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A new high-throughput tool to screen mosquito-borne viruses in Zika virus endemic/epidemic areas

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Abstract

Mosquitoes are vectors of arboviruses affecting animal and human health. Arboviruses circulate primarily within an enzootic cycle and recurrent spillovers contribute to the emergence of human-adapted viruses able to initiate an urban cycle involving anthropophilic mosquitoes. The increasing volume of travel and trade offers multiple opportunities for arbovirus introduction in new regions. This scenario has been exemplified recently with the Zika pandemic. To incriminate a mosquito as vector of a pathogen, several criteria are required such as the detection of natural infections in mosquitoes. In this study, we used a high-throughput chip based on the BioMark™ Dynamic arrays system capable of detecting 64 arboviruses in a single experiment. A total of 17,958 mosquitoes collected in Zika-endemic/epidemic countries (Brazil, French Guiana, Guadeloupe, Suriname, Senegal, and Cambodia) were analyzed. Here we show that this new tool can detect endemic and epidemic viruses in different mosquito species in an epidemic context. Thus, this fast and low-cost method can be suggested as a novel epidemiological surveillance tool to identify circulating arboviruses.

1. Introduction

The World Health Organization stated in February 2016 that Zika infection was considered as a public health emergency of international concern (1) opening a new chapter in the history of vector-borne diseases. Arboviruses are viruses transmitted among vertebrate hosts by arthropod vectors. Successful transmission of an arbovirus relies on a complex life cycle in the vector, which after midgut infection and dissemination, is released in saliva for active transmission to the vertebrate host (2). Arboviruses belong to nine families: Asfarviridae, Flaviviridae, Orthomyxoviridae, Reoviridae, Rhabdoviridae, the newly recognized Nyamiviridae (order Mononegavirales) and the families Nairoviridae, Phenuiviridae and Peribunyaviridae in the new order, Bunyavirales. Most arboviruses possess an RNA genome and are mainly transmitted by mosquitoes (3). While acute infections in vertebrate hosts are typically self-limiting, arboviruses establish persistent infections in arthropods granting to the vector a central role as a viral reservoir (4).

Arboviruses circulate primarily within an enzootic cycle involving zoophilic vector species and non-human hosts. Recurrent spillovers cause occasional infections of humans initiating an epidemic cycle. Arboviruses such as dengue (DENV; *Flavivirus*, Flaviviridae), chikungunya (CHIKV; *Alphavirus*, Togaviridae), Zika (ZIKV; *Flavivirus*, Flaviviridae) and, Yellow fever virus (YFV; *Flavivirus*, Flaviviridae) do not need to amplify in wild animals to cause outbreaks in humans, which act simultaneously as amplifier, disseminator and source of infection for the major vectors, the anthropophilic mosquitoes *Aedes aegypti* and *Aedes albopictus* (5). Thus, the success of these viruses comes from their feature to be mainly transmitted by human-biting mosquitoes strongly adapted to urban environments. The establishment of a new epidemic cycle is undoubtedly related to the introduction of a viremic vertebrate host (humans, animals) acting as a vehicle for importation of the virus into environments receptive to viral amplification.

Other arboviruses such as West Nile virus (WNV; *Flavivirus*, Flaviviridae) remain circulating within an enzootic cycle with sporadic spillovers causing human cases.

Many regions experience simultaneous circulation of different arboviruses (6, 7), and co-infections in vectors were reported (8). These coinfections can present an opportunity for viruses to exchange genetic material. Impacts of such genetic events on virulence for vertebrate hosts are still unknown (9). Thus, being able to detect a wide range of arboviruses in thousands of field-collected mosquitoes in a single experiment can be a valuable tool to predict arboviral emergences in human populations. Indeed similar methods were developed with success to screen tick-borne pathogens (bacteria, parasites and viruses) and allowed the detection of expected and unexpected pathogens in large scale epidemiological studies (10, 11). Therefore, we developed a high throughput system based on real-time microfluidic PCR which is able to detect 96 mosquito-borne viruses in 96 samples within one single run. With this method, we have screened: (1) mosquitoes infected artificially using a feeding system to validate our tool, (2) mosquitoes collected in countries endemic for the major human arboviruses (e.g., Senegal, Cambodia, Brazil), and (3) mosquitoes collected during the Zika and Yellow fever outbreaks in the Americas (French Guiana, Guadeloupe, Brazil, Suriname). This method allowed detecting epidemic viruses (ZIKV, CHIKV, YFV) but also unexpected viruses (e.g. Trivittatus virus, TVTV, *Orthobunyavirus*, Bunyaviridae) underlining the need of such a tool for early detection of emerging mosquito-borne viruses.

2. Materials and methods

2.1. Mosquitoes

To test the ability of our assays to detect viruses present in pools of mosquitoes, 47 batches of three infected mosquitoes of the species, *Ae. aegypti* and *Ae. albopictus* (infection performed by artificial feeding system), were provided by the Institut Pasteur (Paris). Six different viruses, single or double infections, were tested in a pilot study.

In ZIKV-endemic and -epidemic regions from South America, Africa, and Asia (Brazil, French Guiana, Guadeloupe, Suriname, Senegal, Cambodia), adult mosquitoes were collected, identified using morphological characters and dissected to separate abdomen from the remaining body parts (RBP) (See Tables 1-6 for details). Abdomens of the same species were grouped by pools of 20-30 individuals in cryovials, and RBP were stored individually at -80°C until further analysis.

2.2. RNA extraction

Total RNAs were extracted from each pool using the Nucleospin RNA II extraction kit (Macherey-Nagel, Germany). Pools were ground in 350 µL Lysis Buffer and 3.5 µL β-mercaptoethanol using the homogenizer Precellys®24 Dual (Bertin, France) at 5,500 rpm for 20 sec. Total RNA per pool was eluted in 50 µL of RNase free water and stored at -80°C until use.

When pools of abdomens were positive for virus, the RBP (head/thorax) of individual mosquitoes composing each pool were homogenized in 300 µL of DMEM with 10% fetal calf serum using the homogenizer Precellys®24 Dual (Bertin, France) at 5,500 rpm for 20 sec. Then total RNAs were extracted from 100 µL of homogenates using the Nucleospin RNA II extract kit (Macherey-Nagel, Germany) and 200 µL were conserved at -80°C for attempts to isolate the virus. Total RNA per sample was eluted in 50 µL of RNase free water and stored at -80°C until use.

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134 **2.3. Reverse Transcription and cDNA pre-amplification**

135 RNAs were transcribed to cDNA by reverse transcription using the qScript cDNA Supermix
136 kit according to the manufacturer's instructions (Quanta Biosciences, Beverly, USA). Briefly,
137 the reaction was performed in a final volume of 5 µL containing 1 µL of qScript cDNA
138 supermix 5X, 1 µL of RNA and 3 µL of RNase free water; with one cycle at 25°C for 5 min,
139 one cycle at 42°C for 30 min and one final cycle at 85°C for 5 min.
140 For cDNA pre-amplification, the Perfecta Preamp Supermix (Quanta Biosciences, Beverly,
141 USA) was used according to the manufacturer's instructions. All primers were pooled to a final
142 concentration of 200 nM each. The reaction was performed in a final volume of 5 µL containing
143 1 µL Perfecta Preamp 5X, 1.25 µL pooled primers, 1.5 µL distilled water and 1.25 µL cDNA,
144 with one cycle at 95°C for 2 min, 14 cycles at 95°C for 10 sec and 3 min at 60°C. At the end of
145 the cycling program, the reactions were 1:5 diluted. Pre-amplified cDNAs were stored at -20°C
146 until use.

147

148 **2.4. Assay design**

149 Mosquito-borne viruses (MBV), their targeted genes and the corresponding primers/probe sets
150 are listed in Table 7. For a total of 64 viruses including 149 genotypes/serotypes, primers and
151 probes were specifically designed. Each primer/probe set was validated using a dilution range
152 of several cDNA positive controls (when available) (Table 7), by real-time PCR on a
153 LightCycler® 480 (LC480) (Roche Applied Science, Germany). Real-time PCR assays were
154 performed in a final volume of 12 µL using the LightCycler® 480 Probe Master Mix 1X (Roche
155 Applied Science, Germany) with primers and probes at 200 nM and 2 µL of control cDNA
156 (virus reference material) or DNA (Plasmid). Thermal cycling conditions were as follows: 95°C

for 5 min, 45 cycles at 95°C for 10 sec and 60°C for 15 sec and one final cooling cycle at 40°C for 10 sec.

2.5. High-throughput real-time PCR

The BioMark™ real-time PCR system (Fluidigm, USA) was used for high-throughput microfluidic real-time PCR amplification using the 96.96 dynamic arrays (Fluidigm, USA). These chips dispensed 96 PCR mixes and 96 samples into individual wells, after which on-chip microfluidics assemble PCR reactions in individual chambers prior to thermal cycling resulting in 9,216 individual reactions. Real-time PCRs were performed using FAM- and black hole quencher (BHQ1)-labeled TaqMan probes with TaqMan Gene Expression Master Mix in accordance with manufacturer's instructions (Applied Biosystems, France). Thermal cycling conditions were as follows: 2 min at 50°C, 10 min at 95°C, followed by 40 cycles of 2-step amplification of 15 sec at 95°C, and 1 min at 60°C. Data were acquired on the BioMark™ real-time PCR system and analyzed using the Fluidigm real-time PCR Analysis software to obtain C_t values (see Michelet et al. 2014 for more details (12)). Primers and probes were evaluated for their specificity against cDNA reference samples. One negative water control was included per chip. To determine if factors present in the sample could inhibit the PCR, *Escherichia coli* strain EDL933 DNA was added to each sample as an internal inhibition control, using primers and probe specific for the *E. coli eae* gene (13).

2.7. Validation of the results by real-time PCR, virus isolation and genome sequencing

When cDNA of pools of abdomens were detected positive for viruses, the cDNAs of RBP (head/thorax) of individual mosquitoes composing each pool were screened by real-time PCRs on a LightCycler® 480 (LC480) (Roche Applied Science, Germany). Real-time PCR assay targeting the virus of interest (see primers/probe sets in Table 7) was performed in a final

volume of 12 µL using the LightCycler® 480 Probe Master Mix 1X (Roche Applied Science, Germany), with primers and probes at 200 nM and 2 µL of control DNA. Thermal cycling conditions were as follows: 95°C for 5 min, 45 cycles at 95°C for 10 sec and 60°C for 15 sec and one final cooling cycle at 40°C for 10 sec.

When a positive sample was confirmed, virus isolation was attempted in Vero and C6/36 cells. Then, total RNA was extracted using the Nucleospin RNA II extract kit (Macherey-Nagel, Germany) following the manufacturer instructions and full genome sequencing was attempted. For ZIKV, twelve overlapping amplicons were produced using the reverse transcriptase Platinum Taq High Fidelity polymerase enzyme (Thermo Fisher Scientific) and specific primers (Table 8). PCR products were pooled in equimolar proportions. After Qubit quantification using Qubit® dsDNA HS Assay Kit and Qubit 2.0 fluorometer (ThermoFisher Scientific) amplicons were fragmented (sonication) into fragments of 200 bp long. Libraries were built adding barcode for sample identification, and primers to fragmented DNA using AB Library Builder System (ThermoFisher Scientific). To pool the barcoded samples equimolarly, a quantification step by the 2100 Bioanalyzer instrument (Agilent Technologies) was performed. An emulsion PCR of the pools and loading on 520 chip was done using the automated Ion Chef instrument (ThermoFisher Scientific). Sequencing was performed using the S5 Ion torrent technology (Thermo Fisher Scientific) following manufacturer's instructions. Consensus sequence was obtained after removing the thirty first and last nucleotides of each read, trimming reads depending on quality (reads with quality over >99%) and length (reads over 100 pb were kept) and mapping them on a reference (KY415987, most similar sequence after Blastn) using CLC genomics workbench software 11.0.1 (Qiagen). A *de novo* contig was also produced to ensure that the consensus sequence was not affected by the reference sequence.

