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A combinatorial probe-coding multiplex RT-qPCR assay for simultaneous detection and differentiation of dengue, Zika, and chikungunya viruses in human and mosquito samples

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Abstract

Arboviruses such as Zika virus (ZIKV), dengue virus (DENV), and chikungunya virus (CHIKV) remain significant public health threats in tropical and subtropical regions. These viruses are transmitted primarily by *Aedes* mosquitoes and often co-circulate in the same geographic areas, leading to outbreaks with overlapping clinical presentations and cross-reactivity in serological testing, particularly between ZIKV and DENV. These aspects create diagnostic challenges, and the development of molecular diagnostic methods is critically necessary. Here, we developed a diagnostic kit based on the Multicolor Combinational Probe Coding (MCPC) concept to simultaneously detect six viral targets (ZIKV, DENV1–4, and CHIKV) and an internal control (IC), by encoding targets with four spectrally distinct dyes. Using a panel of 660 characterized human serum or plasma samples, and 9 artificial DENV-4 samples, we obtained diagnostic sensitivity and specificity above 94% and 99%, respectively, for all targets. The assay enables the simultaneous analysis of up to 91 samples, substantially increasing testing throughput compared to commercially available products. The test was also applied to mosquito specimens of *Aedes* spp., *Anopheles* spp., *Haemagogus* spp., and *Culex* spp., demonstrating its utility as a robust tool not only for emergency response but also for arbovirus surveillance in public health settings.

Introduction

Arboviruses such as Zika virus (ZIKV), dengue virus (DENV), and chikungunya virus (CHIKV) remain major public health threats in tropical and subtropical regions, especially in the Americas, Asia, and Africa (Bettis et al., 2022; World Health Organization, 2019, 2024a, 2025). These viruses are transmitted primarily by *Aedes* mosquitoes and often co-circulate in the same geographic areas, leading to outbreaks with overlapping clinical presentations, including fever, rash, arthralgia, and conjunctivitis (Leon Juarez et al., 2022; Pan American Health Organization, 2022; Rückert et al., 2017).

In recent decades, these arboviruses have demonstrated a marked increase in global incidence, geographic expansion, and disease burden (Naveca et al., 2023). Epidemiological data reveal unprecedented surges in dengue cases in endemic regions of the Americas, while chikungunya and Zika have expanded beyond their traditional tropical range (César et al., 2015; World Health Organization, 2024a). Recent global mapping efforts have demonstrated a striking convergence in environmental suitability for these arboviruses, with CHIKV and ZIKV transmission increasingly overlapping regions already endemic for DENV. It is estimated that approximately 5.66 billion people currently live in areas environmentally suitable for DENV, CHIKV, and ZIKV transmission (Lim et al., 2025).

The clinical similarities among ZIKV, DENV, and CHIKV illness pose a significant diagnostic challenge, particularly during the early stages of infection when overlapping symptoms frequently occur (Sena et al., 2024; Siew et al., 2025). This resemblance increases the risk of clinical misclassification, inappropriate patient management, and adverse outcomes. Furthermore, laboratory confirmation is hindered by serological cross-reactivity of IgM and IgG antibodies, especially between DENV and ZIKV,

compromising both diagnostic accuracy and the effectiveness of epidemiological surveillance (Chan et al., 2022; Endale et al., 2021; Piantadosi & Kanjilal, 2020).

To promote integrated surveillance and early detection strategies, considering the shared vectors and common environmental drivers of these expanding diseases, in 2022 the WHO launched the Global Arbovirus Initiative. This initiative is structured around six pillars aimed at optimizing resources, strengthening national and regional systems, and enhancing global resilience in the face of the growing risk of arboviruses with epidemic or pandemic potential (World Health Organization, 2024b). In the context of this study, Pillars 2 (reducing local epidemic risk) and 5 (enhancing innovation and new approaches) are particularly relevant.

Although multiple molecular diagnostic tools are currently available for ZIKV, CHIKV, and DENV (Álvarez-Díaz et al., 2021; Cabral-Castro et al., 2016; Cuellar-Quimbaya et al., 2024; Panmei et al., 2024; Waggoner et al., 2016), most kits divide the targets into two or more separate reactions. Therefore, a major limitation of most multiplex assay configurations lies in the limited sample throughput per run, which can lead to operational overload in healthcare systems during periods of high testing demand. In the present work, we developed a highly multiplexed RT-qPCR protocol capable of simultaneously distinguishing six viral targets and an internal control, by employing a Multicolor Combinational Probe Coding (MCPC) approach described by (Huang et al., 2007, 2011). MCPC expands the multiplexing capacity of qPCR assays by employing unique combinations of fluorescence signals rather than assigning a single fluorophore to each target. This strategy enables the implementation of complex multiplex protocols on standard thermocyclers commonly available in healthcare laboratories (Fig. 1). Here, we report an MCPC-based single-tube multiplex RT-qPCR assay optimized for simultaneous detection and discrimination of ZIKV, CHIKV, and DENV1–4 in human and mosquito samples. This work builds on a previously developed assay (IBMP NAT ZDC kit), with improvements in robustness and target discrimination

