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Supplementary Material

VectorDisk: A Microfluidic Platform Integrating Diagnostic Markers for Evidence-Based Mosquito Control

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Table S1. Primers/probes sequences for the assays in the Vector Study.

Oligo	Sequence	Assay (color) Green: FAM-BHQ1 or MGB where denoted Yellow: HEX-BHQ or MGB where denoted Red: ATTO 647N-BHQ2 or MGB where denoted	Final Reaction Concentration (nM)
S7_Fj	CCACCATCGAACACAAAGTTGA	S7-Detox (A-D)	100
S7_R	TGCTGCAAACTTCGGCTATTC	S7-Detox (A-D)	200
S7_P	CCGTGACGTTACGTTCGAATTCCCA	S7-Detox (A-D)	250
P3_Fj	ACAATGTGATTGACGAAACCCT	P3-Detox (A)	400
P3_R	GGATCACATGCTTTGTGCCG	P3-Detox (A)	500
P3_P	ACCCGCGTACCGTCTGTGGACT	P3-Detox (A)	350
M2_F	CTGGCGTTGAATCCAGAGGT	M2-Detox (A)	600
M2_Rj	GATACTTGCGCAGTGATTCATTAAG	M2-Detox (A)	400
M2_P	AGAGAAATCCTGCAAAAGCACAACGGAGA	M2-Detox (A)	250
K1_F	CCGACACGTGGTGATGGATAC	K1-Detox (B)	200
K1_Rj	CGTCGTCGGTCCAGTCAAC	K1-Detox (B)	400
K1_P	CAATCTTCTGATGCAGGCCCGCAA	K1-Detox (B)	300
P4_Fj	CTGGACAACGTTATCAATGAAACC	P4-Detox (B)	400
P4_R	GCACGGTGTAATCACGCATC	P4-Detox (B)	500
P4_P	CCGATCGAGTCACTTTCGCGCG	P4-Detox (B)	300
Z1_Fj	CCCGCAACTGTATCGGTCTG	Z1-Detox (C)	100
Z1_R	TTCGGTGCCAGTGTGATTGA	Z1-Detox (C)	600
Z1_P1	TGATGCTGTCCCGATTTAACTTTTCGGC	Z1-Detox (C)	250
TE2_FJ	CCGGAATTTGTGAAGCTAAACC	GSTE2-Detox (C)	100
TE2_R	GCTTGACGGGGTCTTTCGG	GSTE2-Detox (C)	400
TE2_P	CGGTACGATCATCACCGAGAGCCAC	GSTE2-Detox (C)	300
P1_Fj	ACAGGTGGTGAACGAAACCC	P1-Detox (D)	100
P1_R	GGTGTAATCCTGTCCCGCAA	P1-Detox (D)	500
P1_P	CCGCTCGAAACGACGCTGCG	P1-Detox (D)	300
G16_Fj	GTCCAAGAAGTTGCGTCGGAC	G16-Detox (D)	200
G16_R	TCTTCGATTTGCGTTGACGTG	G16-Detox (D)	200

