

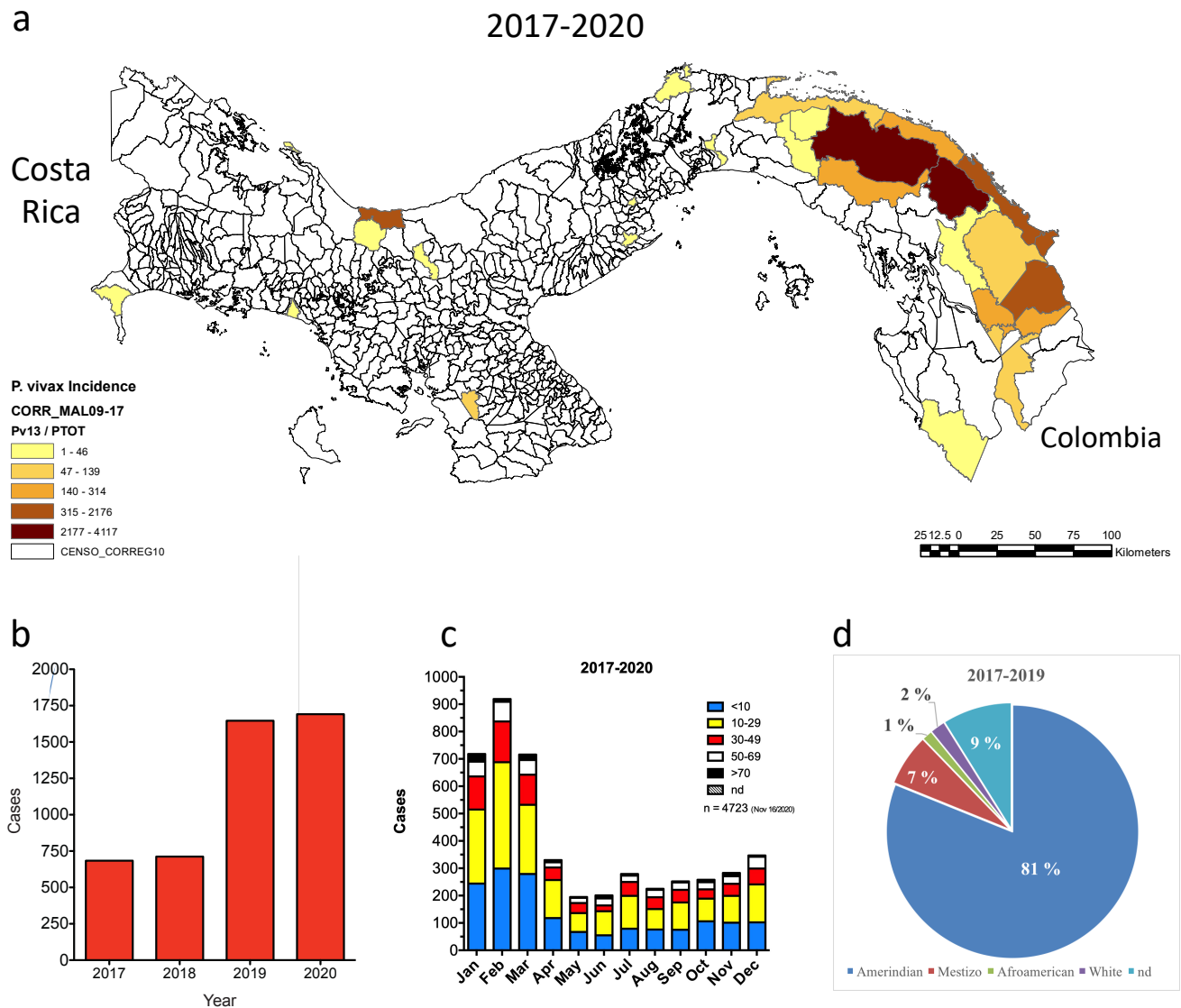


Fig A. Epidemiology of *Plasmodium vivax* malaria in Panama between 2017-2020. a) Map of Panama showing the incidence of *P. vivax* cases by 10,000 population at the corregimiento level for years 2017-2020. b) Number of *P. vivax* cases per year. c) Number of *P. vivax* cases stratified by age for years 2017-2020. d) Percentage of *P. vivax* cases stratified by race and ethnicity for years 2017-2019. Base map downloaded from www.gadm.org under licence CC-BY. www.gadm.org/maps/PAN_1.html