Methods

Control samples

For analytical validation, reference materials were obtained from standardized RNA controls and culture-derived preparations. Lyophilized AMPLIRUN® RNA CONTROLs (Vircell, Granada, Spain) were used for the targets ZIKV (catalog MBC072-R), DENV-1 (catalog MBC055-R), and CHIKV (catalog MBC099-R). Quantified culture-derived preparations from ZeptoMetrix® (Buffalo, USA) were employed for DENV-2, DENV-3, and DENV-4 (catalogs 0810089CF, 0810090CF, and 0810555CF, respectively). Different suppliers were used according to the availability of standardized reference materials for each viral target.

Lyophilized RNAs were resuspended as per the suppliers' instructions. RNA concentrations for ZIKV, DENV-1, and CHIKV were determined by digital PCR using singleplex reactions. For DENV-2, DENV-3, and

DENV-4, RNA concentrations were used as declared by the supplier and considered reference values for analytical validation.

Assay components

The assay (IBMP NAT ZDC One) was formulated using three main components: a reaction mastermix, an oligomix, and a set of controls. The mastermix consisted of a proprietary reaction buffer containing dNTPs and enzymes required for reverse transcription and PCR amplification. The oligomix included primers and probes designed for all viral targets and internal control. In addition, one negative control and four positive controls were included in the assay workflow.

The negative control consisted of a synthetic DNA molecule containing the sequence of the human RNA fragment targeted by the internal control primers and probe. Positive controls comprised synthetic DNA fragments corresponding to the predicted amplicons for each viral target, generated *in silico* and reconstituted in the same formulation as the negative control. These controls were used exclusively to monitor assay performance and were not intended for quantitative calibration.

Oligomix development and combinatorial probe implementation

Genomic sequences of ZIKV, CHIKV and DENV were retrieved from the NCBI Virus database, restricting the search to the taxids 12637 (DENV), 64320 (ZIKV) and 37124 (CHIKV). Sequences longer than 9,000 nucleotides were classified by serotype and genotype (Fonseca et al., 2019) using Genome Detective tools (Vilsker et al., 2019). The number of sequences included for each viral strain is provided in Table S1.

After exclusion of unassigned sequences, remaining entries were aligned to identify conserved regions suitable for primer and probe design. Primers were selected from regions showing complete coverage within each target species and minimal mismatch with non-target species. Degenerate bases were avoided during primer design; instead, sequence variants were incorporated explicitly when necessary, allowing improved fine-tuning of annealing temperatures and reducing the risk of nonspecific amplification. A total of 41 oligonucleotides were designed, including one primer pair and a CY5-labeled probe targeting the β-actin gene as an internal control. In addition, 24 primers and 14 probes labeled with single or dual combinations of FAM, JOE, or ROX fluorophores were designed for the detection of six viral targets. Multiple primers were included to ensure coverage of relevant viral genotypes, including DENV-2 genotype II, three CHIKV genotypes (Asian and East-Central-South African – ECSA, West African – WA, and Asian), and both African and Asian lineages of ZIKV.

RNA extraction

RNA extraction from primary samples (n = 312) were performed using the Extracta 32 automated system (Loccus, São Paulo, Brazil), following procedures appropriate for the biological matrices recommended for the assay. In partner laboratories (n = 348), RNA extraction was carried out using commercial kits from different manufacturers. In samples showing no amplification of viral targets, extraction efficiency was considered adequate when amplification of the internal control (CY5 channel) was observed with a Ct value ≤ 36.

Repeatability and Reproducibility Tests

Repeatability (Table S2) and reproducibility (Table S3) were assessed to evaluate the assay precision. Dilution curves were prepared using commercial RNA from the six viral targets combined with synthetic human RNA and additional components to simulate endogenous material. Four concentration points were tested, each in four technical replicates for each target. Assays were performed by three independent operators, using three different real-time PCR instruments, on three separate days.