G16_P	CTGCAGGCCGACATCATTTTGAAGC	G16-Detox (D)	300
PLAS_F	GCTTAGTTACGATTAATAGGAGTAGCTTG	Plasmodium-DNA	800
PLAS_R	GAAAATCTAAGAATTTCACCTCTGACA	Plasmodium-DNA	600
FALCIP_P	TCTGAATACGAATGTC	falcip-Plasmodium *MGB	300
OVM_P	CTGAATACAAATGCC	ovm-Plasmodium *MGB	300
kdr_F	CATTTTTCTTGGCCACTGTAGTGAT	kdr	500
kdr_R	CGATCTTGGTCCATGTTAATTTGCA	kdr	200
kdr_wt_P	CTTACGACTAAATTTC	wild type-kdr *MGB	500
kdr_mut-w_P	ACGACAAAATTTC	west-kdr *MGB	500
kdr_mut-e_P	ACGACTGAATTTC	east-kdr *MGB	500
Kdr+_F	TGGATCGCTAGAAATGTTCATGACA	super kdr (1575)	500
Kdr+_R	CGAGGAATTGCCTTTAGAGGTTTCT	super kdr (1575)	200
Kdr+ wt_P	ATTTTTTTCATTGCATTATAGTAC	wild type-super kdr *MGB	300
Kdr+_mut_P	TTTTTCATTGCATAATAGTAC	mutant-super kdr *MGB	400
iAChe-F	GGCCGTCATGCTGTGGAT	iAChe	500
iAChe-R	GCGGTGCCGGAGTAGA	iAChe	200
iAChe_wt_P	TTCGGCGGCGGCT	wild type-iAChe *MGB	300
iAChe_mut_P	TTCGGCGGCAGCT	mutant-iAChe *MGB	400
ID_F	GTGAAGCTTGGTGCGTGCT	Species ID	300
ID_R	GCACGCCGACAAGCTCA	Species ID	300
Ag+_P (short)	AGCGGAACAC	gambiae-Species ID *MGB	250
Aq +_P (short)	AGCGGGACAC	q/m-Species ID *MGB	250
Aa+_P	ACATAGGATGGAGAAGG	arabiensis-Species ID *MGB	250
Molecular Forms_F	TCGCCTTAGACCTTGCGTTA	Molecular Forms	800
Molecular Forms_R	CGCTTCAAGAATTCGAGATAC	Molecular Forms	400
Molecular Forms AgM_P	ACCGCGCCGCCATACGTAGGA	M-Molecular Forms	400
Molecular Forms AgS_P	ATGTCTAATAGTCTCAATAGT	S-Molecular Forms *MGB	300
PLP1_F	CCTTTTAGGGTTTGGTATATCCTCTTC	Plasmodium falciparum-infective RNA gene-2	500
PLP1_R	GAGCAGCTTTTCATTCCTGGT	Plasmodium falciparum-infective RNA gene-2	500
PLP1_P	TCAGGGAGAATCAATTC	Plasmodium falciparum-infective RNA gene-2 *MGB	300

Table S2. Primers/probes sequences for the assays in the NA Study.

Oligo	Sequence	Assay	Final Reaction Concentration (nM)
WN_F1	GTGATCCATGTAAGCCCTCAGAA	WNV- Lineage 1 (FAM) WNV- Lineage 2 (HEX)	600
WN_R1	GTCTGACATTGGGCTTTGAAGTTA		600
WN_P1	**FAM**-AGGACCCCACATGTT-MGB		300
WN_P2	**HEX**-AGGACCCCACGTGCT-MGB		300
ZIKA F	CAGACTGCGACAGTTCGAG	ZIKV-5'-UTR	600
ZIKA R	AGAAACTCTCGYTTCCAAATCC		600
ZIKA P	**ATTO 647N**- CCTGTTGATACTGTTGYTAGCTYTCGCTTC-BHQ		300
DENV-1,2,3,4 F1	GACTAGAGGTTAGAGGAGACCCCC	DENV serotypes 1-4 as one	500
DENV-1,2,3 R1	CATTCCATTTTCTGGCGTTCT		500
DENV-4 R2	CAATCCATCTTGCGGCGCTCT		500
DENV-1 P1	**FAM**-CTGTCTCTACAGCATCATTCCAGGCA-BHQ		250
DENV-2,3 P2	**FAM**-CTGTCTCCTCAGCATCATTCCAGGCA-BHQ		250
DENV-4 P3	**FAM**-CTGTCTCTGCAACATCAATCCAGGCA-BHQ		250

Table S3. Microfluidic protocol to operate the VectorDisk. As Hahn-Schickard develops point-of-need tests on a platform basis, several steps correlate with the processing on the RespiDisk published elsewhere [1].