3. Results

One hundred and forty-nine primer/probe sets were designed to detect 64 MBV (Table 7). Among them, 95 sets of primers/probe specifically identified their corresponding positive control samples (37 viral RNA) *via* Taqman RT-real-time PCRs or Taqman real-time PCRs on a LightCycler 480 apparatus. Resulting C_t values varied from 8 to 42 depending on sample type and nucleic acid concentration. Unfortunately, 54 designs were not tested due to the lack of RNA positive control.

To avoid sensitivity problems, cDNA pre-amplification was included in the assay. This step enabled detection of all positive controls (95 primer/probe sets tested on 37 viral RNAs) via Taqman real-time PCRs on a LC480 apparatus. The specificity of each primers/probe set was then evaluated using 37 MBV positive controls on the BioMark^{TM} system (Fig. 1). Results demonstrated high specificity for each primer/probe set after pre-amplification (Fig. 1). Indeed, 91 assays (among the 149 developed) were only positive for their corresponding positive controls. Four designs demonstrated cross-reactivity with a virus from the same species or genus: DENV-1 assay amplified also DENV-2, DENV-2 assay cross-reacted with DENV-3 and DENV-4, DENV-4 cross-reacted with DENV-3, one WNV assay amplified Usutu virus (USUV). Specificity of 54 assays was not fully tested in the absence of their respective positive controls. Nevertheless, those designs did not show any cross-reaction with RNA positive controls from other viruses.

Laboratory-infected mosquitoes

Forty-seven batches containing each, three infected mosquitoes, were screened with the high-throughput technic developed. The system was able to identify the six viruses present in different mosquitoes (Fig. 2). Indeed, seven batches were infected by DENV-1, four by DENV-

3, four by DENV-4, 3 by CHIKV, five by WNV, 13 batches by ZIKV and 10 batches were coinfected by CHIKV and DENV-2. As for the specificity test, DENV-1, DENV-2 and DENV-3 assays demonstrated cross-reactions.

Field-collected mosquitoes from endemic and epidemic areas

A total of 17,958 field-collected mosquitoes in six countries from the African, American and Asian continents were screened for arbovirus.

Endemic areas

Senegal

In Senegal, 934 arthropods including 6 sandflies and 928 mosquitoes (25 males and 909 females) from 21 species and five genera (detailed in Table 1), were collected in the Kedougou area (Southeastern Senegal) from August to November 2017. Moreover, 402 larvae were also collected in the same area from August 2017 to January 2018 and reared until adult emergence in insectarium (188 males and 214 females obtained). Mosquitoes were grouped by species and sex; 231 and 112 pools were respectively analyzed for MBVs. YFV was detected in one pool of 20 females of *Aedes furcifer* and was confirmed in head/thorax from 1 *Aedes furcifer* female by RT-real-time PCR. Virus was identified as YFV from West Africa lineage currently circulating in Senegal (38). Virus isolation was attempted but without any success.

Cambodia

In Cambodia, 492 mosquitoes (73 males and 419 females) from 28 species and 5 genera (detailed in Table 2), were collected in one area at two periods, the dry season in May 2019 and the rainy season in November 2018. Mosquitoes were grouped by species and sex into 109 pools and were analyzed for MBVs. No virus was detected (Table 2).

Endemic/epidemic area, Brazil

In Brazil, 7,705 mosquitoes (889 males and 6,816 females) belonging to 22 species and 15 genera (detailed in Table 3), were collected in 15 areas from January 2016 to May 2017. Mosquitoes were then grouped into 647 pools and were analyzed for MBVs. In total, three different viruses (YFV, CHIKV and Trivittatus virus (TVTV)) were preliminary detected in six pools (in 4, 1 and 1 respectively). Only the presence of YFV was confirmed in the head/thorax from individual mosquitoes, from three species (*Ae. scapularis*, *Ae. taeniorhynchus* and *Hg. leucocelaenus*) by RT real-time PCR corresponding to YFV strains currently circulating in Brazil (37). Attempts to isolate the virus were made but remained unsuccessful.

Epidemic areas

Guadeloupe

In Guadeloupe, 150 mosquito pools corresponding to 2,173 mosquitoes (884 males and 1,289 females), from five species (*Ae. aegypti*, *Culex quinquefasciatus*, *Anopheles albimanus*, *Cx. bisulcatus*, *Cx. nigripalpus*, 54 *Culex.* spp.) collected from May to June 2016 were screened for 64 MBVs. ZIKV was found in two pools of *Cx. quinquefasciatus* females and nine pools of *Ae. aegypti* (eight pools of females and one pool of males) (Table 4). ZIKV was detected only in the head/thorax of individual *Ae. aegypti* females from the eight positive pools by a RT-real-time PCR. Virus was isolated on Vero cells and full genome sequencing identified the Asian genotype (GenBank Accession Numbers: MN185324, MN185325, MN185327, MN185329, MN185330, MN185331, MN185332).

French Guiana

In French Guiana, 3,942 mosquitoes (1,098 males and 2,844 females) from seven species (*Ae. aegypti, Ae. scapularis, Ae. taeniorhynchus, Cx. quinquefasciatus, Ma. titillans, Cq.*

venezualensis, and *Cq. albicosta*) were collected in the Cayenne area from June to August 2016 and grouped into 248 pools to be screened. Three pools of *Ae. aegypti* and one pool of *Cx. quinquefasciatus* were detected positive for ZIKV (Table 5). After screening individual head/thorax from those pools, only pools of *Ae. aegypti* were confirmed positive. ZIKV was isolated and fully sequenced; it belonged to the Asian genotype (GenBank Accession numbers: MN185326 and MN185328).

Suriname

In Suriname, from March to May 2017, four species/genus of mosquitoes (2,256 *Ae. aegypti*, 29 *Culex* spp., 5 *Haemogogus* spp., 20 undetermined species) representing 2,310 adults, were grouped into 77 pools and screened. No virus was detected (Table 6).

4. Discussion

In this study, we developed and validated a new high-throughput virus-detection assay based on microfluidic PCRs able to detect unambiguously 64 MBVs in mosquitoes. Only four primer sets demonstrated cross-reactivity with viruses from the same genus or serotype. Subsequently, we used this newly developed assay to perform a large epidemiological survey screening in six countries/territories during the last Zika pandemic. This new method has allowed the detection of (i) three human infecting arboviruses ZIKV, YFV and CHIKV in mosquitoes and (ii) other unexpected viruses such as TVTV.

The efficiency of our tool was the first requirement; we used artificially infected mosquitoes to detect different viruses (DENV1-4, CHIKV, WNV, ZIKV) offered in single and dual infections.

Our assays can target unambiguously DENV with however cross-reactions between serotypes. Caused by one of the four serotypes (DENV1-4), dengue is the most important arboviral disease worldwide (14). It is widely accepted that a subsequent infection with a second serotype can produce more severe symptoms (15). This situation becomes challenging when multiple serotypes co-circulate (16). Mosquitoes co-infected with different DENV serotypes are occasionally detected (17). *Aedes aegypti* and *Ae. albopictus* are urban vectors of DENV responsible for most epidemic outbreaks in Asia, Latin America, the Caribbean and Pacific islands (14). Co-infected *Aedes* mosquitoes are capable of transmitting multiple arboviruses during one bite (9). Dual DENV detections in mosquitoes may be a sign of co-circulation of DENV and then, may help in predicting co-infections in humans. Diagnosis of dengue infections cannot be based on clinical symptoms as dengue disease shares common symptoms with other arboviral diseases (18). To discriminate dengue serotypes, viral isolation and viral RNA detection remain the gold standard methods but should be performed during patient viremia (within five days after the onset of fever). Less constraining and costly, mass viral screening of mosquitoes in surveillance and epidemic contexts can be an advantageous substitute.

In the same way, WNV assays cross-reacted with the phylogenetically-related USUV. WNV is a flavivirus responsible of neuro-invasive disease in Europe and North America (19). Diagnosis of WNV infection remains challenging and human cases are usually underestimated. On the other hand, USUV has spread over Europe during the last twenty years causing bird mortalities and some rare human cases (20). Human infections are rare and often asymptomatic and neurological disorders can be described (20). While WNV circulates in Europe since 1960s, USUV shares the same geographical distribution and also the same vectors, *Culex pipiens*. Our tool did not succeed in distinguishing the two viruses and therefore, it needs more improvements.

By screening 17,958 mosquitoes collected in six countries/territories for 64 different MBVs, we succeeded in detecting ZIKV, YFV, CHIKV and TVTV in mosquitoes.

The Zika outbreak was unexpected; the first human cases outside endemic regions in Africa were reported in Yap island in 2007 where the outbreak was poorly publicized despite the two third of the population affected (21). Few years later, ZIKV hit French Polynesia (22) where the first notification of severe symptoms associated to ZIKV infections were done, Guillain-Barre syndrome (23) and microcephaly in new-born (24). After, ZIKV reached the American continent in 2015 (25), phylogenetic analysis indicated that the circulating ZIKV belonged to the Asian clade (26, 27). Our tool was able to detect ZIKV in pools of abdomen of *Ae. aegypti* and *Cx. pipiens* from Guadeloupe and French Guiana. However when analyzing disseminated viral particles in head and thorax, only *Ae. aegypti* was found infected corroborating the main role of this species in ZIKV transmission and excluding *Cx. quinquefasciatus* and *Cx. pipiens* as a vector (28).

Our mass screening tool has detected YFV in five mosquito species: *Aedes scapularis, Aedes taeniorhynchus, Haemagogus janthinomys, Haemagogus leucocelaenus,* and *Sabethes chloropterus.* The species *Hg. janthinomys* and *Hg. leucocelaenus* are considered as the main vectors of YFV in Brazil (29, 30) while *Aedes scapularis, Aedes taeniorhynchus* and *Sabethes chloropterus* only play a secondary role (31). Other viruses preliminary detected in Brazilian mosquitoes were TVTV and CHIKV. While CHIKV continues to cause sporadic cases in Brazil after the massive outbreak in 2015, TVTV was first isolated from *Aedes trivittatus* in USA in 1948, and has never been detected outside North America where it is mainly distributed (32). Consequences of TVTV infections on humans remain unknown (33). Nevertheless, the presence of CHIKV and TVTV in tested mosquitoes was not confirmed.

357 This study demonstrates the feasibility of high-throughput screening methods to detect diverse
358 MBVs in field-collected mosquitoes. Performing 9,216 real-time PCRs in one run took four
359 hours, and the cost was around $10 per reaction from sample homogenization to virus detection
360 by real-time PCR (10, 11). Another main advantage of our tool is the adaptability of the system
361 by adding new sets of primers and probes targeting newly emergent viruses in contrast to arrays
362 with fixed panels of probes. Indeed, because the number of YFV cases was unusually high since
363 January 2016 (34), we added specific detections of YFV strains circulating in South America
364 to screen field-collected mosquitoes from Brazil, French Guiana, Suriname and Guadeloupe. In
365 conclusion, our method designed to specifically identify MBVs in mosquitoes can be used to
366 screen other types of samples such as human and/or animal blood or organs (35). We
367 demonstrated the usefulness of this new screening method which represents a powerful, cost-
368 effective and rapid system to track MBVs all around the world and could be easily customized
369 to any viral emergence.
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387 **6. Author contributions**

388 All the authors are involved as partners in the H2020 ZIKAlliance project. SM and ABF
389 designed the experiments. SM and ABF wrote the paper. All the authors performed the
390 experiments, and/or collected mosquitoes, and/or extracted RNAs , and/or performed
391 confirmation tests like conventional and real-time PCR and virus isolation attempts. All the
392 authors reviewed the manuscript.

393

Table 1. Mosquito and sandflies species, number of mosquitoes collected and number of pools analyzed in Senegal.