Limit of Detection (LoD)

The 95% hit-rate Limit of Detection (LoD95%) was determined using three real-time PCR platforms: the 7500 Real-Time PCR System and QuantStudio 5 (Thermo Fisher Scientific), and CFX96 Real-Time PCR System (Bio-Rad). Sixteen experiments were conducted for the 7500 and QuantStudio 5 platforms, including repeatability and reproducibility assays as well as six validation assays with six different production batches of assay reagents.

Analytical specificity

Analytical specificity was assessed using a panel of organisms selected based on phylogenetic relatedness or potential overlap in clinical presentation with the target arboviruses. Genetic material was obtained from commercial reagents or from previously characterized clinical samples and viral cultures provided by reference institutions. The panel of non-target organisms included analytes selected to evaluate potential cross-reactivity under the standardized assay conditions.

Human and mosquito samples

Assay validation was performed using 660 RNA samples obtained from human serum or plasma and 9 artificial RNA samples Clinical specimens were provided by multiple reference laboratories located in different regions of Brazil, including the Laboratório Central de Saúde Pública Prof. Gonçalo Moniz (LACEN-BA), Laboratório Central de Saúde Pública do Distrito Federal (LACEN-DF), Instituto Adolfo Lutz (IAL), Instituto Oswaldo Cruz (IOC/Fiocruz), Instituto Leônidas e Maria Deane (ILMD/Fiocruz), Instituto Evandro Chagas (IEC), Fundação Ezequiel Dias (FUNED), Instituto Carlos Chagas (ICC/Fiocruz), Laboratório Central do Estado do Paraná (LACEN-PR), Laboratório Central de Saúde Pública Dr. Giovanni

Cysneiros (LACEN-GO), and Instituto Aggeu Magalhães (IAM/Fiocruz). The specimens had been previously characterized using either the IBMP NAT ZDC assay or validated in-house RT-qPCR protocols (Table S4). Because DENV-4 was underrepresented among clinical samples, artificial RNA materials were included to supplement validation for this target. Seven of these artificial samples were obtained from quality control program provided by Controllab (Rio de Janeiro, RJ, Brazil), and two were commercial reference materials: a commercial RNA from Vircell® (catalog MBC058-R; 100 copies/µL) and a viral culture fluid from ZeptoMetrix® (catalog 0810555CF; 90 copies/µL) (Table S5).

RNA extracted from mosquito pools belonging to the genera *Aedes, Anopheles*, *Haemagogus*, and *Culex*, representing different ecological and geographic contexts in Brazil, was tested to evaluate assay performance in entomological surveillance contexts.

Statistical Analysis

The 95% limit of detection (LoD95) for each viral target was estimated by Probit regression using Python scripts executed in the Google Colab environment. Detection outcomes obtained at five concentration levels were analyzed using a generalized linear model with a binomial distribution and Probit link function, with the natural logarithm of target concentration included as the independent variable. The LoD95 was defined as the concentration corresponding to a 95% probability of detection and was estimated by inverse prediction from the fitted Probit model using the 95th percentile of the standard normal distribution. The analysis was performed using the statsmodels and SciPy Python libraries, and 95% confidence intervals were estimated from the fitted model parameters.

Diagnostic performance was evaluated using MedCalc Statistical Software (MedCalc Software Ltd., Ostend, Belgium) by comparing the results of the index assay with those of the reference method. For each viral target, contingency tables were constructed, and samples were classified as true positive (TP), false positive (FP), true negative (TN), or false negative (FN). Clinical sensitivity, clinical specificity, positive predictive value (PPV), negative predictive value (NPV), and overall diagnostic accuracy were calculated using standard contingency-table formulas. Sensitivity was calculated as TP/(TP + FN), specificity as TN/(TN + FP), PPV as TP/(TP + FP), NPV as TN/(TN + FN), and accuracy as (TP + TN)/(TP + TN + FP + FN). Ninety-five percent confidence intervals (95% CIs) for sensitivity, specificity, and accuracy were calculated using the exact Clopper–Pearson method, whereas 95% CIs for PPV and NPV were estimated using the standard logit method implemented in MedCalc.

Ethical Statement

The study was conducted in accordance with applicable ethical principles and was approved by the Research Ethics Committee of the *Instituto Oswaldo Cruz, Fundação Oswaldo Cruz* (CAAE No. 90249218.6.1001.5248; Ethics Committee Approval No. 6.824.149).

Results

Assay configuration and signal interpretation

The final assay configuration comprised 41 oligonucleotides, including primers and probes targeting six viral targets and a β-actin–based internal control. The combinatorial labeling strategy enabled unambiguous interpretation of fluorescence signal patterns across the four available detection channels, allowing discrimination of all viral targets within a single reaction. The internal control signal was used to validate sample processing in reactions without detectable viral amplification.

