

Supplemental material

Supplemental tables

Table S1: Mosquitoes collected from different region of Texas and Chicago used in this study

Pool ID	Location	Collection date	Trap type	mosquito species	sample type	# per pool
SA2016Cu#7	San Antonio, TX	6/26/16	BG	<i>Cx. quinq.</i>	Mosquito	7
SA2016Cu#8	San Antonio, TX	7/2/16	BG	<i>Cx. quinq.</i>	Mosquito	6
SA2016Aae#11	San Antonio, TX	8/22/16	BG	<i>Ae. aegypti</i>	Mosquito	13
SA2016Aae#20	San Antonio, TX	8/22/16	BG	<i>Ae. aegypti</i>	Mosquito	20
SA2016Aalbo#22	San Antonio, TX	10/15/16	BG	<i>Ae. albopictus</i>	Mosquito	16
SA2016Aalbo#23	San Antonio, TX	10/15/16	BG	<i>Ae. albopictus</i>	Mosquito	20
LRGV16-184	LRGV, TX	10/27/16	AGO	<i>Cx. quinq.</i>	Mosquito	8
LRGV16-159	LRGV, TX	10/19/16	AGO	<i>Cx. quinq.</i>	Mosquito	17

LRGV16-157	LRGV, TX	10/19/16	AGO	<i>Ae. aegypti</i>	Mosquito	6
LRGV16-166	LRGV, TX	10/24/16	AGO	<i>Ae. aegypti</i>	Mosquito	6
LRGV16-163	LRGV, TX	10/24/16	AGO	<i>Ae. aegypti</i>	Mosquito	13
LRGV16-155	LRGV, TX	10/19/16	AGO	<i>Ae. albopictus</i>	Mosquito	1
LRGV16-162	LRGV, TX	10/24/16	AGO	<i>Ae. albopictus</i>	Mosquito	1
CSCx13-259	College Station, TX	6/5/16	BG	<i>Cx. quinq.</i>	Mosquito	50
CSCx13-352	College Station, TX	6/5/16	BG	<i>Cx. quinq.</i>	Mosquito	50
CSWNV10-7163	Chicago, IL	8/13/10	BG	<i>Culex</i> sp.	Mosquito	20
CSWNV10-9531	Chicago, IL	9/2/10	BG	<i>Culex</i> sp.	Mosquito	50
CSAL-1104	College Station, TX	6/20/16	BG	<i>Ae. albopictus</i>	Mosquito	2
CSAL-1270	College Station, TX	6/26/16	BG	<i>Ae. albopictus</i>	Mosquito	3
CSAL-1781	College Station, TX	7/12/16	BG	<i>Ae. albopictus</i>	Mosquito	4
ZIKV-CTL	Colony	11/9/16	Aspirator	<i>Ae. aegypti</i>	Spiked	10
ZIKV-CTL	Colony	11/9/16	Aspirator	<i>Ae. aegypti</i>	Spiked	10

31	LRGV, TX	11/28/16	BG	<i>Ae. aegypti</i>	Mosquito	24
34	LRGV, TX	12/21/16	Aspirator	<i>Ae. aegypti</i>	Mosquito	9
16-306	LRVG, TX	11/15/16	AGO	<i>Ae. aegypti</i>	Mosquito	10
16-363	LRVG, TX	11/23/16	BG	<i>Ae. aegypti</i>	Mosquito	13
16-391	LRVG, TX	11/30/16	AGO	<i>Ae. aegypti</i>	Mosquito	9
16-462	LRGV, TX	12/7/16	AGO	<i>Ae. aegypti</i>	Mosquito	6
16-419	LRGV, TX	12/2/16	Aspirator	<i>Ae. albopictus</i>	Mosquito	2
16-555	LRGV, TX	12/20/16	AGO	<i>Culex</i> sp.	Mosquito	6
16-568	LRGV, TX	12/21/16	BG	<i>Culex</i> sp.	Mosquito	10
Ae_MG_1	LRGV, TX	10/10/17	Aspirator	<i>Ae. aegypti</i>	MG	23
Ae_SG_1	LRGV, TX	10/10/17	Aspirator	<i>Ae. aegypti</i>	SG	23
CU_MG_1	LRGV, TX	9/22/17	Aspirator	<i>Culex</i> sp.	MG	23
CU_SG_1	LRGV, TX	9/22/17	Aspirator	<i>Culex</i> sp.	SG	23
DENV-2 CTL	Colony	10/10/17	Aspirator	<i>Ae. aegypti</i>	Spiked	10