Stage collected	Collection Site	GPS coordinates	Urban Rural Sylvatic	Mosquito species	Number of arthropods screened	Virus detected through microfluidic system	Type of confirmation performed
Adult	Baraboye	12°41'11.2"N/12°24'39.2"W	Rural	*Ae. furcifer, Ae. dalzieli, Ae. taylori, Ae. vittatus, An. coustani, An. funestus, Ma. uniformis,* sandflies	97	-	-
	Ngari	12°38'07.3"N/12°14'59.5"W	Rural	*Ae. furcifer, Ae. dalzieli, Ae. aegypti, Ae. argenteopunctatus, Ae. hirsutus, Ae. mcintoshi, Cx. quinquefasciatus, Ae. vittatus, An. coustani, An. funestus,*	96	-	-
	Silling	12°32'36.5"N/12°16'18.7"W	Rural	*Ae. luteocephalus, An. gambiae*	3	-	-
	Tenkoto	12°40'23.1"N/12°16'37.1"W	Rural	*Ae. furcifer*	5	-	-
	Velingara	12°27'33.9"N/12°03'17.3"W	Rural	*Ae. aegypti, Ae. dalzieli, Ae. furcifer, Ae. luteocephalus, Ae. vittatus, An. coustani*	43	-	-
	Kedougou (C1F)	12°39'42.1"N/12°16'05.2"W	Sylvatic	*Ae. aegypti, Ae. furcifer, Ae. luteocephalus, Ae. taylori, Ae. unilineatus, Ae. vittatus, An. coustani, An. funestus, An. nili, Cx. perfuscus, Ma. africana, Ma. uniformis*	151	-	-
	Kedougou (D1F)	12°36'43.9"N/12°14'50.7"W	Sylvatic	*Ae. aegypti, Ae. africanus, Ae. dalzieli, Ae. furcifer, Ae. luteocephalus, Ae. taylori, Ae. unilineatus, Ae. vittatus, An. coustani, An. funestus, An. nili, Cx. annulioris, Cx. bitaeniorhynchus, Cx. poicilipes, Cx. perfuscus, Ma. africana, Ma. uniformis*	278	YFV*	YFV confirmed by PCR
	Kedougou (E2F)	12°29'21.2"N/12°06'06.2"W	Sylvatic	*Ae. aegypti, Ae. africanus, Ae. argenteopunctatus, Ae. dalzieli, Ae. furcifer, Ae. luteocephalus, Ae. taylori, Ae. unilineatus, Ae. vittatus, An. coustani, An. funestus, Cx. poicilipes, Ma. uniformis*	261	-	-

	8				**934**		
Larvae	Dalaba	12°33'25.6"N/12°10'41.0"W	Rural	*Ae. aegypti*	24	-	-
	Ngari	12°38'07.3"N/12°14'59.5"W	Rural	*Ae. aegypti, Ae. vittatus*	18	-	-
	Kedougou (D1F)	12°36'43.9"N/12°14'50.7"W	Sylvatic	*Ae. aegypti, Ae. bromeliae, Ae. furcifer, Ae. longipalpis, Ae. luteocephalus, Ae. taylori, Ae. unilineatus, Ae. vittatus*	237	-	-
	Kedougou (E2F)	12°29'21.2"N/12°06'06.2"W	Sylvatic	*Ae. aegypti, Ae. bromeliae, Ae. longipalpis, Ae. luteocephalus, Ae. neoafricanus, Ae. taylori, Ae. unilineatus, Ae. vittatus, Er. chrysogaster*	123	-	-
	4				**402**		

Ae., Aedes; An., Anopheles ; Cx., Culex; Er., Eretmapodites ; Ma., Mansonia ; YFV, Yellow fever virus.

* YFV detected in one pool of *Ae. scapularis*, one pool of *Ae. furcifer* and confirmed in one female of this pool.

Table 2. Mosquito species, number of mosquitoes collected and number of pools analyzed in Cambodia.

Collection Site	GPS coordinates	Urban Rural Sylvatic	Mosquito species	Number of mosquitoes screened	Virus detected through microfluidic system	Type of confirmation performed
Mondulkiri, Cambodia	12°10'28.4" / 106°53'40.9"	Sylvatic	*Ae. aegypti, Ae. albopictus, Ae. gardnerri imitator, Ae. prominens, An. barbirostris, An. indefinitus, An. jamesii, An. kochi, An. mimulus complex, An. philippinensis, An. roperi, An. umbrosus, An. vegus, Ar. annulipalpis, Ar. dolichocephalus, Ar. flavus, Ar. foliatus/kuchingensis, Ar. moultoni, Ar. subalbatus, Ar. theobaldi, Cx. bitaeniorhynchus, Cx. brevipalpis, Cx. fuscocephala, Cx. perplexus/whitei, Cx. sitiens, Cx. vishnui complex, Hz. catesi, Hz. demeilloni*	492	-	-

Ae., Aedes; An., Anopheles; Ar., Armigeres; Cx., Culex; Hz., Heizmannia.

Table 3. Mosquito species, number of mosquitoes collected and viruses detected in Brazil.

Collection Site	GPS coordinates	Urban Rural Sylvatic	Mosquito species	Number of mosquitoes screened	Virus detected through microfluidic system	Type of confirmation performed
Belo Horizonte	19°51'59.29"S/44° 0'43.51"W	Urban Forest	*Ae. albopictus, Ae. aegypti, Cx. quinquefasciatus, Sa. albiprivus*	17	-	-
Casimiro de Abreu	22°26'33.31"S/42°12'30.34"W	Sylvatic	*Ae. scapularis*	24	-	-
Domingos Martins	20°17'12.48"S/40°50'14.35"W	Sylvatic	*Ae. albopictus*	7	-	-
Goiânia	6°40′16.32″S/49°22′49.93″W	Urban Forest	*Aedeomiya, Ae. aegypti, Ae. albopictus, Aedes sp., Coquillettidia sp., Culex sp., Hg. leucocelaenus, Limatus sp., Mansonia sp., Orthopodomyia sp., Psorophora sp., Sabethes sp., Wyeomyia sp.*	689	-	-
Guapimirim	22°28'56.31"S/42°59'26.36"W	Sylvatic	*Ru. frontosa*	10	-	-
Macaé	22°18'17.54"S/42° 0'8.80"W	Sylvatic	*Ae. scapularis, Wyeomyia sp.*	25	-	-
Manaus	3°00′12.78″S/59°55’37.86”W	Urban Forest	*Ae. aegypti, Ae. albopictus, Aedes sp., Culex sp., Hg. leucocelaenus, Limatus sp., Orthopodomyia sp., Psorophora sp., Sabethes sp., Trichoposopum sp., Uranotenia sp., Wyeomyia sp.*	3939	CHIKV*	Isolation, conventional and real-time PCR failed to confirm the result
Maricá	22°55'24.44"S/42°42'27.88"W	Sylvatic	*Ae. aegypti, Ae. albopictus, Ae. scapularis, Ae. taeniorhynchus, Culex sp., Cx. nigripalpus, Hg. janthinomys, Hg. leucocelaenus, Li. durhamii, Ru. humboldti*	198	YFV§	YFV confirmed by PCR
Miguel Pereira	22°29'3.21"S/43°18'15.98"W	Sylvatic	*Sh. fluviatilis*	12	-	_

Nova Friburgo	22°24'46.35"S/42°18'58.57"W	Sylvatic	*Ru. humboldti*	2	CHIKV[£]	Isolation, conventional and real-time PCR failed to confirm the result
Queluz	22°41'52.33"S/44°43'43.91"W	Sylvatic	*Wy. pilicauda*, *Wy. confusa*	6	-	-
Rio de Janeiro	22°52'45.7"S/43°18'10.0"W	Urban	*Ae. aegypti*, *Ae. albopictus*, *Cx. quinquefasciatus*	261	-	-
	22°56′6.57″S/43°26′42.19″W	Urban Forest	*Ae. aegypti*, *Ae. albopictus*, *Aedes sp. , Coquillettidia sp., Culex sp., Hg. leucocelaenus*, *Mansonia sp., Psorophora sp., Runchomyia sp., Sabethes sp., Trichoposopum sp., Wyeomyia sp.*	2447	TVTV[$]	Isolation, conventional and real-time PCR failed to confirm the result
Serra	20° 6'46.89"S/40°11'12.53"W	Sylvatic	*Ae. albopictus*, *Cx. quinquefasciatus*	26	-	-
Simonésia	19°55'12.06"S/41°54'20.23"W	Sylvatic	*Ae. albopictus*, *Cq. venezuelensis*, *Hg. janthinomys*, *Hg. leucocelaenus*, *Sa. albiprivus*	41	-	-
Teresópolis	22°26'58.56"S/42°59'5.43"W	Sylvatic	*Ru. frontosa*	1	-	-
15				**7705**		

Ae., Aedes; Cq., Coquillettidia; Cx., Culex; Hg., Haemagogus; Li., Limatus; Ru., Runchomyia; Sa., Sabethes; Sh., Shannoniana; Wy., Wyeomyia; CHIKV, Chikungunya virus; YFV, Yellow fever virus; TVTV, Trivittatus virus.

*CHIKV detected in one pool of *Cx. erraticus*; [§] YFV detected in one pool of *Ae. scapularis*, one pool of *Ae. taeniorhynchus*, one pool of *Hg. leucocelaenus;* [£] CHIKV detected in one pool of *Ru. humboldti* ; [$] TVTV detected in one pool of *Cx. nigripalpus*

Table 4. Mosquito species, number of mosquitoes collected and virus detected in Guadeloupe.

Collection Site	GPS coordinates	Urban Rural Sylvatic	Mosquito species	Number of mosquitoes screened	Virus detected through microfluidic system	Type of confirmation performed
Gosier	16° 12' 21.229"N/61° 29' 31.438"W	Urban	*Ae. aegypti, Cx. quinquefasciatus, An. albimanus*	399	-	-
Deshaies	16° 18' 24.973"N/61° 47' 39.556"W	Urban/Periurban	*Ae. aegypti, Cx. quinquefasciatus, An. albimanus, Cx. bisulcatus, De. magnus*	306	ZIKV*	Confirmed by real-time PCR on head-thorax of individual mosquitoes, isolation of the virus and full genome sequencing
Petit Bourg	16° 11' 29.476"N/61° 35' 25.753"W	Urban/Periurban	*Ae. aegypti, Cx. quinquefasciatus, Cx. nigripalpus, Culex sp.*	422	ZIKV*	Confirmed by real-time PCR on head-thorax of individual mosquitoes, isolation of the virus and full genome sequencing
Le Moule	16° 19' 52.342"N/61° 20' 37.41"W	Urban/Periurban	*Ae. aegypti, Cx. quinquefasciatus, Culex sp.*	202	ZIKV*	Confirmed by real-time PCR on head-thorax of individual mosquitoes, isolation of the virus and full genome sequencing
Saint François	16° 15' 5.141"N/61° 16' 26.825"W	Urban	*Ae. aegypti, Cx. quinquefasciatus*	356	ZIKV*	Confirmed by real-time PCR on head-thorax of individual mosquitoes, isolation of the virus and full genome sequencing
Sainte Anne	16° 13' 31.613"N/61° 23' 9.377"W	Urban/Periurban	*Ae. aegypti, Cx. quinquefasciatus, Cx. nigripalpus, Culex sp.*	325	ZIKV*	Confirmed by real-time PCR on head-thorax of individual mosquitoes, isolation of the virus and full genome sequencing
Baie Mahault	16° 16' 3.979"N/61° 35' 13.337"W	Urban	*Ae. aegypti, Cx. quinquefasciatus*	19	-	-
Le Lamentin	16° 16' 17.36"N/61° 37' 59.754"W	Urban/Periurban	*Ae. aegypti, Cx. quinquefasciatus*	27	-	-

Goyave	16° 7' 26.447"N/61° 34' 40.253"W	Urban/Periurban	*Ae. aegypti, Cx. quinquefasciatus, Culex sp.*	86	-	-
Morne-à-L'eau	16° 19' 53.832" N/61° 27' 25.855"W	Urban	*Ae. aegypti, Cx. quinquefasciatus*	19	-	-
Pointe-à-Pître	16° 14' 54.499"N/61° 32' 18.888"W	Urban	*Ae. aegypti, Cx. quinquefasciatus*	5	-	-
Saint-Claude	16° 1' 36.077"N/61° 42' 6.703"W	Urban/Periurban	*Ae. aegypti*	1	-	-
Petit Canal	16° 22' 49.03"N/61° 29' 14.384"W	Urban/Periurban	*Culex sp.*	6	-	-
13				**2173**		

Ae., Aedes; An., Anopheles; Cx., Culex; De., Deinocerites.