Repeatability and Reproducibility

The repeatability analysis demonstrated high consistency across all viral targets, with low variability across technical replicates. Relative standard deviation (RSD%) remained below 11% for all dilution points, with a maximum observed variation of 10.84%. Reproducibility analysis showed even lower variability, with RSD% values below 4% across independent runs. Detection rates exceeded 90% at concentrations ranging from 10 to 30 copies/reaction, whereas no amplification was observed at concentrations of ≤5 copies per reaction.

Analytical sensitivity and specificity

Analytical sensitivity was assessed by determining the 95% hit-rate limit of detection (LoD95%) for each viral target. The estimated LoD95% values were ≥14.81 copies/reaction (95% CI: 7.33-40.88) for ZIKV; ≥48.00 copies/reaction (95% CI: 12.85-398.05) for DENV1; ≥22.93 copies/reaction (95% CI: 9.81-79.59) for DENV2; ≥39.95 copies/reaction (95% CI: 12.42-246.86) for DENV3; ≥52.09 copies/reaction (95% CI: 16.82-276.82) for DENV4; and ≥82.87 copies/reaction (95% CI: 18.79-854.52) for CHIKV (Figure 3; Table S6).

All analytical specificity tests with unintended targets showed non-detectable results, indicating the absence of spurious cross-detection events (Table S7).

Diagnostic performance metrics

Diagnostic sensitivity, specificity, positive predictive value, and negative predictive value were calculated for each viral target using 660 human clinical samples and 9 artificial RNA samples. The assay showed consistently high diagnostic performance across all targets, with sensitivity values ranging from 94.6% to 100% and specificity values exceeding 99.5% (Table 1).

Table 1 – Diagnostic performance of the assay using human clinical samples

Virus	Sensitivity (%) (95% CI)	Specificity (%) (95% CI)	PPV (%) (95% CI)	NPV (%) (95% CI)	Accuracy (%) (95% CI)
ZIKV	100 (88.1–100)	100 (99.4–100)	100 (88.1–100)	100 (99.4–100)	100 (99.5 - 100)
DENV1	97.2 (92.1–99.4)	99.5 (98.5–99.9)	97.2 (91.9–99.1)	99.5 (98.4–99.8)	99.1 (98.1 - 99.7)
DENV2	98.4 (95.3–99.7)	99.8 (98.9–100)	99.5 (96.2–99.9)	99.4 (98.1–99.8)	99.4 (98.5 - 99.8)
DENV3	94.6 (81.8–99.3)	100 (99.4–100)	100 (90.0–100)	99.7 (98.8–99.2)	99.7 (98.9 - 100)
DENV4	100 (75.3–100)	100 (99.4–100)	100 (75.3–100)	100 (99.4–100)	100 (99.5 - 100)
CHIKV	96.8 (92.0–99.1)	99.8 (99.0–100)	99.2 (94.4–99.9)	99.3 (98.1–99.7)	99.3 (98.3 - 99.8)

95% CI: 95% confidence interval, PPV: positive predictive value, NPV: negative predictive value.

RNA extracted from mosquito pools belonging to the genera *Aedes*, *Anopheles*, and *Haemagogus*, as well as *Culex* spp., was tested using the assay. Internal control amplification was observed across all insect samples, allowing evaluation of assay performance in entomological matrices.

Finally, one of the outcomes achieved through the application of the MCPC strategy was a substantial increase in the number of samples that could be tested per plate, representing twice the number processed compared to other protocols.

Discussion

The development of a multiplex RT-qPCR assay capable of simultaneously detecting and differentiating dengue, Zika, and chikungunya viruses is particularly relevant in the current epidemiological context, characterized by their overlapping geographic distribution and increasing incidence in tropical and subtropical regions (Cabral-Castro et al., 2016; Segura et al., 2021). In such settings, high testing demand and limited laboratory capacity frequently challenge timely diagnosis and surveillance efforts.

The substantial clinical overlap among arboviral infections and the limitations of serological assays highlight the importance of molecular diagnostics for early and accurate case confirmation (Lim et al., 2025). Although several RT-qPCR assays for these arboviruses have been described, many rely on parallel reactions or partially multiplexed formats, which may limit analytical throughput during periods of sustained transmission (Álvarez-Díaz et al., 2021; Waggoner et al., 2016). Importantly, the recent re-emergence of DENV-3 in Brazil after more than 15 years of limited circulation, including the introduction of a new genotype III lineage into the Americas, highlights that dengue molecular surveillance should extend beyond virus detection to serotype differentiation, as this information is essential for tracking

changes in circulating serotypes, identifying viral introductions, and anticipating populations at increased risk of future outbreaks and severe disease (Naveca et al., 2023).