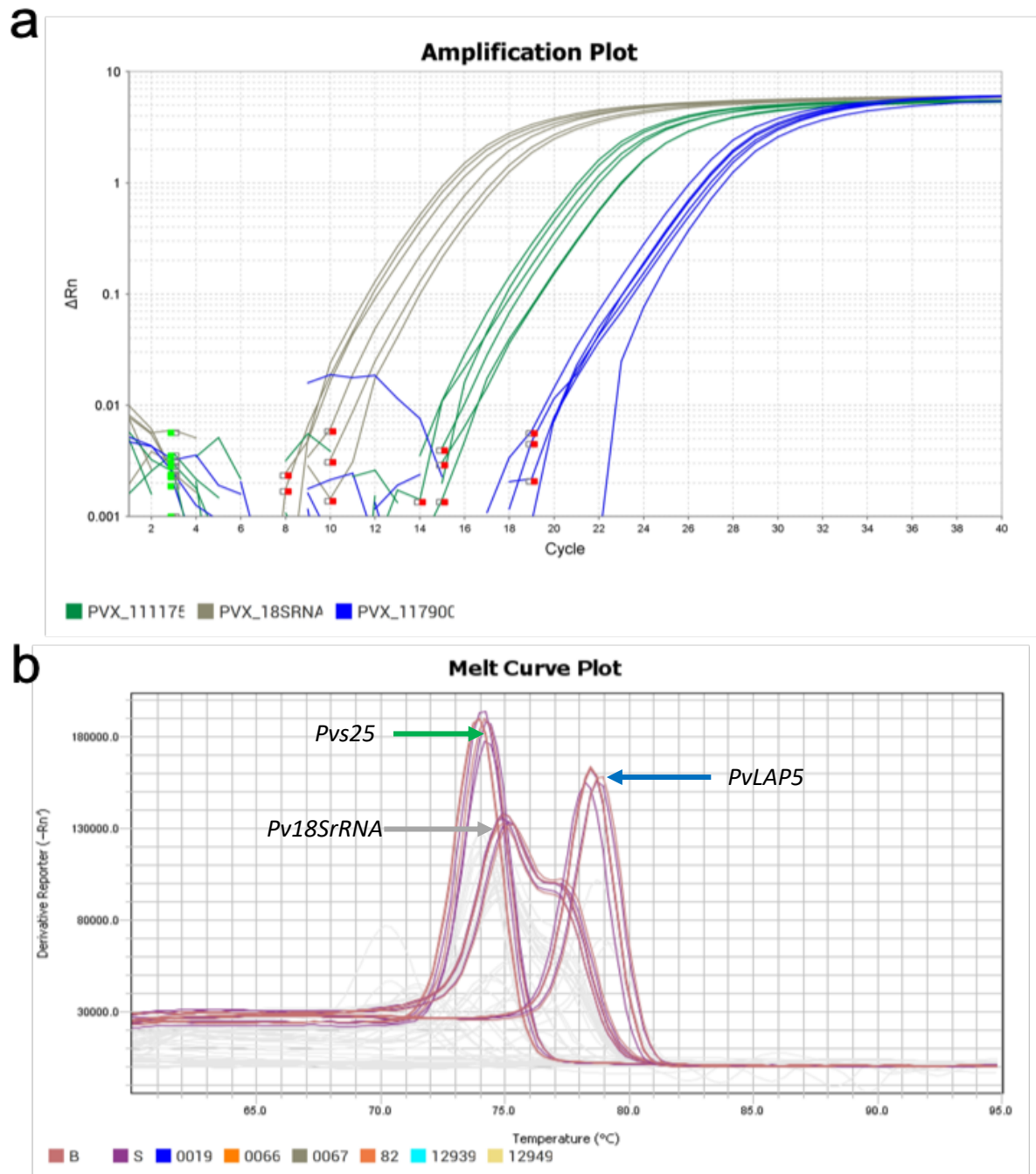


Fig B. Typical amplification and melt curves plots of a qRT-PCR assay for the detection of gametocyte stage-specific markers PVX_111175 (*Pvs25*), PVX_117900 (*PvLAP5*) and constitutive gene *Pv18SrRNA*. a) Amplification curves plot of *P. vivax* positive human controls B and S; b) Melt curves plot of positive *P. vivax* human controls B and S. Each assay was run in triplicate.

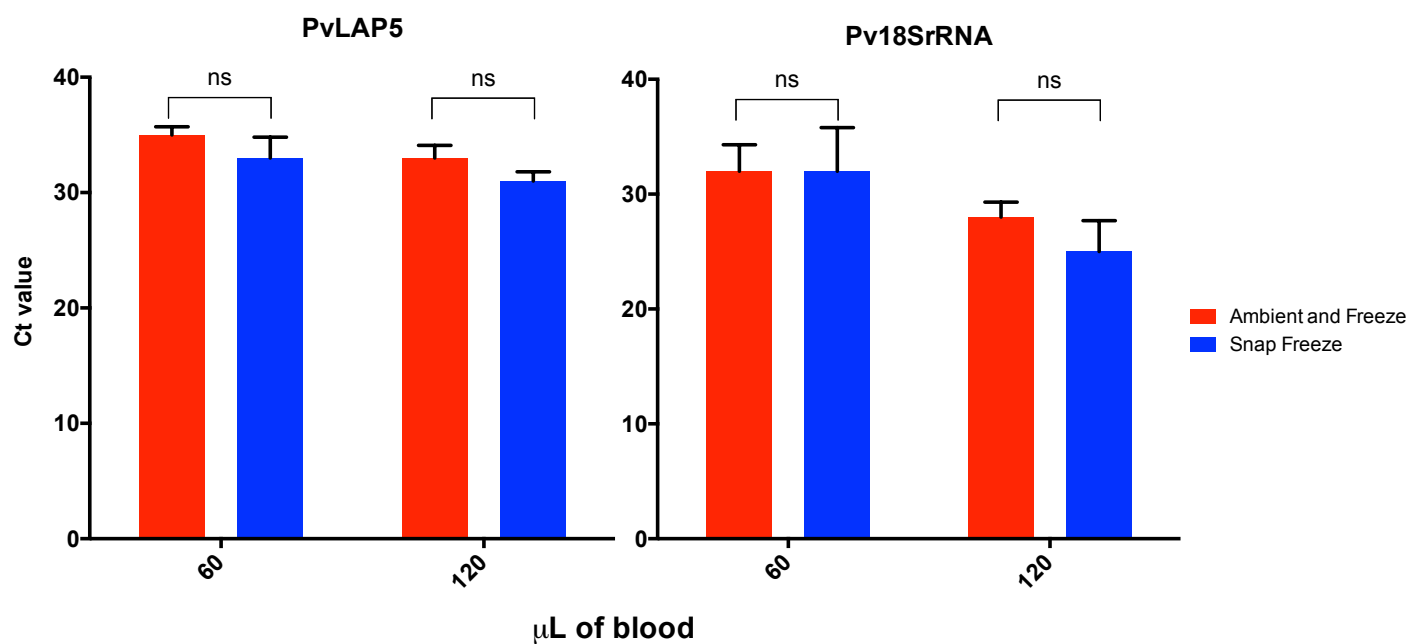


Fig C. Optimization of blood volume and sample preservation conditions for detection of *P. vivax* by qRT-PCR. Gene expression Ct values of gametocyte stage-specific markers *PvLAP5* and constitutive gene *Pv18SrRNA* qRT-PCR assays using 60 or 120 μL of Aotus *P. vivax* SAL-1 infected blood preserved in 500 μL of RNAp under different environmental conditions. Parasitemia of Aotus blood donor 51,080 parasites x μL. ns = non-significant (two-way ANOVA).