*ZIKV detected in 2 pools of *Cx. quinquefasciatus* and 9 pools of *Ae. aegypti*, and confirmed in 9 females *Ae .aegypti* (one female per pool).

Table 5. Mosquito species, number of mosquitoes collected and virus detected in French Guiana.

Collection Site	GPS coordinates	Urban Rural Sylvatic	Mosquito species	Number of mosquitoes screened	Virus detected through Microfluidic system	Type of confirmation performed
Cayenne	4°55'53.08"N/ 52°18'55.99"W	Urban	*Ae. aegypti, Ae. scapularis, Cx. quinquefasciatus, Ma. titillans, Cq. venezualensis, Cq. albicosta*	1928	ZIKV*	Confirmed by real-time PCR on head-thorax of individual mosquitoes, isolation of the virus and full genome sequencing
Remire-Montjoly	4°53'34.01"N/ 52°16'34.32"W	Urban/Periurban	*Ae. aegypti, Ae. scapularis, Cx. quinquefasciatus, Ma. titillans, Cq. venezualensis*	1078	_	-
Matoury	4°50'52.22"N/ 52°19'41.58"W	Urban/Periurban	*Ae. aegypti, Ae. taeniorhynchus, Cx. quinquefasciatus, Ma. titillans, Cq. venezualensis, Cq. albicosta*	936	ZIKV*	Confirmed by real-time PCR on head-thorax of individual mosquitoes, isolation of the virus and full genome sequencing
3				**3942**		

Ae., Aedes; Cq., Coquillettidia ; Cx., Culex; Ma., Mansonia.

*ZIKV in 1 pools of *Cx. quinquefasciatus* and 3 pools of *Ae. aegypti*, and confirmed in 3 females *Ae.aegypti* (one female per pool)

Table 6. Mosquito species, number of mosquitoes collected and virus detected in Suriname.

Collection Site	GPS	Urban Rural Sylvatic	Mosquito species	Number of mosquitoes screened	Virus detected through Microfluidic system	Type of confirmation performed
Paramaribo	5°51'54.2"N/55°11'33.4"W	Urban (Paramaribo)	Undetermined	4	-	-
Roti shop	5°51'59.616"N/55°6'20.952W	Urban (Paramaribo)	*Ae. aegypti*	68	-	-
Car mechanic	5°50'35.8"N 55°06'56.7"W	Urban (Paramaribo)	*Ae. aegypti*	96	-	-
Kwikfit car mechanic	5°50'38.1"N 55°07'23.3"W	Urban (Paramaribo)	*Ae. aegypti*	567	-	-
Family home	5°50'33.3"N 55°07'17.5"W	Urban (Paramaribo)	*Ae. aegypti*	296	-	-
Outpatient clinic	5°50'30.3"N/55°7'8.615"W	Urban (Paramaribo)	*Ae. aegypti*	103	-	-
Chi min restaurant	5°49'54.408"N/55°8'24.683W	Urban (Paramaribo)	*Ae. aegypti*	78	-	-
Albertine retirement home	5°48'49.572"N/55°11'27.6"W	Urban (Paramaribo)	*Ae. aegypti*	661	-	-
Medisch Opvoedkundig Bureau (MOB)	5°49'43.212"N/55°10'41.375"W	Urban (Paramaribo)	*Ae. aegypti*	376	-	-
Brownsweg	5°0'57.384"N/55°10'2.172"W	Rural/Sylvatic	*Ae. aegypti*, *Culex* sp., Undetermined	21	-	-
Brownsberg	4°56'36.24"N/55°10'6.6"W	Rural/Sylvatic	*Ae. aegypti*, *Culex* sp., *Haemagogus* sp., Undetermined	40	-	-
11				**2310**		

Ae., Aedes; Cx., Culex; Hg., Haemagogus.

Table 7. List of mosquito-borne viruses, targets, primers/probe sets, and positive controls.

Family	Genus	Species	Primers/probe	Sequence (5'-3')	Target	Length (bp)	Positive controls
Flaviviridae	Flavivirus	Banzi	Banzi_F	TCT GTG CCA AAC CAG CTT AG	NS3	71	Cell culture strain SA H 336
			Banzi_R	CTC GGA TCT CAG GAG GAT TG			
			Banzi_P	TGA GAA GCT GGT TCA GTC CAT TGA CAC ACT			
		Bussuquara	BussuqV_F	GAA GGT CTT GGA GAT GGT GG	NS5	72	Cell culture strain BeAn 4073
			BussuqV_R	GTCTGTACGGGCACAACACT			
			BussuqV_P	AGC CGT GGC TTA AGA ACA AAC CTG AAT TCT			
		Dengue	Deng_1_F	AAC CCA TGG AAG CTG TAC GC	3' UTR	120	Cell culture strain Indonesia and Guiana
			Deng_1_R	CTA GTC CTT ACC ACC AGG GT			
			Deng_1_P	ACA GCT TCC CCT GGT GTT GGG CC			
			Deng_2_F	AGA AGA GAA GAG GAA GAG GCA	NS5	138	Cell culture strain D2
			Deng_2_R	TGG CCT GAC TTC TTT TAA CGT C			
			Deng_2_P	CTT GGA CGG GGC TCA CAG GTA GC			
			Deng_3_F	GAA GAG ATT CAG GAA GGA GGA	NS5	144	Cell culture strain D3
			Deng_3_R	GGC CTG ACT TCT TCT TTT AAC G			

			Deng_3_P	TCC TTG GAC GGG GCT CAC AGG C			
			Deng_4_F	TGG ATT CAG GAA GGA GAT AGG	C	124	Cell culture strain D4
			Deng_4_R	GTT CGC CAT CTC TTG TTG ACA			
			Deng_4_P	CCG CAT GCT GAA CAT CTT GAA CGG GA			
		Japanese Encephalitis	JEV_F	GCT TAG CGC TCA CAT CCA CT	NS2a/Ns2b	88	Cell culture strain SA14
			JEV_R	CAC CCT CTC TTC TTG TTT GGG			
			JEV_P	TGC AGA CCA TTA GTC CGG CAG CTA TAG T			
			JEV_IIIa_F	TGG ACG TCC GCA TGA TCA AC	E	162	
			JEV_IIIa_R	CCT TGT TTG CAC ACA TAG CTA C			
			JEV_IIIa_P	TAT CAG CTC GCT TCT CGT TGT GGG CTT			
			JEV_IIIb_F	ACT GAC ATC TCG ACG GTG G	E	131	
			JEV_IIIb_R	CAT GTG TCA ATG CTT CCC TTC			
			JEV_IIIb_P	CTC GGT GCC CCA CGA CTG GAG AA			
			JEV_II_F	GAA GGA GCT AGT GGA GCT AC	E	156	
			JEV_II_R	TGA AGC GTG ATA GCA GTA GCT			
			JEV_II_P	TTG GGT GGA CCT GGT GTT AGA AGG AGA TA			
			JEV_I_F	GTT ACT GCT ATC ACG CTT CAG	E	163	
			JEV_I_R	GTC AAT GCT TCC TTT CCC GAA			
			JEV_I_P	TCA CTG ACA TTT CAA CGG TGG CTC GAT G			
			JEV_IV_F	TTG AAA GGT GCC CAA AGA TTG G	E	188	
			JEV_IV_R	TAT TCC CAT CCA GAG TAG CAG A			
			JEV_IV_P	CAG CGT TGG GAG ATA CAG CTT GGG ATT			

			JEV_V_F	GGA TGG ATG CTT GGC AGC AA	prM/E	170	
			JEV_V_R	GAG GCA ACT GTC TCC TTC CA			
			JEV_V_P	CAA CGG CCA GCG TGT GGT GTT CAC			
		Ilheus	IlheusV_F	GGA AGT GCC ATT TTG CTC CC	NS5	77	Cell culture strain PE 20545
			IlheusV_R	AGC GGC ATG GAA CTA CGA TG			
			IlheusV_P	TAC GTC CAT CCT TCA TCA ACA GCT CGT TG			
		Kedougou	KedV_F	AAG CAC CAA CGG CAG AAG TG	E	85	Cell culture strain Dak Ar
			KedV_R	TGG CTG AAG TCC AAG CCT G			
			KedV_P	ATT GAG CCT CGC ACG TCAT GGT CAT AGT			
		Kokobera virus groupe	Kok_I_F	CAT CAA GGT ACC AGT GAA CGT	NS5	144	Cell culture strain MRM
			Kok_I_R	AAC TCC TCG GAA CTC AAC TCT			
			Kok_I_P	CTT CTC ACT CTC ATA CCT CTT CTG TGT TCC			
			Kok_II_F	GTT GTG ATA GTC AGG CCT GAA	3'NCR	86	
			Kok_II_R	CCA CGA CAC TGG AGC ATC A			
			Kok_II_P	CTC CAG GCC GGA TGC AGG CAG			
			Kok_Bai_F	ACA GAA GAG GTA TGA GAG TGA G	NS5/3'NCR	109	
			Kok_Bai_R	TCA GGT GGC TTT TCA GGC CT			
			Kok_Bai_P	AAG AGA GTT GAG TTC CGA GGA GTT CTG TAG			
			Kok_Tor_F	TGC GGA TCT CTC ATC GGC TA	NS5/3'NCR	183	
			Kok_Tor_R	TCA CCA TCA TTC CCT CTT ACA G			
			Kok_Tor_P	AAC CCC TGT GAA CTT GAC ACT TTC CTC C			
			Kok_Map_F	AAG AGA AGA TCT ATG GTG TGG C	NS5	81	

			Kok_Map_R	CTC CAC AGT TCT TCG GAT GTT			
			Kok_Map_P	TCG CTC ATT GGA CAC AGG CCC AGA A			
			Kok_Strat_F	AAG GCA GAA GGC CGC ATC AT	E	177	
			Kok_Strat_R	AGT CTC CCA AGT CTG CCG T			
			Kok_Strat_P	CCA GAA GGA GAA TTT GGA GTA CAC AGT GC			
		Koutango	Kout_I_F	GAG ATT CCT CGA GGG AGA G	NS5	200	Cell culture strain DAK AR D
			Kout_I_R	ACA AGT TGC ACG GCC AGG TT			
			Kout_I_P	CAC CGC TTA CTA GCC CGA GCA ATC ATT			
			Kout_II_F	CAG AGC ATC AGG TAA CAT CGT	NS5	190	
			Kout_II_R	TGA GCC TCT CGA TTC TCC TC			
			Kout_II_P	CCA CGC TGT GAG CAT GAC CAG TCA A			
		Murray Encephalitis	EnM_gI_F	TTA CCT ACA CTG ATC TAG TGC G	NS1/NS2a	176	Cell culture strain 3329
			EnM_gI_R	CCA GGA CGA GAT TCT CTT GAT T			
			EnM_gI_P	AGT CCA TCT ACT GCG TGT CAA GCT GG			
			EnM_gII_F	ATG TGC GAT GAC ACC ATC ACT T	prM	117	
			EnM_gII_R	TCG TGC ATC TTC CAT AGT TCA C			
			EnM_gII_P	ACG AAT GTC CGA AAT TGG AAA GTG GAA ACG A			
			EnM_gIII_F	ACG ATG AGT CCA CTC TGG TAA	NS1	114	
			EnM_gIII_R	TTC CTC AAG ACC TCC TGG GT			
			EnM_gIII_P	AG TCA AGG GTC CAA GCA TTC AAT GGA GAC A			
			EnM_gIV_F	TTG CGA GCA GTA CAC TCA AAC T	E	95	
			EnM_gIV_R	TGC ACA TCC CAT AAG TAG TTC C			