By applying a MCPC strategy (Huang et al., 2007, 2011), the assay described herein expands the multiplexing capacity of real-time PCR beyond the conventional one-target–one-fluorophore paradigm. This approach enables discrimination of multiple viral targets within a single reaction tube using standard four-channel instruments widely available in public health laboratories.

The analytical sensitivity achieved for all viral targets was within the range reported for established singleplex and multiplex RT-qPCR assays. The LoD estimates were comparable to other described assays (Pabbaraju et al., 2016; Waggoner et al., 2016), indicating that increased multiplexing did not result in a substantial loss of sensitivity, a common limitation reported for highly multiplexed assays (Segura et al., 2021; Varghese et al., 2023).

Clinical validation was performed using a large and geographically diverse set of samples obtained from reference laboratories across different regions of Brazil. The consistent diagnostic performance observed across viral targets supports the applicability of the assay under heterogeneous epidemiological and laboratory conditions, which is particularly relevant for integrated surveillance networks in endemic settings (Wallau et al., 2023).

A relevant feature of the assay is its ability to differentiate all four dengue virus serotypes within a single reaction. Given the frequent co-circulation of multiple dengue serotypes and the emergence and spread of distinct viral lineages, including dengue virus serotype 2 genotype II (Cosmopolitan), serotype-level resolution remains important for molecular surveillance and epidemiological investigations (Gräf et al., 2023). In this context, the inclusion of primers designed to detect multiple chikungunya virus genotypes, including the Asian, East/Central/South African, and West African lineages, as well as both African and Asian lineages of zika virus, supports broader diagnostic coverage in settings characterized by ongoing viral diversification and repeated introduction events (de Araújo et al., 2025; Gräf et al., 2023; Nunes et al., 2015; Tsetsarkin et al., 2014).

Within this context, the applicability of the assay to both human clinical samples and mosquito-derived RNA support its potential integration into combined clinical and entomological surveillance workflows. The use of a single molecular assay across different biological matrices may facilitate coordinated surveillance strategies in endemic regions, where human case detection and vector monitoring are complementary components of arbovirus control programs. Although documented co-infections involving dengue, Zika, and chikungunya viruses are relatively uncommon (Ahmed et al., 2024; Rico-Mendoza et al., 2019), their occurrence underscores the importance of integrating molecular diagnostic data from both human and vector samples with epidemiological and genomic information, particularly during outbreak investigations (Fonseca et al., 2019; Vilsker et al., 2019; Wallau et al., 2023).

From an operational perspective, the combinatorial probe-coding strategy enabled an increased number of samples to be processed per 96-well plate compared with other conventional multiplex RT-qPCR

formats (Ribeiro et al., 2025; Stone et al., 2023). This increase in analytical throughput may be particularly relevant during arbovirus outbreaks, when laboratory surge capacity and turnaround time represent critical constraints, and was achieved without compromising analytical sensitivity or diagnostic performance.

From an operational perspective, the combinatorial probe-coding strategy adopted in this assay enables an increased number of samples to be processed per 96-well plate using standard real-time PCR platforms. This characteristic is particularly relevant in endemic and outbreak-prone settings, where both clinical diagnosis and entomological surveillance may generate high testing volumes over short timeframes. By allowing high-throughput molecular screening without compromising analytical sensitivity or diagnostic performance, the assay may support laboratory surge capacity as part of integrated arbovirus surveillance and response strategies (Cabral-Castro et al., 2016; Rico-Mendoza et al., 2019; Segura et al., 2021; Vogels et al., 2019).

Taken together, these findings indicate that MCPC approaches can be effectively applied to multiplex RT-qPCR assays for arbovirus detection in settings characterized by viral diversity, co-circulation, and heterogeneous surveillance demands. By combining serotype-level resolution, broad lineage coverage, applicability to both human and vector samples, and increased analytical throughput on widely available platforms, the assay described here illustrates how molecular diagnostic strategies can be adapted to support integrated clinical and surveillance workflows in regions with recurrent arbovirus transmission.

Conclusions

In conclusion, this study demonstrates that a combinatorial probe-coding strategy can be effectively applied to multiplex RT-qPCR assays for the detection and differentiation of major arboviruses. By combining robust analytical performance, broad lineage coverage, applicability to both human and vector samples, and increased analytical throughput on widely available platforms, this approach supports integrated diagnostic and surveillance strategies in regions with recurrent arbovirus transmission.