DENV-2 CTL	Colony	10/10/17	Aspirator	<i>Ae. aegypti</i>	Spiked	10
DENV-2 CTL	Colony	11/27/18	Aspirator	<i>Ae. aegypti</i>	Spiked	10
DENV-2 CTL	Colony	11/27/18	Aspirator	<i>Ae. aegypti</i>	Spiked	10
DENV-2 CTL + MAYV CTL	Colony	11/27/18	Aspirator	<i>Ae. aegypti</i>	Spiked	10
DENV-2 CTL + MAYV CTL	Colony	11/27/18	Aspirator	<i>Ae. aegypti</i>	Spiked	10
RVFV CTL	Colony	11/27/18	Aspirator	<i>Cx. qinq.</i>	Spiked	10
NEGATIVE CTL	Colony	10/10/17	Aspirator	<i>Ae. aegypti + Cx. qinq.</i>	Spiked	10+10

LRGV: Lower Rio Grande Valley, AGO: Autocidal Gravid Ovitrap, BG: Biogent, F

MG: midgut

SG: salivary gland

Cx. qinq. = *Cx. quinquefasciatus*

Table S2: Primers and probes use in the qPCR assay for LLMDA validation

Genus	Virus	Target	Primer name	Sequence 5' to 3'	Reference
Flavivirus	ZIKV	NS5	ZIKV_835F	TTGGTCATGATACTGCGATTGC	(1)
	ZIKV	NS5	ZIKV_911R	CCTCCACAAAGTCCCTATTGC	(1)
	ZIKV	NS5	ZIKV_860_probe	CGGCATACAGCATCAGGTGCATAGGAG	(1)
	WNV	ENV	WNENV_F	TCAGCGATCTCTCCACCAAAG	(2)
	WNV	ENV	WNENV_R	GGGTCAGCACGTTTGTTCATTG	(2)
	WNV	ENV	WNENV_probe	TGCCCCGACCATGGG	(2)
	CxFLAV	ENV	CxFV_E_F	CACGCCGAACGGACTTCT	(3)
	CxFLAV	ENV	CxFV_E_R	TCCATTGGCCGCATATATC	(3)
	CxFLAV	ENV	CxFV_FA_probe	TTTCG CACCGGAGCAGCCG	(3)

Table S3: Primers used for LLMDA validation of field samples using conventional PCR or qPCR assay

Classification	Specie	Target	Primer name	Sequence 5' to 3'	Product size (bp)	Reference
Virus	CFAV	E	CFAV_E F	AATGAGACCTGTTCGCTTAG	340	(4)
Flavivirus	CFAV	E	CFAV_E R	CGTTTGTCAATCAAGGCAG	340	(4)
	CxFLAV	NS5	CxFV_9131 F ^a	TTGTGGTTCTTGCTGGACCAAGTG	206	(5)
	CxFLAV	NS5	CxFV_9337 R ^a	ATTCTCCCAACCTGGTTCTTCCCA	206	(5)
Bacteria	<i>Wolbachia</i> group A	wspA	QAdir1 ^b	GGG TTG ATG TTG AAG GAG	-	(6)
<i>Wolbachia</i>	<i>Wolbachia</i> group A	wspA	QArev2 ^b	CAC CAG CTT TTA CTT GAC C	-	(6)
	<i>Wolbachia</i> group B	wspB	183F ^b	AAG GAA CCG AAG TTC ATG	-	(6)
	<i>Wolbachia</i> group B	wspB	QBrev2 ^b	AGT TGT GAG TAA AGT CCC	-	(6)