			EnM_gIV_P	CAC CTC AGG CCA TCT CAA GTG CCG			
			EnM_gV_F	GGA AGA ATG GTG ACG GCT AAT	E	127	
			EnM_gV_R	TGA TTG ATC TGC TTG TCT CCC			
			EnM_gV_P	CTG CCT ACC ACG ATG TAT GAG TCT CC			
		Rocio	RocioV_F	GAA AGG AAG CCT GCA GAC CT	E	72	Cell culture strain 5P H34 675
			RocioV_R	TCA CGC TGG ATG GTC ATT CC			
			RocioV_P	ATA GCT TTC TGC GTG CAT CCA AAT TTA ACG C			
		Saint Louis Encephalitis	StLouisEV_F	TGCTGATGTTGATTGCCCCG	prM	125	Cell culture strain MSI-7
			StLouisEV-R	GTGACACAGCTTCCTCCTTC			
			StLouisEV-P	AGTACCAAGTCAATCCATGTTGCCCCG			
		Spondweni	Spond_I_F	CTG GTT AAG CAG AGA GAA CTC	NS5	156	Cell culture strain SM-6 V-1s
			Spond_I_R	TTC TAG GTC GCA TTT CGT GAT G			
			Spond_I_P	TGG AGG GGG AGT TGA GGG CTT GG			
			Spond_II_F	AGG AGT GTG AAT GCC ACC AG	NS5	190	
			Spond_II_R	TGC CAA GTG GAT CCA TAT TCC T			
			Spond_II_P	CCA GCT GCT CAT GCA CAG AAT GGA CAT			
		Usutu	UV_F	CAC GCA ACA TGG GAA AAA CC	prM	96	Cell culture strain SAAR-1776
			UV_R	GCA TCC AGT TTG GGG CAT TC			
			UV_P	TGC TGG ATT AGA GCC ATG GAT GTC GGG TA			
		Wesselsbron	WessV-F	GTG TCT CCT GGA AAT GGA TGG	NS5	79	Cell culture strain SAH-177
			WessV-R	CAT CAA CAG CCA CAT TTG CG			
			WessV_P	ATG ATC AGA GAA ACG GCC TGC CTC AGT			

		West Nile	WN_F	AAG TTG AGT AGA CGG TGC TGC	3'NTR	92	Cell culture strains UG956 D117 + IS98 + MRM16
			WN_R	AGA CGG TTC TGA GGG CTT AC			
			WN_P	CGA CTC AAC CCC AGG AGG ACT GG			
			WN_1A_F	GTT GGC TCT CTT GGC GTT CT	C	194	Cell culture strain IS98
			WN_1A_R	GCA ATT CCG GTC TTT CCT CC			
			WN_1A_P	TCA GGT TCA CAG CAA TTG CTC CGA CC			
			WN_1B_F	GAA GTT AGC AGT CTA CGT TAG G	prM	194	Cell culture strain Kunjin MRM16
			WN_1B_P	TAT GGA AGA TGC ACC AAG ACA CGA CAC TC			
			WN_1B_R	GCA TAT CCA GGG TTT CTC AAG			
			WN_1C_F	TCA TGG TTG CGA CGT TCG TG	NS2a	188	NA
			WN_1C_P	AAG GCT AGG TGG ACG AAC CAG GAG AA			
			WN_1C_R	AAG TGT TGG TAA ACG TGA TGG C			
			WN_3_F	ATT TGA AGA ACC ACA TGC CAC G	E	195	Cell culture strain Rabensburg
			WN_3_P	AAG CAA TCG GTG GTC GCC TTA GGT TCT			
			WN_3_R	TGC GCA TAC TCC ATA GGT CG			
			WN_4_F	GAT TGT GAA CCC AGG TCA GG	E	137	NA
			WN_4_P	CGT TGA TGT GGA CGC CTT CTA CGT GAT			
			WN_4_R	TGT TCC TCC AGT TCG TGT TTC			
			WN_2.1_F	GAG CTG TTT CTT AGC ACG AAG	C	137	Cell culture strain UG956 D117
			WN_2.1_P	ATC TCG ATG TCT AAG AAA CCA GGA GGG C			
			WN_2.1_R	CAG ACT CAG CAT AGC CCT CT			
			WN_2.2_F	CAT GGA GAA AGT ACA CTG GCT A	prM	183	

			WN_2.2_P	ATA AGA AAG GAG CTT GGC TGG ACA GCA C			
			WN_2.2_R	GCA GTA GGA TAG CGA ACA CG			
		Yellow fever	YF_F	CTG TCC CAA TCT CAG TCC AAG	prM	69	Cell culture strain 17D
			YF_R	AAC GTT TTC CAC CCC ATA GC			
			YF_P	AGA GGA GCC AGA TGA CAT TGA TTG CTG GT			
			FJ_AO_4_F	TGA TGA AGT GCT GAT TGA GGT G	E	142	
			FJ_AO_4_R	TCC GCG CCT TTC ATG GTC T			
			FJ_AO_4_P	AAC CCA CCC TTT GGA GAT AGC TAC ATC AT			
			FJ_AO_3_6_F	AGA CCC GGC AAG AAA AAT GGA	M	169	
			FJ_AO_3_6_R	AGC CAA GAC CAG TAG GGC AA			
			FJ_AO_3_6_P	TGA CTG GAA GAA TGG GTG AAA GGC AAC TC			
			FJ_AO_1_F	CCA TGA GCT TGT TTG AGG TTG A	E	185	
			FJ_AO_1_R	GCA TTC CAG AGT AGC TTT TCC A			
			FJ_AO_1_P	CCA GAC AAA GAT CCA GTA CGT CAT CAG AG			
			FJ_AC_AE_F	TGG TCG AAA AGC TCA GGG TAA	C	116	
			FJ_AC_AE_R	GAA GGG CCA GGT CTG TTT C			
			FJ_AC_AE_P	AAC CCT GGG CGT CAA TAT GGT AAG ACG			
			FJ_AmS_2_F	CAC ATT CCA GGA TAC AAG GTC	NS1	158	
			FJ_AmS_2_R	AAT GAT CTT CCC ACT GTC GGT			
			FJ_AmS_2_P	CAG ACA AAT GGG CCT TGG ATG CAG GT			
			FJ_AmS_1_F	AGC TGA GAT GGG AGC CAA TC	NS3	170	
			FJ_AmS_1_R	TCT CTG TTA GGG TTC CTT CCA A			

			FJ_AmS_1_P	TCT GCG TGG AGA GAG TGT TGG ATT GTA G			
		Zika	Zika2_F	AAT GAC ACA TGG AGG CTG AAG	NS1	70	Cell culture strain MR766
			Zika2_R	TGT GTG AGA CTT TGG CCA TTC			
			Zika2_P	AGG GCC CAC CTG ATT GAG ATG AAA ACA TGT			
			Zika_III_F	TGG CAG TGC TGG TAG CTA TG	NS1/NS2a	78	
			Zika_III_R	GTG GCA CCC ATC AAA ATT GCA			
			Zika_III_P	AGC TTA GCC AGG TCA CTC ATT GAA AAT CCT			
			Zika_IV_F	CCA ACT GGG AGA ACC ACC T	NS5	102	
			Zika_IV_R	GGT CGT TCT CCT CAA TCC AC			
			Zika_IV_P	ACT CTA TTC CAC ACC ATG AGC ATG TCC TC			
			Zika_VI_F	GCA TCA GGT GCA TAG GAG TC	prM/E	194	
			Zika_VI_R	TTG ATG CCT CAT AGC AGT AGG A			
			Zika_VI_P	AGC AAT AGG GAC TTT GTG GAA GGT ATG TCA			
			Zika_VII_F	GCA ATC AAG CCA TCA CTG GG	C	138	
			Zika_VII_R	GTC TCT TCT TCT CCT TCC TAG			
			Zika_VII_P	CAT TGA TTA TTC TCA GCA TGG CAG CCA GAT C			
			Zika_VIII_F	AAG ATC CTA CTG CTA TGA GGC A	E	198	
			Zika_VIII_F	GGA GCA TGC AAA CTT AGC GC			
			Zika_VIII_F	TCA ATA TCA GAC ATG GCT TCG GAC AGC C			
Phenuiviridae	Phlebovirus	Rift Valley fever	RVFV_F	ACAAAAAGCGGGTGGGGATAG	Nsm/Gn	82	Cell culture strain ZH548
			RVFV_R	GCAATCCCTGCCATGGTTTC			
			RVFV_P	CGGTGTGAGAGACGAAGAGACGCTAAG			

			RVF_SegS_F	GTT GAT TTG CAG AGT GGT CGT	NSs	134	
			RVF_SegS_R	GCG AAC CTC GTG ACT AGG A			
			RVF_SegS_P	CGA TGG TGC ATG AGA AAG ACA CAA CAG G			
Peribunyaviridae	Orthobunyavirus	Batai	batai_1_F	GAA TGG GAG GTT ACG CTT AAC	N	134	NA
			batai_1_R	GTA CCT GGC AAG GAA TCC AC			
			batai_1_P	CTT GGG GGC TGG AAG GTT ACT GTA TTT AAT A			
			batai_2_F	GGG CAG ATG GTG AGG AGA T	N	188	
			batai_2_R	TGC TAA CCG TCC ATG TCC CT			
			batai_2_P	TTA CCT CTC ATT CTT CCC AGG CTC GGA			
		Bunyamwera	Bunyam_1_F	CGG TAC CTA CTT GAG AAG ATT C	N	121	NA
			Bunyam_1_R	TAC ACC TCT TCT CCA TCT GAC			
			Bunyam_1_P	TGA AAG TGA GTG AAC CAG AAA AGC TGA TCA TC			
			Bunyam_2_F	TTG TTA CAG CCG GTG GTA GTA	Gn	192	
			Bunyam_2_R	GAC TTA AAC CAG CCA GTG CTA A			
			Bunyam_2_P	TGG TGG TTC CTG TCA CTT CAA AAT GGT TCA			
			Bunyam_3_F	ACA TCA CTC TTC GGT GCA GG	NSm	200	
			Bunyam_3_R	TGG ATC CTC TAG CAG CCC A			
			Bunyam_3_P	TCT GAC CAT CAT TTT TGC AGG AGT AGC ATT G			
		Bwamba	Bwa_S_F	TAT CCA GGG GCA CTC AAT ACA A	N	124	Cell culture strain BWA
			Bwa_S_R	TCC ACC TCC CAG TTT CCA AAT T			
			Bwa_S_P	ATA CCG CTA GGA CAT TCT TCC TCA ATG CC			
		Cache Valley	CacheV_S_F	GAT GGT CTT ACC CTC CAC AG	N	194	NA