Table A. Primer sequences of *Plasmodium vivax* constitutive and gametocyte stage specific markers of a qRT-PCR assay.

Gene Name	Gene ID	Chromosome	Pf ortholog	Primer sequence (5'-3')	Primer efficiency
LCCL domain-containing protein (LAP5)	PVX_117900	12	PF3D7_1451600 (LCCL domain-containing protein, LAP5)	CGCGCGTTTTGTAGGGAGCC GGCGGTACTCCGTCAGTTTCTTCA	94.82%
Ookinete surface protein (Pvs25)	PVX_111175	6	PF3D7_1031000 (25 kDa ookinete surface antigen precursor, Pfs25)	GGCAAAGTCCCAATCCAGA GCCTTCATACACTGGCACT	97.99%
Pv18SrRNA			-	GCTTTGTAATTGGAATGATGGGAAT ATGCGCACAAAGTCGATACGAAG	113.60%

Table B. Epidemiologic and qRT-PCR data of *Plasmodium vivax* constitutive marker Pv18SrRNA and gametocyte specific genes PVX_111175 (*Pvs25*) and PVX_117900 (*PvLAP5*) from microscopic negative controls collected in Panama during 2017-2019.

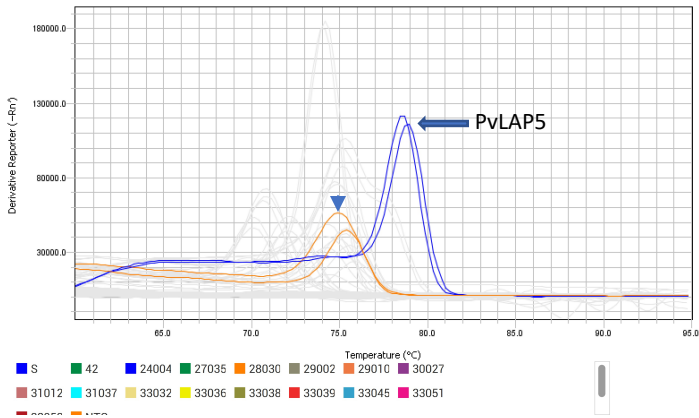
Id	Code	Gender	Age	Province	Days	Parasitemia %	PvLAP5	Pvs25	Pv18SrRNA	Result
1	C18001	M	59	Veraguas	0	Neg.	37	40	36	0
2	C18002	M	56	Cocle	0	Neg.	35	37	36	0
3	C18003	M	38	Guna Yala	0	Neg.	38	36	40	0
4	C18004	M	63	Veraguas	0	Neg.	40	37	40	0
5	C18005	M	59	Panama	1	Neg.	37	40.	37	0
6	C19006	M	nd	Darien	1	Neg.	40	40	40	0
7	C19007	M	52	Panama	1	Neg.	36	35	37	0
8	C19008	M	65	Panama	1	Neg.	40	40	40	0
9	C19009	F	nd	Panama	1	Neg.	36	38	40	0
10	C19010	F	nd	Panama	1	Neg.	40	40	36	0
11	C19011	F	24	Panama	1	Neg.	36	36	37	0
12	C19012	M	32	Panama	0	Neg.	40	40	36	0
13	C19013	F	51	Panama	0	Neg.	40	37	36	0
14	C19014	M	29	Panama	0	Neg.	40	37	40	0
15	C19015	M	37	Panama	0	Neg.	40	37	40	0
16	C19016	M	62	Panama	0	Neg.	40	40	38	0
Median (range)			52 (24, 65)	0(0, 1)		GM (95% CI)	38.39 (37.36, 39.45)	38.08 (37.12, 39.01)	38.02 (37.05, 39.01)	