^a: to be use as a nester PCR after FU2-CFD2

^b: real-time quantitative SYBR PCR assay

Table S4: Summary of LLMDA probe hit detection

Sample	Species	Location	LLMDA detection	Score	Probes detected/observed
1	<i>Cx. quinq.</i>	San Antonio, TX	<i>Wolbachia pipientis</i> wAlbB	199.7/199.7	55/59
2	<i>Cx. quinq.</i>	San Antonio, TX	ND	ND	ND
3	<i>Ae. aegypti</i>	San Antonio, TX	CFAV	77/77	21/21
			<i>Klebsiella pneumoniae</i>	25.1/25.1	10/13
5	<i>Ae. aegypti</i>	San Antonio, TX	FAIL RUN	FAIL RUN	FAIL RUN
6	<i>Ae. albopictus</i>	San Antonio, TX	<i>Wolbachia wVit of Nasonia vitripennis</i>	169.6/169.6	50/56
7	<i>Ae. albopictus</i>	San Antonio, TX	<i>Wolbachia pipientis</i> wAlbB	51.8/51.8	39/59
8	<i>Cx. quinq.</i>	LRGV, TX	<i>Wolbachia pipientis</i> of <i>Cx. quinquefasciatus</i>	129.4/129.4	46/58

9	<i>Cx. quinq.</i>	LRGV, TX	ND	ND	ND
10	<i>Ae. aegypti</i>	LRGV, TX	ND	ND	ND
11	<i>Ae. aegypti</i>	LRGV, TX	Avian endogenous retrovirus	74.9/74.9	23/23
12	<i>Ae. aegypti</i>	LRGV, TX	ND	ND	
13	<i>Ae. albopictus</i>	LRGV, TX	ND	ND	ND
14	<i>Ae. albopictus</i>	LRGV, TX	<i>Wolbachia pipientis wAlbB</i>	205.2/205.2	56/59
15	<i>Cx. quinq.</i>	College Station, TX	ND	ND	ND
16	<i>Cx. quinq.</i>	College Station, TX	ND	ND	ND
17	<i>Culex spp</i>	Chicago, IL	WNV	115.3/115.3	58/79
			CxFLAV	74.7/74.7	19/19
18	<i>Culex spp</i>	Chicago, IL	<i>Wolbachia pipientis of Cx. quinquefasciatus</i>	95.5/95.5	42/58
20	<i>Ae. albopictus</i>	College Station, TX	ND	ND	ND
21	<i>Ae. albopictus</i>	College Station, TX	<i>Comamonadaceae</i>	52.8/52.8	21/26

22	<i>Ae. albopictus</i>	College Station, TX	ND	ND	ND
23	<i>Ae. aegypti</i>	Colony	<i>Erwinia pyrifoliae</i>	14.8/14.8	23/37
			ZIKV	ND	3/27
			<i>Comamonadaceae</i>	65.7/65.7	23/26
25	<i>Ae. aegypti</i>	Colony	<i>Erwinia pyrifoliae</i>	44.5/44.5	27/37
			ZIKV	ND	3/27
			CFAV	77/77	22/22
26	<i>Ae. aegypti</i>	LRGV, TX	<i>Xanthomonadaceae</i>	87.4/87.4	25/25
			FAIL RUN	FAIL RUN	FAIL RUN
27	<i>Ae. aegypti</i>	LRGV, TX	FAIL RUN	FAIL RUN	FAIL RUN
28	<i>Ae. aegypti</i>	LRVG, TX	ND	ND	ND
29	<i>Ae. aegypti</i>	LRVG, TX	ND	ND	ND
30	<i>Ae. aegypti</i>	LRVG, TX	CFAV	77/77	21/21
			<i>Comamonadaceae</i>	51.5/51.5	21/26
31	<i>Ae. aegypti</i>	LRGV, TX	ND	ND	ND