			CacheV_S_R	TAA GAA CAT CTC TGA GCC AGG			
			CacheV_S_P	ACT CAG TGG ATA CCT TGC CAG GTA CCT A			
		California Encephalitis (Snowshare, Chatanga, La Crosse viruses)	CalifEV_1_F	GCA TCA ACA GGT GCA AAT GGA T	N	172	NA
			CalifEV_1_R	TCG CCA AAT TTA GGA CTT GCC			
			CalifEV_1_P	TTG ATC CTG ATG AAG GGT ATA TGG CAT TCT G			
			CalifEV_2_F	GGC AGA GGT ATG GTT CAC TAA	N	186	
			CalifEV_2_R	GTC CTA AAC AAT TTG CCT GCC			
			CalifEV_2_P	CTG CTG AAA AGT GGA TGT CCC AAA AGA CC			
		C Groupe_Apeu	GpC_Ape_F	CCC AAA CTA CCA AGA GTG TAA C	G2	102	NA
			GpC_Ape_R	GCA AGC ATA CGT CTT TGG GAT A			
			GpC_Ape_P	AGC ATA AGG TCT CGG TCG GGC TTG AAA			
		C Groupe_Restan	GpC_Rest_F	GTA AGA GCA GAG GAT CAG CAT T	G2	175	
			GpC_Rest_R	TAT ACT CCT GAC ATT GCC CAG A			
			GpC_Rest_P	TTG CCT TGC AGT AAT TCT AGC CAC ATT ACT C			
		C Groupe_Murutucu	GpC_Muru_F	CTA GGA TTC TCT GCA AGA GTA G	G3	110	
			GpC_Muru_R	TGT TGT ATG ACA GCT GCA TTG C			
			GpC_Muru_P	AGG TTC TGC ATT TTG CCT CGC TGT GAT ATT A			
		C Groupe_Oriboca	GpC_Oribo_F	CTA GAA CTG TAT GCT GAC ACA G	N	198	
			GpC_Oribo_R	TCT CTC CCT TTT GAG CTC TGT A			
			GpC_Oribo_P	AAC ACC GCC CAG AGA TTG AAG AGA AAA TCA			
		C Groupe_Marituba and Apeu	GpC_Mari_F	GTT CTT CCT CCG TGC GAA TG	NSs	140	
			GpC_Mari_R	CCA TCA GCG ACC GTA TTT GC			

			GpC_Mari_P	AGG CTA AAC AGA AGC TCC GTA AGA GTT CG			
		C Groupe_Nepuyo	GpC_Nepu_F	GAG TGC ATC TAC TTT TGA CCC TA	NSs	147	
			GpC_Nepu_R	AGC GAC CTT TGC CGT ACT C			
			GpC_Nepu_P	AA CAG GCG TAC CAG AGT TTT ATC GAT AAC C			
		C Groupe_Itaqui	GpC_Itaq_F	AT TGG TCT GAG TGT AAC CCT G	N / NSs	194	
			GpC_Itaq_R	CTG TTG TCG TAC CCA TAA CCT			
			GpC_Itaq_P	TGG AGA CAG CCA ACG GCC CGA TC			
		C Groupe_Caraparu	GpC_Cara_I_F	TTC TGC CGT CGT ACG AGT CT	N / NSs	189	
			GpC_Cara_I_R	GTA ACC TGA TAT GCG ATG CAA C			
			GpC_Cara_I_P	TCT ACA TCA ACG CGG CGA AGG TCA AAG			
			GpC_Cara_II_F	TGA GAG TGA CTA CGG TTC CC	N / NSs	187	
			GpC_Cara_II_R	CGT AGT CAG GCA GTG GAA TG			
			GpC_Cara_II_P	AAT TGG AGT CCG CTA TTG TTC GAG TCT TCT A			
		Germiston	Germi_segS_F	GGG CCA GAA GTC TAC CTG T	N	196	NA
			Germi_segS_R	TCC ACT TCT CCA AGC TTA GTT G			
			Germi_segS_P	CCT TCT TCC CAG GTG CTG AAA TGT TCC T			
		Guama Group_Catu	Guama_I_F	TCA GAG AGG ATG AAG AAG CAC	G2	105	NA
			Guama_I_R	ACA GGA TGA AAG AAG AGC CTC			
			Guama_I_P	AGA GAG TCT GGA ATG TGC CAC GGA TAT AAA T			
		Guama Group_Catu and Guama	Guama_II_F	GTC GCA CAA GGG AGA AAT AAC	N	154	
			Guama_II_R	GCT GTA CCA AAC TTA AGA GTG G			
			Guama_II_P	TTC ATC CCT AGT GAG GCT TAC GCT GTC			

		Guama Group_Bimiti	Guama_III_F	ACT TCA GAA TGC TCC CAT TGG	N	173	
			Guama_III_R	GGA GCT TAG ACA CTA GTG TCA			
			Guama_III_P	CAA TTG GGA TTT ACA GAG TAC AGC AGA AAC AG			
		Guama Group_Moju	Guama_IV_F	AGT TCA GAA TGC TTC CCC TAG	N	102	
			Guama_IV_R	ATA TCG CCA TAC TGC TGT CTG A			
			Guama_IV_P	CAA TAG GCA TTT ACA GAG TCC AGC AGA AAC A			
		Guama Group_Mahogany Hammock	Guama_V_F	GGA TCA CCT GGG AAC ATG GT	N	196	
			Guama_V_R	CAG GAC CAT CCA TTG GCT TG			
			Guama_V_P	CTG GCA TGT CTC CAT ACT GCT GGC G			
		Guaroa	Guaroa_S1_F	GTT TAA CCC GGA GCT CCA ATA	N	217	NA
			Guaroa_S1_R	AAT CAT CGA GGA CTG GAC TGT T			
			Guaroa_S1_P	TGC TAC ATT TAA ACG TAC AAA CAC AAC AGG GC			
			Guaroa_S2_F	TAT AGC TGC ATC AAA CGG GAT C	N	146	
			Guaroa_S2_R	GCC TCC ATC ATC TTT TTC TGG			
			Guaroa_S2_P	ACA TGG GAA GAT GGA CCA GAG GTT TAT CT			
			Guaroa_S3_F	GGA GAC TTT CAA ATT CTA CCC C	N	185	
			Guaroa_S3_R	ATT AGC TTT CTT CCA GCC CAG			
			Guaroa_S3_P	TGA TGA GAC AAC AGA AAG AGC AGC ATT GAT C			
		Ilesha	Ile_L_F	GCA CAT GGC GAT TTT ACA CTG A	RdRp	108	Cell culture strain ILEP1
			Ile_L_R	TCT TGG TGG CAT AGA ACT GAT G			
			Ile_L_P	CTG CAC CAT GGT GCA CAA CAG AGA CT			
		Inkoo	Inkoo_S_F	TTG ACG TAG ACC TAC TGA TGG	RdRp	149	NA

			Inkoo_S_R	GTG GGA TCT CTA ATG TCA ATG G			
			Inkoo_S_P	CAA GAC ATG ACT ACT TCG GGA GAG AGT TG			
		Jamestown Canyon	Jamestown_F	AAG CCA AAG CTG CTC TCG CT	N	166	NA
			Jamestown_R	AAC CCA TCT GGC TAG ATA TCC			
			Jamestown_P	CGT AAA CCG GAG CGG AAA GCT ACT C			
		Keystone	Key_segS_F	AGG GTA TGT GGC ATT TAT GGC	N	120	NA
			Key_segS_R	CCA CTC TCC AAA CTT AGG TGT A			
			Key_segS_P	TAA CCA TGG GGA GTC GAT CAG TCT GTC			
		La Crosse	LaCrosse_1_F	GCT GCA AGC CCA GTG TAT CA	Gn	196	NA
			LaCrosse_1_R	TGC CAA TCA GAG ACT AGC CAT			
			LaCrosse_1_P	AAG GTG TTT CCA AGA TGG GGC TAT AGT GAA G			
			LaCrosse_2_F	TTC CAA GAT GGG GCT ATA GTG	Gn	175	
			LaCrosse_2_R	AGT CGT GCC AAT CAG AGA CC			
			LaCrosse_2_P	AAG CAA AAC CCA TCC AAA GAG GCA GTC AC			
			LaCrosse_3_F	AAT CAG AGG TGC CTG CAT TAG	Gc	185	
			LaCrosse_3_R	GGC CAG TAC ACA ATT CAT CAT G			
			LaCrosse_3_P	CTG GGA CAT CTA TCG GGT TCA AAA TCA ATT C			
			LaCrosse_4_F	CCA TTC ACA GAG TGT GGC AC	Gn	134	
			LaCrosse_4_R	CGA CTT GCA CAT GAC TCT GG			
			LaCrosse_4_P	ACA TTG TGT CTG TGG TGC TCG CTA TGA TAC			
			LaCrosse_5_F	TGT CTG TGG TGC TCG CTA TG	Gn/Nsm	104	
			LaCrosse_5_R	TTG CAC ATG ACT CTG GCA GC			

			LaCrosse_5_P	ATA CTT CCG ATA GAA TGA AAC TGC ACA GAG C			
			LaCrosse_6_F	GCC CAG TGT ATC AAA GGT GTT T	Gn	97	
			LaCrosse_6_R	GCT AAC ATC ATC TTT CAG GCA C			
			LaCrosse_6_P	CCA AGA TGG GGC TAT AGT GAA GCA AAA CC			
			LaCrosse_7_F	CTC ACA TTT GCA AGA GAG AGG	Gc	123	
			LaCrosse_7_R	CAC TTC TTC GGT CTC CTT CC			
			LaCrosse_7_P	ACA AGT TCA TGG GGA TGC GAA GAG TTT GG			
			LaCrosse_8_F	ATC CAA AGA GGC AGT CAC GG	Gn	199	
			LaCrosse_8_R	GAT CAT CAC CAA CCT CTA TCA C			
			LaCrosse_8_P	AGG TGT GCC TAA AGG ATG ATG TCA GTA TGA T			
		Ngari	Ngari_segS_F	TTC ATG ATG TCG CTG CTA ACA C	N	79	NA
			Ngari_segS_R	CCC AAG GTT AAG TGT AAC TTC C			
			Ngari_segS_P	CAG CAG TAC TTT TGA CCC AGA GGT CG			
		Nyando	Nyando_1_F	CGC TCG GAT CTT CTT CCTCA	N	154	NA
			Nyando_1_R	TGT AAG ATC CGT GTC GCT GAT A			
			Nyando_1_P	ATG CCC GGA AAG CCA AAG ATC AAC TCT CT			
			Nyando_2_F	CAG AGC CGA AGG TTG GTC TT	N	182	
			Nyando_2_R	TGC CAG CTT GTG CAA CAC TAT T			
			Nyando_3_P	AAA TTT GGA ACA TGG CAG GTG GAA GTG GTC			
		Oropouche	Oropou_F	GTG GTA ACC TCT TCA AGG AGA	Gn	181	NA
			Oropou_R	TAG CAC TGG ATT GCA CTC AGA			
			Oropou_P	TGA ACT TGA GTG TAG GAC TTG GCG AAA TAT G			

		Snowshoe hare	Snow_segS_F	GAC GAT CTT ACC ATC CAC AGA	N	151	NA
			Snow_segS_R	CCG CTA TCC CAT CTC ACT C			
			Snow_segS_P	TTG TCA GGA TAT TTA GCC AGA TGG GTT CTT G			
		Tacaiuma	Tacaiuma_F	CCA TTA GCT GAG GTT GCT GG	N	139	NA
			Tacaiuma_R	TCC TTT TTC ACC CTA GCT ATG C			
			Tacaiuma_P	TGT CTC ATG GGC TAA TTC TAC GCC AGA AAT			
		Tahyna	Tahy_S_F	ATT TGG CTA GAT GGG TGC TAG	N	156	Cell culture strain 92
			Tahy_S_R	GTC CCA CCT GAT CCC ATT AG			
			Tahy_S_P	ACT CAG CTA TGG GGT TGA TAA CGG TTG TT			
		Trivattatus	Trivatta_F	CCA TCA ACA GGT GCA AAC GG	N	199	NA
			Trivatta_R	TGA TTA TTG ACC ACC TCC ACC			
			Trivatta_P	ATT TGA TCC CGA TGC AGG GTA TGT GGC			
Reoviridae	Orbivirus	Orungo	Orungo_1_F	GTG ATT CGG GCA AAC TGC GT	RdRp	171	NA
			Orungo_1_R	GAA CTC GCA TAA TTG CGC AGA A			
			Orungo_1_P	TTT AAA GGT ATG CAA GTT GTG GTA GAG TCG AC			
	Seadornavirus	Banna	BannaV_F	ATCCGGTGTCGTCACTTGG	VP7	110	Cell culture strain BAV
			BannaV_R	TCAAAGCCCACACACTCAGTG			
			BannaV_P	ACCGTGACTACACCATGCCACTACCAAATG			
Rhabdoviridae	Vesiculovirus	Jurona	Jurona_F	AGT TGA TGA TTA CAG AGG ACC C	N	189	NA
			Jurona_R	TTC TGC CTG ATT GCT TCT AAG C			
			Jurona_P	ATA CCG GAT GGC AAG TCA AGC AGT GG			
Togaviridae	Alphavirus	Barmah Forest	Barmah_F	GTG CCC AGG TCC GAA GTT A	E2	110	NA