Step[a]	#	Action description	Rotation frequency [Hz]	Rotation acceleration [Hz s^{-1}]	Temperature [°C]	Duration[b] [s]
Sample addition	0-1	Add 180 µL sample to the sample inlet using a pipette. Seal with tape[c]	0	N/A[d]	N/A	N/A
	0-2	Spin sample into lysis chamber	20	10		0
1st TCR[e] actuated valving [2]	1-1	Heating for valve actuation			60	10
	1-2	Spin	20	5		2
	1-3	Set valving frequency	9	5		5
	1-4	Cooling down to activate valve			35	
	1-5	Load compression chamber	40	5		70
Centrifugo-pneumatic inward pumping [3]	2-1	Inward pumping	5	8		2
	2-2	Increase temperature to empty compression chamber			60	0
	2-3	Air pressure equilibration in microfluidic network	25	5		5
TCR actuated mixing [4, 5]	3-1	Cool down system for bubble mixing			40	15
	3-2	Rotation frequency reduction for bubble mixing	5	8		2
	3-3	Heating to initiate bubble mixing			60	0
	3-4	Rotation frequency for cooling	25	5		5
	3-5	Loop: Repeat 4× steps 3-1 to 3-4				
2nd TCR actuated valving [2]	4-1	Set rotation frequency for valving	9	5		10
	4-2	Cool down for valving			40	
Aliquoting and transfer into reaction chambers [6]	5-1	Metering	12	0.5		1
	5-2	Metering	16	0.2		1
	5-3	Empty valve	30	5		5
	5-4	Transfer into reaction chambers	45	5		5
	5-5	Transfer into reaction chambers	5	5		5
	5-6	Repeat steps 5-4 & 5-5 3x				

RT-PCR reaction & detection[f]	6-1	Set rotation frequency	25	0.1		5
	6-2	RT reaction			44/50	600/900
	6-3	Set PCR denaturation temperature			97/95	10/3
	6-4	Set PCR annealing and extension temperature			60/60	30/30
	6-5	Sequential (chamber 1 → 12) detection in FAM, HEX, ATTO 647N				
	6-6	PCR reaction loop: Repeat steps 6-3 to 6-5 40x / 45x				

[a]: If no value is stated for a parameter, it remains constant as stated before.

[b]: 'Duration' refers to the time during which a set of parameters is kept constant, before the next protocol step is executed. The time starts when the given parameters (frequency, acceleration, temperature) are reached.

[c]: Diagnostic tape # 9795R, 3M, USA.

[d]: N/A: Parameter not applicable and/or not controlled by the device.

[e]: Temperature change-rate.

[f]: For steps 6-2 to 6-4 and 6-6, the two values separated by '/' refer to the Vector- and NA-Study, respectively.

Table S4. Statistical analysis of the Ct values derived from five pools of mosquitoes tested with the VectorDisk during the Vector Study (intra-pool variation). The last three columns refer to the inter-pool variation after performing a weighed averaging. Ct, Std, and CV represent the average, standard deviation and coefficient of variation, respectively, upon statistical analysis of the data. 'n.a.' means that there was not statistically sufficient data to calculate Std or CV. The column title cells, 'Pool #1…#5' have been colored accordingly in order to correspond to the colors of manuscript Figure 1 and Table 3. [a]: The numbers of this column indicate the VectorDisk reaction chambers. The reactions that are expected and detected negative (TN) are not shown (Table 3 in manuscript).