Declarations

Author contributions

M. K. T. conceptualized the study, performed experiments, and analyzed the data. T. J. conceptualized the study, performed experiments, and analyzed the data. A. A. conceptualized the study and revised the manuscript. L. M. C. prepared the first draft and finalized the manuscript. F. G. A., A. B. F. L, V. S. T. R. performed experiments and analyzed the data, F. M. P., A. P. S. C., M. M. R., A. Y. M., C. D. S. R., C. C. S., F. N., A. M. B. F., L. M. N. C., B. T. D. N., A. C. R. C., P. S. A., N. T. R., A. K., C. Z., C. N. D. S., A. A. F., N. F. Q. M., T. C. M., E. A.N.A., C. N. L. M., L. K. S. performed experiments and revised the manuscript, F. K. M.

acquired the funding and revised the manuscript, and I. N. R. conceptualized the study and revised the manuscript. All authors read and approved the final version of the manuscript.

Competing interests

The authors declare no competing interests.

Data availability

The datasets analyzed during the current study are available from the corresponding author, Irina Nastassja Riediger (irina.riediger@ibmp.org.br), on reasonable request.

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Figures

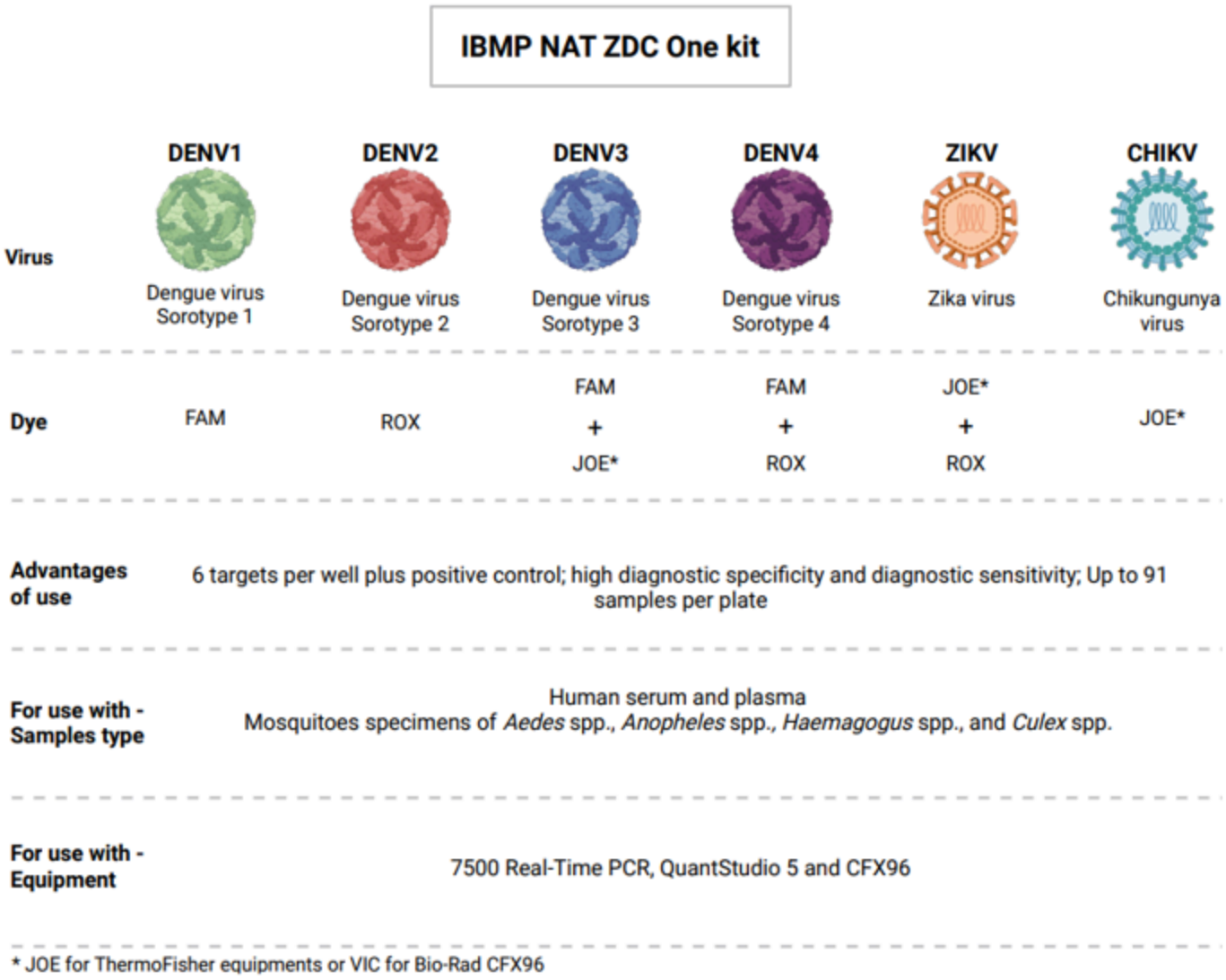
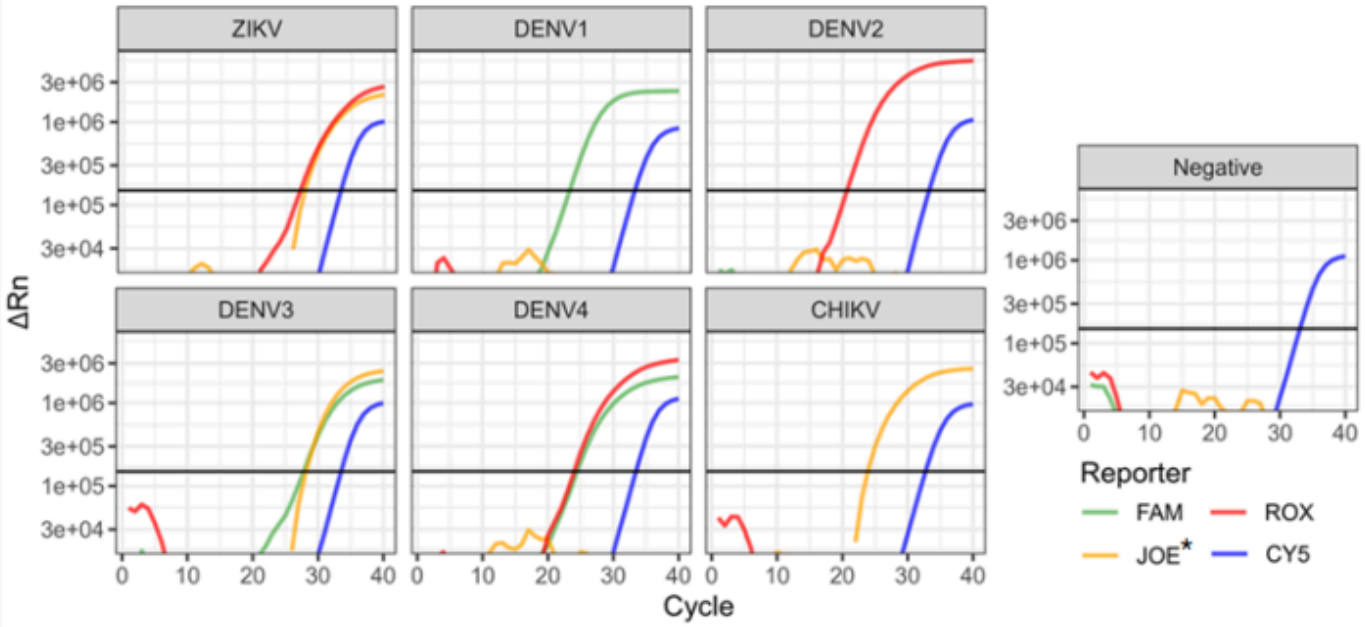