			Barmah_R	TCT GTA TGT CAC TTG CGG TTC A			
			Barmah_P	CGG AGG TGA AAG GAA AGA TCC ATG TGC			
		Chikungunya	chik_F_SG	TGG AAT GGC TGG TTA ACA AGA TAA	NSP2	114	Cell culture strain LR2006_OPY
			chik_R_SG	CTC CGC GGA CAC CTA ACG			
			chik-P2	ACG GCC ACC ACG TGC TCC TGG T			
			Chik_WAfri_F	CGA ACT ACA TAT CCG GCA CC	NSP4	129	
			Chik_WAfri_R	TGA TTT GGT ACG ACG CAA CTG T			
			Chik_WAfri_P	AGT GTA CTC ACC CCC AAT CAA TAT CCG AC			
			Chik_Asia_F	TGA TCA AAT GAC CGG CAT CCT	NSP1	104	
			Chik_Asia_R	CGT TGC GTT CTG CCG TTA AC			
			Chik_Asia_P	TGC TAC AGA AGT CAC GCC GGA GGA TG			
			Chik_IndECSA_F	AGG AAG TCC ACG AGG AGA AG	NSP3/NSP4	158	
			Chik_IndECSA_R	TTA GTC TCT GGA TGA TTG CTG C			
			Chik_IndECSA_P	TGT TAC CCA CCT AAG CTG GAT GAA GCA AAG			
			Chik_ECSA_F	ACA CAA CCC CGT TCA TGT ACA	NSP1	191	
			Chik_ECSA_R	CTA CTG AGA ACA GCA CAC GG			
			Chik_ECSA_P	ATG CCA TGG CGG GTG CCT ACC C			
		Eastern Equine Encephalitis	EEEV_F	GAA GTG GCC TGG AGC TTT TG	NSP3	83	Cell culture strain H178/99
			EEEV_R	GCA TGG ATG ACG TTC GGA GA			
			EEEV_P	ATG CTT GAC GAG GTG CGC TTT ACC AGT			
			EEEV_1_F	TGT ATT GCC TCT AAG GCC GC	NSP1	184	
			EEEV_1_R	GTT CGT ACA CCT TTC AGC GC			

			EEEV_1_P	CTG GTA GTA AAT GGA AGT CGG TGC ATG CA			
			EEEV_2_F	GTT CAT GTT GAC TTA GAC GCA G	NSP1	149	
			EEEV_2_R	ACC TGG TCT GTA TCC ACT TCT			
			EEEV_2_P	ACA GCC CAT TCG TCA AGT CAC TGC AAA G			
		Mayaro	Maya_I_F	ATG AGC GAA GCC TAC GTG G	E1	162	Cell culture strain TC625
			Maya_I_R	ATC GTT ACG GCA TGG TCA CC			
			Maya_I_P	AGC GCG CTG ACG TGT GTA AAC ACG A			
			Maya_II_F	CAG CCG GCA ATA TCC ACG T	E1	194	
			Maya_II_R	GTC TAG TGA ATG CGC TGT CG			
			Maya_II_P	CCC CTA TAC CCA GAC ACC ATC TGG TT			
		O'Nyong-Nyong	ONN-9237F	ACA AGT GCA AAT GTG ACG GC	E2	125	Cell culture strain Dakar 234
			ONN-9362R	GCG GTG AAT TGT ATT GCC ATT T			
			ONN-9307P	GTT TGT AAC CGC TGT GTG GCA TTG GTC T			
		Ross River	RRV - 9,239 F	CAA TGC CAT GCT GCC GTT AC	E2	72	Cell culture strain 5281v
			RRV - 9,328 R	TTT GCC CCT CCT AGC TGT C			
			RRV - 9307 P	ATC AGC CCT GGG AAC AAA TGG AGA GGT			
		Semliki forest	SemFV-9240F	CGT CAT CCA CGG CAA AAG AG	E2	72	Cell culture strain 1745
			SemFV-9310R	TGC GGT AGG AAA AGA GCG T			
			SemFV-9261P	TGACACTGCACCTTCACCCAGATCATC			
			Seml_II_F	AAC AGC TGA AGA CAA GCA GGA	NSP4	179	
			Seml_II_R	AAC GCC TTA ATG TCC CTC GC			
			Seml_II_P	CGA AGA CAG GCG ACG AGC ACT GAG			

		Sindbis	SinFP	GGT TCC TAC CAC AGC GAC G	NSP1	75	Cell culture strain Egypt 339
			SinRP	TGA TAC TGG TGC TCG GAA AAC			
			SinP	TTG GAC ATA GGC AGC GCA CCG GCT			
			Sindbis_I_F	GAA GGT AGA CGC CTA CGA AC	E1	152	
			Sindbis_I_R	ATG TAC TCT TGG TTG GTG GAA G			
			Sindbis_I_P	ATG CGA CCA CTG TTC CAA ATG TGC CAC			
			Sindbis_2_3_F	GGT AGA CGC CTT CGA ACA TG	6k	152	
			Sindbis_2_3_R	GTG ACG TAC TCC AGG TTC GT			
			Sindbis_2_3_P	CGA CCA CTG TCC CAA ATG TGC CGA G			
			Sindbis_IV_F	AAT TCG AGG TAG TAG CAC AGC	NSP1	176	
			Sindbis_IV_R	GCA GAC GCA GTG ATA GTG GT			
			Sindbis_IV_P	AGG CCA CAC CAA ATG ACC ATG CTA ATG C			
			Sindbis_V_F	GTA GGA ATT AGG AAC ACT CTC G	NSP4	162	
			Sindbis_V_R	CTG ACT ATT TAG GAC CGC CG			
			Sindbis_V_P	CAG TTG CCG TAT CGA CCA GGT ACG A			
			Sindbis_VI_F	TAA CAG TGA AGA CGT GGT CAC	NSP2	198	
			Sindbis_VI_R	CTC CGT CTC TTG TTC CTT CAT A			
			Sindbis_VI_P	CGC TCT GGC CAG AAA GTT CGT CCG			
		Una	Una_1_F	ACG TGC ACA GCA TCG TGC A	E1	155	NA
			Una_1_R	TAG TGC AAC TGC TGT CAG CG			
			Una_1_P	CGC CAC CAA AGG ACC ACA TCG TAC C			
			Una_2_F	CCG CAG TAA GTG CCT TAC AC	E1	187	

			Una_2_R	TGA TTA ATA GCA CTG CGA CCG			
			Una_2_P	CAT ATG CAC TCA CAC CAG GCG CAG T			
			Una_3_F	CGT ATG CTC TCA CAC CAG G	E1	184	
			Una_3_R	ACG GAG GCA GTA CGA AAT GAT			
			Una_3_P	TGC GGT GAT TCC CAT GAC AGT TGG ACT			
			Una_4_F	CAC GAT CAT GCG GCT GCTT	E1	139	
			Una_4_R	ATG AAT CGG GTC CCT GCG A			
			Una_4_P	ATA AGG CAC ATA CCG CTT CAA TGA AGG CC			
		Venezuelan Equine Encephalitis	VEEV -5306 F	CATCCTTGACACCCTGGAGG	NSP3	89	Cell culture strain TC83 vaccine
			VEEV -5394 R	GAAACTCCATGCTCCTTGCG			
			VEEV - 5345 P	AAGTAAGAGTTAGTCTCGGCTGACGCTG			
			VEEV_1_F	GCT ACG ACC GCA AAC CAA C	E3/E2	192	
			VEEV_1_R	CAG CTT CCT ACA GCA CAC CT			
			VEEV_1_P	TGA AAC CTT GGC TAT GCT CAG CGC CA			
			VEEV_2_F	GTT ATG ACG AGT TGC TCG AAG	E3/E2	176	
			VEEV_2_R	CAT GCC CTT CGC TCC TTA C			
			VEEV_2_P	CAG TAC TGA AAT GTC CAG GCA GAG GCA A			
			VEEV_3_F	GCT GCC ATA GTC CAA TAG CAA	E2	186	
			VEEV_3_R	CGA GAT GTG TGG AGT GAC AC			
			VEEV_3_P	T TGA GGC AGT GAA GAG CGA CGG GC			
			VEEV_4_F	ACG GAG TAG AGC AAG CGT G	E2	172	
			VEEV_4_R	ACA CTC GCA TTC CAC CAG G			

			VEEV_4_P	CCA AGT CTA CGC ACA TGA TGC ACA GAA C			
			VEEV_5_F	TTT GCT ATG ATC GGA AAC CAG C	C/E3	164	
			VEEV_5_R	AAC TCC CAA CGG CAC ACC T			
			VEEV_5_P	AGA GAC GCT GGC CAT GCT CAG TGC			
			VEEV_6_F	CCC TTA CAT GGC CAG ATG CA	E3/E2	199	
			VEEV_6_R	GTA GCG GTA TCT CTT CAA TGG T			
			VEEV_6_P	TCA GAT GTG CCG TTG GGA GCT GCC			
			VEEV_7_F	AAA CTT ACA CGT CCG TAC ATG G	E3	173	
			VEEV_7_R	CCT CAT AAC TCT GCT CTT AAC G			
			VEEV_7_P	CCA AGT GTG TGC GGT GTG CCG TTG			
			VEEV_8_F	TTG AAG CCG TAT TGA AGT GTC C	E3/E2	100	
			VEEV_8_R	TCG GAC GCA CTT TGC CAT GT			
			VEEV_8_P	AGG TAG GCA GAA GAG ATC CAC GGA AG			
			VEEV_9_F	GGA CTC ATC CGG AAA CTT GAA	E3/E2	147	
			VEEV_9_R	GCA ACG AGC TAG GAG GAA GT			
			VEEV_9_P	AGG AAG AAC AAT GAG GTA CGA CGT GCA AG			
			VEEV_10_F	GCT GTT AGA TGG AGT ACT GAG A	E3/E2	182	
			VEEV_10_R	AAG CGC ACG TAC CCA TCA TG			
			VEEV_10_P	TGT CAA GGG AGG TCC AAG AGG TCC C			
			VEEV_11_F	ATA TAA GCT TAC CAC GCC GTA C	E2	169	
			VEEV_11_R	ATT GCC TGA TGA ATC TAG TCC G			
			VEEV_11_P	ATG GCC AGG TGC TCC AGA TGT GCA G			

			VEEV_12_F	GAT CCA CTG ATG AGC TGT TCA	E3/E2	185	
			VEEV_12_R	TGA CGC TTC CCG ATG GAT C			
			VEEV_12_P	AAG AGT ACA AGC TCA CAC GGC CAT ATA TG			
			VEEV_13_F	GGG ACT CAT TGA CCA TGG AG	E2	190	
			VEEV_13_R	AGG GAG GTG CAT TTC GAC GT			
			VEEV_13_P	TTC AAG AAA GAT ACA GTA ACG CAC TCA TGC TC			
		Western Equine Encephalitis	WEEV- 4,164 F	GGACGGCTAGACTTGTGAAG	NSP3	115	Cell culture strain 47a
			WEEV - 4,278 R	ATGCTCATGTAGGCAGCTGC			
			WEEV - 4,184 P	CACGAACCGCTCATCATACATGCTGTA			
			WEEV_B2_B3_F	GGA GCG TAT ATT TTC TCA TCG G	NSP3	111	
			WEEV_B2_B3_R	GGC GTA ATA CTT CTC ATG GAC			
			WEEV_B2_B3_P	AAA CAG GCC AAG GTC ACC TTC AAC AGA AAT			
			WEEV_B1_A_F	TGT TCA AGA CTT TCA GGC ACT G	NSP2	159	
			WEEV_B1_A_R	TTC TGA GTC TGT GTC CTG AGT			
			WEEV_B1_A_P	AGT GAG AGC GCC ACG ATC GTT TTC AAC			
Beta-actine			beta-actine_F	GCT ACG TCG CCC TGG ACT T	beta-actine	151	Mosquitoes
			beta-actine_R	AGG AAC GAC GGC TGG AAG A			
			beta-actine_P	AGG AAA TGG CCA CCG CTG CCT CGT			
Escherichia coli			eae_F2	CATTGATCAGGATTTTTCTGGTGATA	eae	102	Culture of EDL933 strain
			eae_R	CTCATGCGGAAATAGCCGTTA			
			eae_P	ATAGTCTCGCCAGTATTCGCCACCAATACC			

NA: not available.