Table C. *Plasmodium vivax* qRT-PCR data Ct values for constitutive marker Pv18SrRNA and gametocyte specific genes PVX_111175 (*Pvs25*) and PVX_117900 (*PvLAP5*) from malaria naive Aotus monkeys.

Id	Monkey	Sex	Parasitemia %	<i>PvLAP5</i>	<i>Pvs25</i>	<i>Pv18SrRNA</i>	Result
1	24004	F	Neg.	40	40	40	0
2	27035	M	Neg.	40	40	40	0
3	28030	M	Neg.	35	40	40	0
4	29002	M	Neg.	40	35	40	0
5	29010	F	Neg.	40	40	40	0
6	30027	F	Neg.	40	40	40	0
7	31012	F	Neg.	34	40	40	0
8	31037	M	Neg.	40	40	40	0
9	33032	M	Neg.	40	40	40	0
10	33036	M	Neg.	36	40	40	0
11	33038	F	Neg.	40	40	40	0
12	33039	M	Neg.	40	40	40	0
13	33045	M	Neg.	40	40	40	0
14	33051	F	Neg.	40	40	40	0
15	33053	F	Neg.	40	40	40	0
Mean			GM (95% CI)	38.94 (37.75, 40.18)	39.65 (38.90, 40.41)	40	