Figure 1

Graphical abstract. Overview of the multiplex RT-qPCR assay based on combinatorial probe coding for simultaneous detection and differentiation of arboviruses.
The assay enables discrimination of Zika virus (ZIKV), chikungunya virus (CHIKV), and dengue virus serotypes 1–4 (DENV1–4) within a single reaction tube using multicolor fluorescence signal combinations.

Virus target	FAM	JOE*	ROX	Cy5
ZIKV	✗	✓	✓	–
DENV1	✓	✗	✗	–
DENV2	✗	✗	✓	–
DENV3	✓	✓	✗	–
DENV4	✓	✗	✓	–
CHIKV	✗	✓	✗	–
Negative sample	✗	✗	✗	✓



* JOE for ThermoFisher equipments or VIC for Bio-Rad CFX96

Figure 2

Interpretation of fluorescence patterns for target calling. Upper panel: signal combination expected for each viral species/serotype. Lower panel: Representative qPCR amplification profiles obtained from each target and from a negative sample. The β-actin internal control (IC; co channel, represented in the figure as the blue line) is required to validate reactions in which no viral target is detected; in reactions with detectable viral amplification, IC amplification may be reduced or absent. ZIKV: Zika virus; DENV1:

dengue virus serotype 1; DENV2: dengue virus serotype 2; DENV3: dengue virus serotype 3; DENV4: dengue virus serotype 4; CHIKV: chikungunya virus.