Table 8. Primers used for full genome sequencing of Zika virus.

Name	Sequence (5'-3')	Sense Antisense	Primer concentrations
ZIKV- 1Sbis3	AGTTGTTGATCTGTGTGAGTCAG	Sense 1	10 µM
ZIKV-epidemic-947R	AATCAGCAGTATCATGACCAAGT	Antisense 1	10 µM
ZIKV-epidemic-889S	TTAGCAGCAGCTGCCATCGC	Sense 2	10 µM
ZIKV-epidemic-1893R	GGTACACARGGAGTATGACACG	Antisense 2	20 µM
ZIKV-epidemic-1795S	GCTGGAGCTCTGGAGGCTG	Sense 3	10 µM
ZIKV-epidemic-2753R	ATCCCACAACGACCGTCAGTT	Antisense 3	10 µM
ZIKV-epidemic-2718S	GGAGCTCAACGCAATCCTGGA	Sense 4	10 µM
ZIKV- 3844Rbis	TGTCCAATTAGCTCTGAAGATG	Antisense 4	10 µM
ZIKA_3581S_cam	AGTGCTTGTGATTCTGCTCATGGT	Sense 5	10 µM
ZIKV-epidemic-4581R	GTACCACGCTCCAGCTGCA	Antisense 5	10 µM
ZIKV-epidemic-4535S	TGGTCCTGATGACCATCTGTG	Sense 6	10 µM
ZIKV-epidemic-5610R	GGTGTCCATAATTGGTGAGTTG	Antisense 6	10 µM
ZIKV-epidemic-5432S	TACTACAGCCAATYAGAGTCC	Sense 7	20 µM
ZIKV-epidemic-6545R	CGAGGTTGTCAATGGCTTCCT	Antisense 7	10 µM
ZIKV-epidemic-6404S	CGAGGTGGATGGAYGCCAGAG	Sense 8	20 µM
ZIKA_7511R_cam	CACAAAGTGGAAGTTGCSGCTGT	Antisense 8	20 µM
GP-ZIKV-Cuba-09/2018_6760S	GAGCCAGCCAGAATTGCATG	Sense 8	10 µM
GP-ZIKV-Cuba-09/2018_7893R	CTGCCACATCCAAGATCAATG	Antisense 8	10 µM

GP-ZIKV-Cuba-09/2018_7768S	CTCAAGGACGGTGTGGCAAC	Sense 9	10 µM
GP-ZIKV-Cuba-09/2018_8871R	ATGCTGCATTGCTACGAACC	Antisense 9	10 µM
GP-ZIKV-Cuba-09/2018_8771S	CACTCGTCAGGTTATGAGCATG	Sense 10	10 µM
GP-ZIKV-Cuba-09/2018_10019R	CAGTTGGAACCCAGTCAACTG	Antisense 10	10 µM
ZIKV-7328S	ACGGCAGCTGGCATCATGAAG	Sense 9	10 µM
ZIKV-epidemic-8243R	TGCTGGTGTATGGGCACAACA	Antisense 9	10 µM
ZIKV-epidemic-8166S	AGAAGCACGGACGCTCAGAG	Sense 10	10 µM
ZIKV-epidemic-9171R	CATCCAGTGATCCTCGTTCAAG	Antisense 10	10 µM
ZIKV-epidemic-8963S	CAGTGGAAGCTGTGAACGATC	Antisense 11	10 µM
ZIKV-epidemic-10338R	GTGGATAGGTARTCCATGTAC	Antisense 11	20 µM
ZIKV-epidemic-10248S	TCTCATAGGGCACAGACCGC	Sense 12	10 µM
ZIKV-epidemic-10670R	TCCCTCTTCTGGAGATCCAC	Antisense 12	10 µM

Figure legends

Figure 1. BioMark™ dynamic array system specificity test (96.96 chip). Specificity of primers/probe sets from the Table 7 are presented into two figures 1A and 1B. Each square corresponds to a single real-time PCR reaction, where rows indicate the pathogen in the positive control and columns represent the targets of each primer/probe set. C_t values for each reaction are indicated in color; the corresponding color scale is presented in the legend on the right. The darkest shade of blue and black squares are considered as negative reactions with $C_t > 30$.

Figure 2. Screening of artificially infected mosquitoes through the BioMark™ dynamic array system developed (96.96 chip). Each square corresponds to a single real-time PCR reaction, where rows indicate the batches of mosquitoes tested and columns represent the targets of each primer/probe set. Cross indicate cross-reaction of assays. C_t values for each reaction are indicated in color; the corresponding color scale is presented in the legend on the right. The darkest shade of blue and black squares are considered as negative reactions with $C_t > 30$.

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Fig1.A.

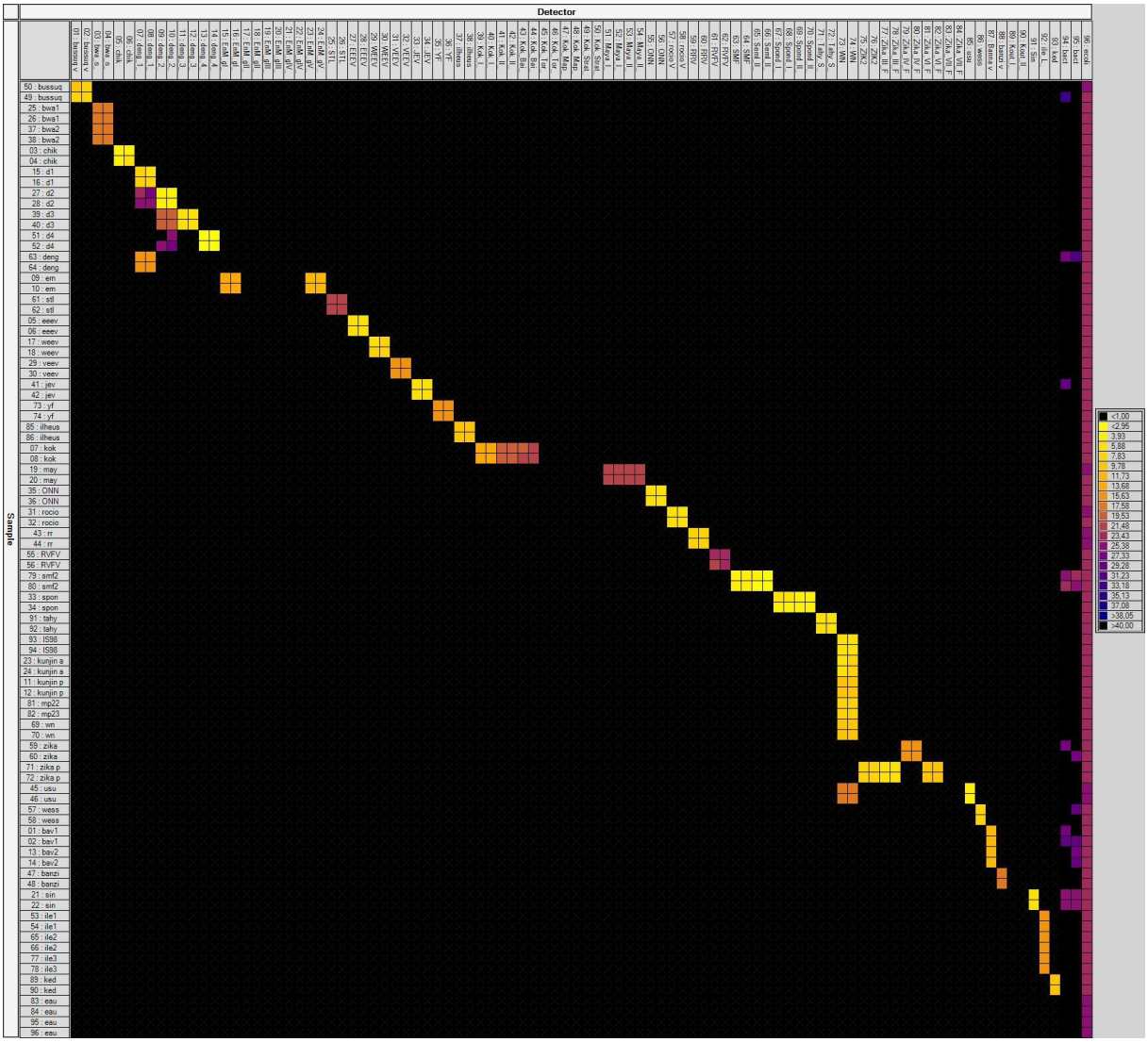

Fig 1.B.

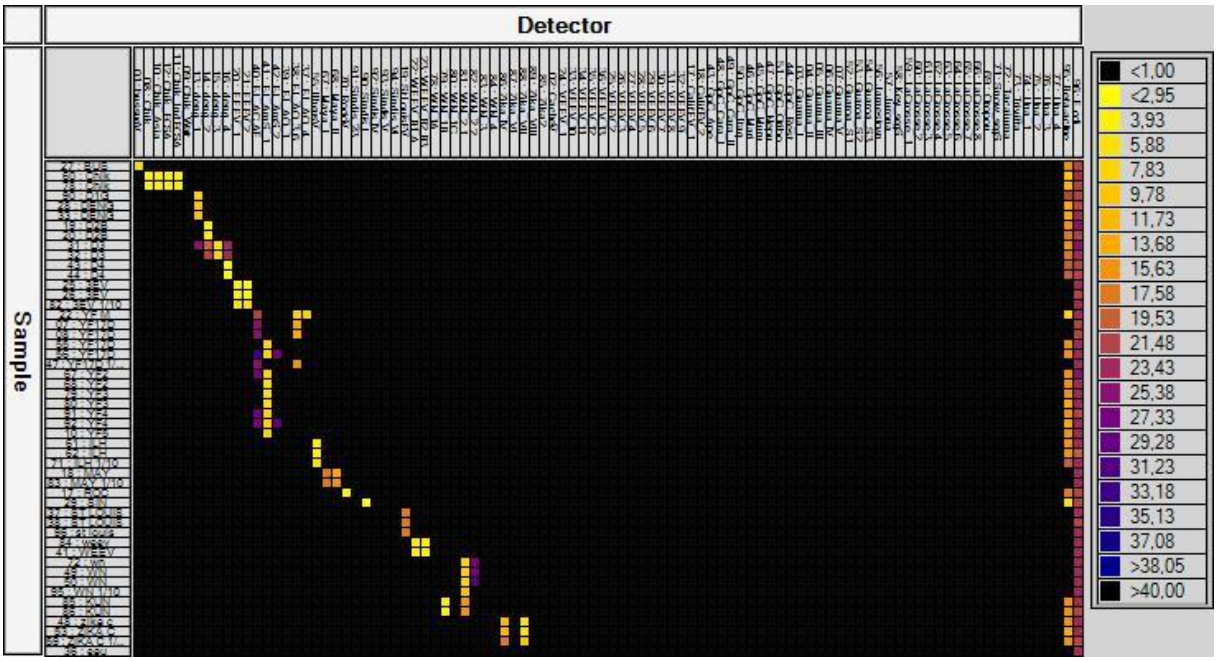

Fig.2.