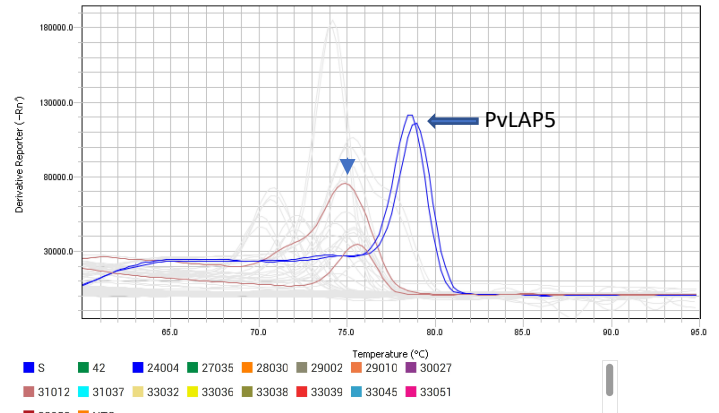
MN28030

Melt Curve Plot



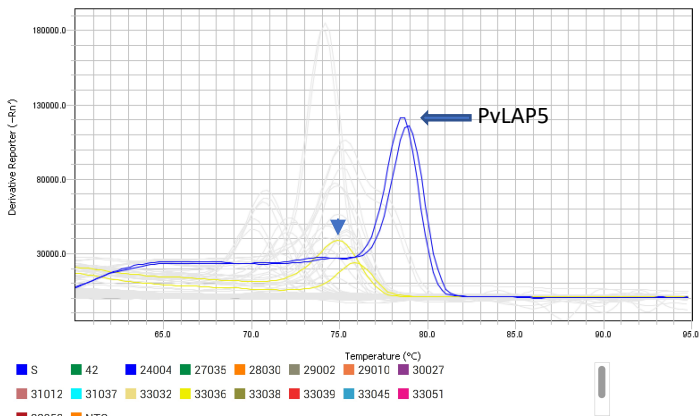
MN31012

Melt Curve Plot



MN33036

Melt Curve Plot



MN29002

Melt Curve Plot

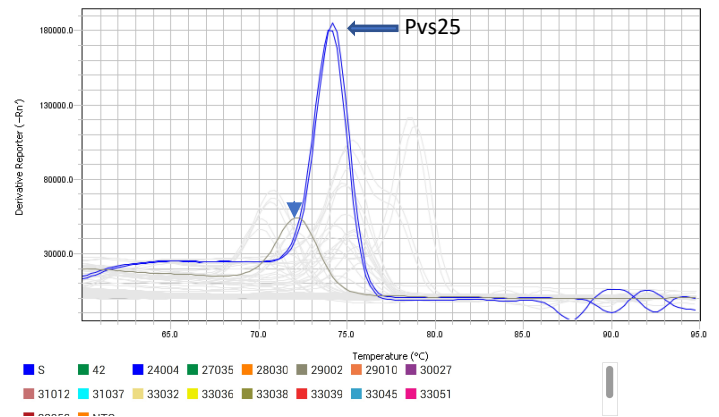


Fig D. Melt curves plots of a qRT-PCR assay for the detection of gametocyte stage-specific markers PVX_117900 (*PvLAP5*) and PVX_111175 (*Pvs25*) in malaria naïve lab-bred Aotus monkeys use as negative controls. Melt curves plots showing non-specific products (primer dimers) (arrow heads) in monkeys MN28030, MN31012, MN33036 and MN29002. Arrows show positive controls: *PvLAP5* and *Pvs25*.