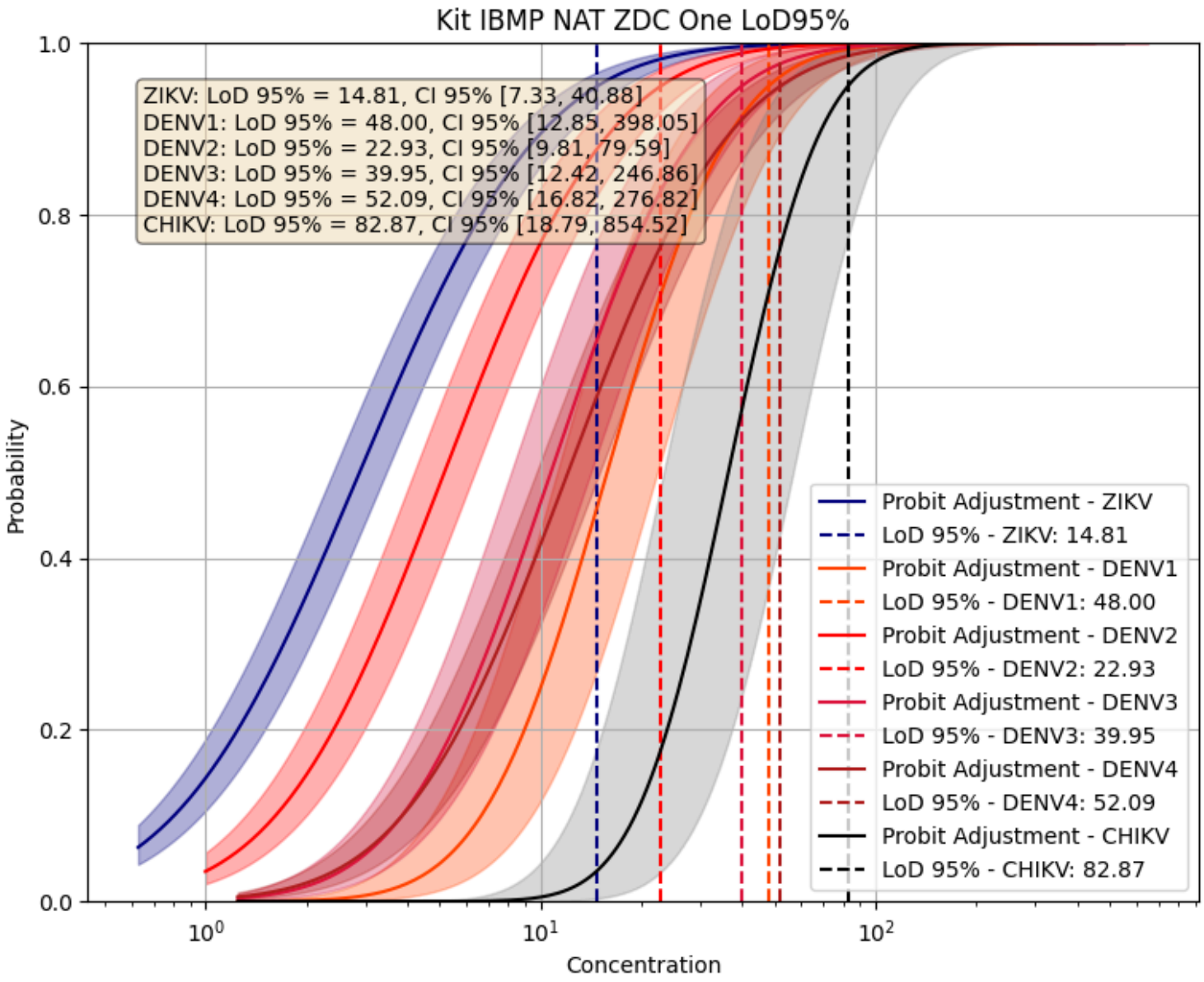


Figure 3

Analytical sensitivity of the IBMP NAT ZDC One assay determined by probit regression analysis. Probit regression curves were fitted to detection data obtained at increasing RNA concentrations (copies per reaction) for each viral target. Solid lines represent model-predicted detection probabilities, and shaded areas indicate the 95% confidence intervals of the fitted models. The estimated 95% hit-rate limit of detection (LoD95%) and corresponding confidence intervals for each target are indicated by dashed lines.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryMaterialZDCOne.zip