Notably, domestic blood screening reagents still demonstrate lower sensitivity compared to CE-approved alternatives, such as the Procleix ArboPlex Assay (LoD<17.5 copies/mL), cobas® CHIKV/DENV (LoD<7.1 IU/mL) and Cobas® Zika (LoD<8.1 copies/mL). Given that the evaluated reagents are currently limited to research use only, manufacturers must significantly enhance product performance before commercial authorization.

DENV, currently the most concerning arbovirus, is transmitted by *Aedes albopictus* and *Aedes aegypti*. Asymptomatic but viremic blood donors pose a significant transfusion safety risk. A systematic review and meta-analysis revealed that NAT screening detected an overall DENV viremic rate of 0.2% among blood donors, with RNA positivity in Southeast Asia at 0.16% (*8*). This study's screening identified a lower prevalence rate of 1.33‰ (6/44,972) among Chinese blood donors in 2023 compared to Southeast Asian rates. As of April 30, 2024, the World Health Organization (WHO) has reported over 7.6 million Dengue fever cases worldwide, including 3.4 million confirmed cases, more than 16,000 severe cases, and over 3,000 fatalities across 90 countries with active transmission (*9*). These findings underscore the necessity for continued arbovirus screening in China's tropical and subtropical regions. However, given China's vast geographical expanse and the regional and seasonal variations in DENV epidemiology, pre-donation health questionnaires may be more cost-effective than universal testing in low-prevalence areas.

In conclusion, this study has successfully established a robust quality control system for NAT screening of DENV/ZIKV/CHIKV in Chinese blood donors. The detection of positive samples in 2023 and the subsequent Dengue outbreak in 2024 emphasize the urgent need to implement comprehensive surveillance and early warning mechanisms, while maintaining readiness in diagnostic capabilities to address emerging health threats.

Conflicts of interest: No conflicts of interest.

Acknowledgements: The staff members at the central blood stations of Nujiangzhou, Baoshan, Lincang, Wenshanzhou, Honghezhou, Pu'er, Xishuangbanna, and Baise for their contributions to the screening program.

Ethics statement: Blood donor screening was conducted in accordance with the "14th Five-Year Plan Work Scheme for Blood Station Blood Safety Monitoring and Risk Early Warning" issued by the National Health Commission. Confirmation testing of reactive samples were approved by the Ethics Committee of Beijing Hospital (2023BJYYEC-443-02).

Funding: Supported by the CAMS Innovation Fund for Medical Sciences (CIFMS) (2021-I2M-1-060).

doi: 10.46234/ccdcw2025.058

[#] Corresponding author: Lunan Wang, lnwang@nccl.org.cn.

[1] National Center for Clinical Laboratories, Institute of Geriatric Medicine, Chinese Academy of Medical Sciences, Beijing Hospital/National Center of Gerontology, Beijing, China; [2] National Center for Clinical Laboratories, Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing, China; [3] Xishuangbanna Central Blood Station, Xishuangbanna City, Yunnan Province, China.
[&] Joint first authors.