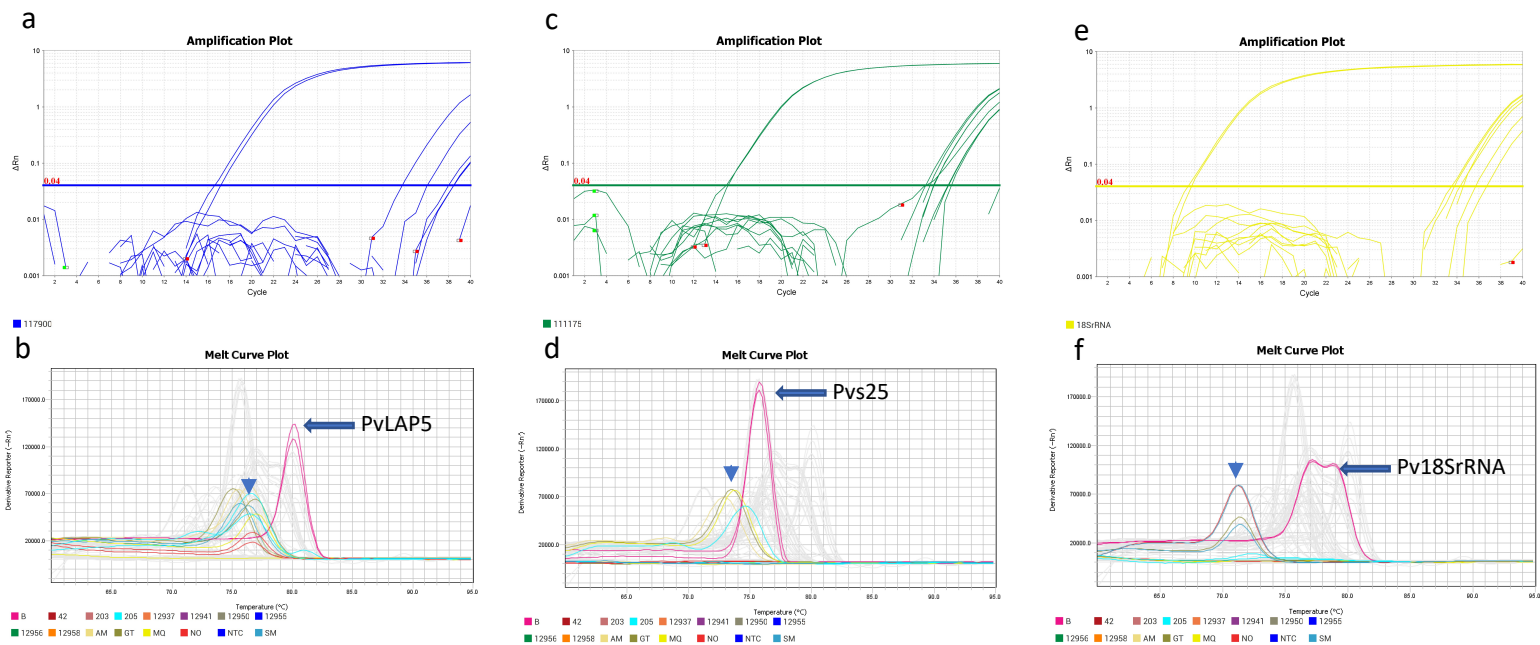


Fig E. Melt curves plots of a qRT-PCR assay for the detection of gametocyte stage-specific markers PVX_117900 (*PvLAP5*) and PVX_111175 (*Pvs25*) and Pv18SrRNA in healthy malaria negative controls. Melt curves plots showing non-specific products (primer dimers) (arrow heads) from six selected samples for illustration. Arrows show positive controls: a–b) *PvLAP5*; c–d) *Pvs25*; e–f) *Pv18SrRNA*.

Table D. Frequency distribution of *Plasmodium vivax* samples collection sites stratified by province and district for years 2017-2020.

Sample collection site			
Province	District	Frequency	(%)
Darién	Cemaco	6	(10)
	Cirilo Guainora	1	(2)
	Manuel Ortega	9	(14)
	Pinogana	10	(16)
Panama	Arraijan	1	(2)
	Chepo	20	(32)
	La Chorrera	1	(2)
	Panamá	2	(3)
Guna Yala	Tubuala	13	(21)
Total		63	(100)

Table E. Demographic and socioeconomic characteristics of selected study participants for the validation of a qRT-PCR assay for the detection of *Plasmodium vivax* gametocytes in field isolates collected from Panama during 2017-2020.

	Frequency	%
Gender		
Male	47	64
Female	26	36
Total	73	100
Race / Ethnicity		
Amerindian	31	42
Mestizo	7	10
Afro	1	1
White	2	3
Other	1	1
nr	31	42
Total	73	100
Place of residence		
Cocle	1	1
Darien	19	26
Guna Yala	20	27
Panama	29	40
Veraguas	2	3
nr	2	3
Total	73	100
Employment		
Yes	8	11
No	19	26
nr	46	63
Total	73	100
Literacy		
Yes	30	41
No	10	14
nr	33	45
Total	73	
House Type		
1	2	3
2	16	22
3	16	22
4	1	1
5	1	1
nr	37	51
Total	73	100

n = 73 survey participants

nr = non-responders

Table F. Epidemiologic and qRT-PCR data of *Plasmodium vivax* constitutive marker *Pv18SrRNA* and gametocyte specific genes *Pvs25* and *PvLAP5* from microscopic positive field isolates collected in Panama during 2017-2020.