32	<i>Ae. albopictus</i>	LRGV, TX	ND	ND	ND
33	<i>Culex</i> spp.	LRGV, TX	<i>Pseudomonas fulva</i>	91.8/91.8	58/91
34	<i>Culex</i> spp.	LRGV, TX	ND	ND	ND
35	<i>Ae. aegypti</i>	LRGV, TX	CFAV	77/77	21/21
36	<i>Ae. aegypti</i>	LRGV, TX	CFAV	77/77	21/21
			<i>Comonadaceae</i>	75.6/75.6	34/26
37	<i>Culex</i> spp.	LRGV, TX	<i>Silanimonas</i>	91.5/91.5	13/16
38	<i>Culex</i> spp.	LRGV, TX	<i>Xanthomonadaceae</i>	87.4/87.4	21/21
41	<i>Ae. aegypti</i>	Colony	Densovirus	59	19/22
42	<i>Ae. aegypti</i>	Colony	Densovirus	59	19/22
			Densovirus	83.8	21/22
43	<i>Ae. aegypti</i>	Colony	Dengue virus type 2	56.7	20/27
			Densovirus	83.8	21/22
44	<i>Ae. aegypti</i>	Colony	Dengue virus type 2	60.7	20/27

			Densovirus	83.8	21/22
45	<i>Ae. aegypti</i>	Colony	Dengue virus type 2	187.1	47/49
			Mayaro virus	78.5	20/25
			Densovirus	92.3	22/22
46	<i>Ae. aegypti</i>	Colony	Dengue virus type 2	224.6	53/54
			Mayaro virus	122.3	25/25
			<i>Wolbachia pipientis wAlbB</i>	277.2	52/55
47	<i>Cx. quinq.</i>	Colony	Rift Valley fever virus	52.8	16/19
	<i>Cx. quinq.</i>				
48	<i>Ae. aegypti</i>	Colony	<i>Wolbachia pipientis wAlbB</i>	268	51/55

1. Lanciotti RS, Kosoy OL, Laven JJ, Velez JO, Lambert AJ, Johnson AJ, Stanfield SM, Duffy MR. 2008. Genetic and serologic properties of Zika virus associated with an epidemic, Yap State, Micronesia, 2007. *Emerg Infect Dis* 14:1232-9.
2. Lanciotti RS, Kerst AJ, Nasci RS, Godsey MS, Mitchell CJ, Savage HM, Komar N, Panella NA, Allen BC, Volpe KE, Davis BS, Roehrig JT. 2000. Rapid detection of West Nile virus from human clinical specimens, field-collected mosquitoes, and avian samples by a TaqMan reverse transcriptase-PCR assay. *J Clin Microbiol* 38:4066-71.
3. Liang W, He X, Liu G, Zhang S, Fu S, Wang M, Chen W, He Y, Tao X, Jiang H, Lin X, Gao X, Hu W, Liu Y, Feng L, Cao Y, Yang G, Jing C, Liang G, Wang H. 2015. Distribution and phylogenetic analysis of *Culex flavivirus* in mosquitoes in China. *Arch Virol* 160:2259-68.
4. Contreras-Gutierrez MA, Guzman H, Thangamani S, Vasilakis N, Tesh RB. 2017. Experimental Infection with and Maintenance of Cell Fusing Agent Virus (Flavivirus) in *Aedes aegypti*. *Am J Trop Med Hyg* 97:299-304.
5. Newman CM, Cerutti F, Anderson TK, Hamer GL, Walker ED, Kitron UD, Ruiz MO, Brawn JD, Goldberg TL. 2011. *Culex flavivirus* and West Nile virus mosquito coinfection and positive ecological association in Chicago, United States. *Vector Borne Zoonotic Dis* 11:1099-105.

6. Mousson L, Martin E, Zouache K, Madec Y, Mavingui P, Failloux AB. 2010. *Wolbachia* modulates Chikungunya replication in *Aedes albopictus*. Mol Ecol 19:1953-64.