No. of lysate pool		Pool #1			Pool #2			Pool #3			Pool #4			Pool #5			Overall		
Green detection channel		**Ct**	**Std**	**CV (%)**	**Ct**	**Std**	**CV (%)**	**Ct**	**Std**	**CV (%)**	**Ct**	**Std**	**CV (%)**	**Ct**	**Std**	**CV (%)**	**Ct**	**Std**	**CV (%)**
3[a]	Molecular Forms (S)	25.5	0.7	2.8	25.7	0.2	0.6	25.5	0.2	1.0	25.5	0.3	1.1	24.9	0.5	2.1	25.4	0.5	1.8
9	Detox (A) RPS7	29.4	0.7	2.4	29.7	1.2	4.1	27.2	0.9	3.5	29.8	1.1	3.8	29.8	0.4	1.4	29.2	1.3	4.5
10	Detox (B) RPS7	28.9	1.4	4.8	29.9	0.8	2.7	26.7	1.6	6.0	30.2	1.2	3.8	29.9	0.8	2.6	28.9	1.7	5.9
11	Detox (C) RPS7	29.4	1.3	4.6	30.5	1.7	5.7	27.0	1.7	6.3	29.8	2.0	6.6	30.2	0.1	0.3	29.1	1.9	6.5
12	Detox (D) RPS7	28.8	1.8	6.3	28.7	2.7	9.4	28.0	0.6	2.1	29.8	1.2	4.1	29.1	0.3	0.9	28.9	1.6	5.7
Yellow detection channel		**Ct**	**Std**	**CV (%)**	**Ct**	**Std**	**CV (%)**	**Ct**	**Std**	**CV (%)**	**Ct**	**Std**	**CV (%)**	**Ct**	**Std**	**CV (%)**	**Ct**	**Std**	**CV (%)**
2	Species ID (Ag)	12.5	0.3	2.6	13.2	0.7	5.5	12.8	0.2	1.7	13.1	0.6	4.8	12.9	0.6	4.8	12.9	0.5	4.1
4	kdr (S-wt)	24.2	0.5	1.9	24.2	0.3	1.1	24.2	0.4	1.8	24.1	0.6	2.4	24.3	0.2	0.8	24.2	0.4	1.5
5	kdr+ (S-wt)	26.1	0.9	3.3	25.9	0.6	2.4	25.8	0.5	2.0	26.2	0.3	1.3	26.1	0.6	2.1	26.0	0.6	2.1
6	iAChE (S-wt)	22.4	0.4	1.6	22.5	0.3	1.5	23.3	0.6	2.8	22.7	0.5	2.0	22.8	0.3	1.1	22.7	0.5	2.3
9	Detox (A) CYP6P3	33.1	0.5	1.6	32.2	1.5	4.8	32.3	1.6	4.9	33.0	1.6	4.7	32.2	0.1	0.2	32.5	1.2	3.6
10	Detox (B) CYP9K1	32.3	n.a.	n.a.	33.9	0.5	1.4	29.0	n.a.	n.a.	33.8	0.2	0.6	31.9	n.a.	n.a.	32.7	2.4	7.3
11	Detox (C) CYP6Z1	31.2	1.1	3.5	31.5	1.6	4.9	31.5	0.8	2.6	33.0	n.a.	n.a.	33.2	1.0	3.1	32.0	1.3	3.9
12	Detox (D) CYP6P1	31.5	1.3	4.1	30.1	n.a	n.a	30.0	0.6	1.9	30.9	1.1	3.5	33.7	n.a.	n.a.	31.0	1.3	4.3
Red detection channel		**Ct**	**Std**	**CV (%)**	**Ct**	**Std**	**CV (%)**	**Ct**	**Std**	**CV (%)**	**Ct**	**Std**	**CV (%)**	**Ct**	**Std**	**CV (%)**	**Ct**	**Std**	**CV (%)**
9	Detox (A) CYP6M2	31.0	n.a.	n.a.	32.5	1.1	3.3	32.3	1.7	5.1	n.a.	n.a.	n.a.	32.7	0.8	2.3	32.4	1.0	3.2
10	Detox (B) CYP6P4	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	31.4	n.a.	n.a.	n.a.	n.a.	n.a.	31.9	n.a.	n.a.	31.6	n.a.	n.a.
11	Detox (C) GSTE2	33.3	1.2	3.6	34.2	1.6	4.6	33.4	2.1	6.3	n.a.	n.a.	n.a.	34.0	2.2	6.4	33.6	1.5	4.4
12	Detox (D) CYP4G16	31.3	0.7	2.1	31.3	0.8	2.7	30.1	0.5	1.7	31.8	1.5	4.7	n.a.	n.a.	n.a.	31.1	1.1	3.5

Table S5. Statistical analysis of the Ct values derived from the VectorDisk results from the NA Study. Ct, Std, and CV represent the average, standard deviation and coefficient of variation, respectively, upon statistical analysis of the data.

Sample	VectorDisk results		
	Ct	Std	CV (%)
WNV-Lineage 1	29.9	1.0	3.2
ZIKV	30.6	1.7	5.4
Plasmodium falciparum	25.9	0.7	2.7
Pf infective stage	31.5	1.3	4.0

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