Submitted: August 07, 2024
Accepted: February 08, 2025
Issued: March 14, 2025

REFERENCES

1. Liu LB, Li M, Gao N, Shen JY, Sheng ZY, Fan DY, et al. Epidemiological and clinical characteristics of the chikungunya outbreak in Ruili City, Yunnan Province, China. J Med Virol 2022;94(2):499 – 506. https://doi.org/10.1002/jmv.27302.
2. Zhu XX, Wang SW, Li YF, Cao Y, Su XM, Zhao XT. Epidemiological characterization of dengue fever — Yunnan province, China, 2010-2021. China CDC Wkly 2024;6(20):457 – 62. https://doi.org/10.46234/ccdcw2024.088.
3. Li L, Li Y, Lu SF, Dong J, Xu HX, Zhang Q, et al. Epidemiological survey and screening strategy for dengue virus in blood donors from Yunnan Province. BMC Infect Dis 2021;21(1):104. https://doi.org/10.1186/s12879-021-05810-8.
4. Munoz-Jordan JL. Diagnosis of Zika virus infections: challenges and opportunities. J Infect Dis 2017;216(suppl_10):S951 – 6. https://doi.org/10.1093/infdis/jix502.
5. Asia Pacific Blood Network (APBN). Dengue and the blood supply. White paper updated January 2019. https://asiapacificbloodnetwork.org/wp-content/uploads/2023/03/apbn_white_paper_dengue_v2019_final.pdf. [2024-7-29].
6. Guzman MG, Harris E. Dengue. Lancet 2015;385(9966):453 – 65. https://doi.org/10.1016/S0140-6736(14)60572-9.
7. Viennet E, Frentiu FD, Williams CR, Mincham G, Jansen CC, Montgomery BL, et al. Estimation of mosquito-borne and sexual transmission of Zika virus in Australia: risks to blood transfusion safety. PLoS Negl Trop Dis 2020;14(7):e0008438. https://doi.org/10.1371/journal.pntd.0008438.
8. Giménez-Richarte Á, Ortiz de Salazar M, Arbona C, Giménez-Richarte MP, Collado M, Fernández PL, et al. Prevalence of Chikungunya, Dengue and Zika viruses in blood donors: a systematic literature review and meta-analysis. Blood Transfus 2022;20(4):267 – 80. https://doi.org/10.2450/2021.0106-21.
9. World Health Organization. Global arbovirus initiative: preparing for the next pandemic by tackling mosquito-borne viruses with epidemic and pandemic potential. World Health Organization. https://www.who.int/publications/i/item/9789240088948. [2024-5-13].

SUPPLEMENTARY MATERIAL

Quality Control Materials

The quality control materials were generated using Moloney Murine Leukemia virus (MMLV)-based pseudoviruses. The process involved two key steps: First, the target regions of Dengue virus (DENV), Zika virus (ZIKV), and Chikungunya virus (CHIKV) genomes — specifically those amplified by all domestic DENV/ZIKV/CHIKV triplex nucleic acid testing (NAT) screening reagents in China — were synthesized and inserted into the MMLV vector backbone PQCXIG(w497-1). Second, the resulting plasmids were packaged with pUMVC and pVSVG in 293T cells. The viral supernatant was harvested 48 hours post-transfection and quantified via digital PCR. For reagent performance evaluation and external quality (EQA), the pseudoviruses were diluted to predetermined concentrations in negative plasma. To evaluate reagent performance, pseudoviruses underwent serial two-fold dilutions from 12,000 copies/mL, generating 12 distinct concentrations.

Limit of Detection

The limit of detection (LoD) evaluation followed the Clinical Laboratory Standards Institute EP17-A2 guideline (Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline-Second Edition). Each quality control sample underwent extraction and testing in octuplicate daily for 3 consecutive days. The positive rate for each dilution was calculated, and the LoD was determined using a probit regression model as the measurand concentration yielding positive results with 95% probability. Result interpretation adhered to manufacturer specifications.

Precision

Precision assessment followed the Clinical Laboratory Standards Institute EP15-A3 guideline (User Verification of Precision and Estimation of Bias; Approved Guideline). Samples at concentrations of 2 LoD and above were tested in quintuplicate daily for 5 consecutive days. Within-run precision (repeatability) and within-laboratory precision were calculated according to guideline specifications.

External Quality Assessments

National Center for Clinical Laboratories prepared a ten-sample panel comprising negative plasma and positive pseudovirus samples. The EQA utilized a 100-point scoring system, with each sample worth 10 points. Blood stations were required to achieve a minimum score of 80 points based on their qualitative results to pass the assessment.

Screening of Blood Samples

Blood screening protocols required equipment-compatible reagents from either Manufacturer A or Manufacturer E. Screening was conducted using Multiplex NAT, processing either six samples (Manufacturer A) or eight samples (Manufacturer E) per pool. Individual donor NAT (ID-NAT) was performed on samples from reactive pools to identify specific reactive specimens.

Genotype Determination

E gene amplification utilized the following primer pairs: F1: 5'-CACATGCCATAGGAACATCCA-3', R1: 5'-ATGAGCCTGTGCACATCACA-3'; F2: 5'-GGAACAGACAAGATTTGCTGGT-3', R2: 5'-TGCCACTTC CACATTTGAGT-3'. The PCR thermal cycling conditions were: 50 °C for 30 min; 94 °C for 2 min; 35 cycles of 94 °C for 30 s, 56 °C for 30 s, 72 °C for 60 s; followed by 72 °C for 7 min, with final hold at 4 °C. Genotype analysis was performed using the NCBI database.