No.	Code	Gametocytes	Province	Gender	Age	Days	RNA	Parasitemia %	PVX_117900	PVX_111175	PVX_18sRNA
1	17001	1	Panama	F	3	5	1	0.26	34	32	33
2	18002	0	Panama	M	5	7	35.4	0.25	32	32	38
3	18003	1	Panama	F	3	7	17.8	0.1	40	33	32
4	18004	1	Panama	F	2	7	16.5	0.34	35	31	34
5	18005	1	Panama	F	0.5	2	32.7	0.05	33	31	36
6	18006	0	Panama	M	2	10	1.3	0.11	34	33	33
7	18007	0	Panama	M	nd	2	1.4	0.25	38	40	35
8	18008	0	Guna Yala	M	18	2	25.9	0.16	33	40	35
9	18009	1	Panama	M	nd	1	10.1	0.37	30	26	18
10	19010	0	Panama	F	nd	1	7.2	0.2	31	26	19
11	19012	0	Guna Yala	F	nd	40	31.9	0.23	36	36	32
12	19013	0	Guna Yala	M	nd	38	8.6	0.24	35	36	27
13	19014	1	Guna Yala	F	nd	38	59.4	0.12	37	37	30
14	19016	0	Guna Yala	M	nd	36	6.3	0.05	38	34	26
15	19017	1	Guna Yala	M	19	31	18.6	0.15	32	31	25
16	19020	1	Panama	M	34	7	5.9	0.66	33	30	22
17	19021	0	Panama	M	33	8	-0.9	0.14	32	29	20
18	19022	1	Panama	F	nd	1	1.6	0.13	32	29	28
19	19025	0	Panama	M	14	4	49.9	0.88	35	34	28
20	19027	1	Panama	M	nd	0	16.1	0.48	33	33	30
21	19028	0	Panama	F	nd	0	15	0.18	37	37	34
22	19029	0	Panama	F	nd	3	7.2	0.15	33	30	22
23	19030	0	Guna Yala	F	29	2	34.1	0.42	40	38	37
24	19031	1	Panama	M	24	3	6.5	0.09	28	26	18
25	19032	1	Panama	F	2	5	3.4	0.35	30	28	20
26	19033	0	Panama	F	53	14	3.9	0.25	40	33	28
27	19034	1	Panama	M	nd	1	13.6	0.38	36	31	22
28	19036	1	Darien	M	15	4	11.1	0.62	31	27	16
29	19039	1	Guna Yala	F	25	6	1.7	0.4	40	32	36
30	19040	1	Guna Yala	M	5	4	17.3	0.82	33	31	29
31	19041	1	Darien	M	27	4	-0.6	0.09	36	33	22
32	19042	0	Darien	F	44	4	4	0.16	37	40	37
33	19043	1	Darien	M	42	1	8.9	0.001	29	25	15
34	19044	1	Darien	M	76	1	20.8	1.35	26	25	21
35	19045	1	Panama	M	71	11	7.6	0.4	35	31	21
36	19046	0	Panama	M	19	11	19.6	0.16	40	33	24
37	19047	1	Guna Yala	M	38	7	4.9	0.28	32	28	20
38	19048	0	Darien	F	0.6	14	2.8	0.08	37	40	38
39	19050	1	Guna Yala	M	7	4	6.4	0.95	33	30	26
40	19051	1	Guna Yala	M	16	8	2.3	0.38	35	36	28
41	19052	1	Guna Yala	M	28	4	3.2	0.32	32	29	25
42	19058	1	Panama	M	50	0	6.4	0.47	31	30	26
43	19059	1	Darien	M	2	7	0	1.04	29	27	21
44	19063	1	Darien	M	58	7	2.8	0.13	31	36	40
45	19064	0	Darien	F	65	3	3.6	0.04	31	30	29
46	19065	1	Darien	F	56	13	1.3	0.75	37	35	37
47	19066	1	Darien	M	51	6	8.7	0.63	34	36	36
48	19067	0	Darien	M	55	9	2.6	0.04	35	32	31
49	19068	0	Darien	F	74	31	-0.5	0.18	34	40	40
50	19069	0	Darien	F	49	16	1.2	0.05	35	35	32
51	19070	0	Panama	M	19	6	nd	0.14	29	28	24
52	19071	0	Darien	F	10	10	3	0.07	40	35	29
53	19072	1	Darien	M	56	6	2	0.06	37	34	32
54	19073	0	Darien	M	55	14	22.9	0.04	38	36	40
55	19075	0	Darien	M	45	6	1.1	0.11	35	40	32
56	19076	0	Darien	F	6	12	1	0.05	40	35	29
57	19077	1	Darien	M	6	12	-0.8	0.29	33	33	26
58	19078	1	Darien	M	12	11	1.8	0.66	31	29	24
59	19079	1	Darien	M	39	7	2.2	0.05	34	31	26
60	19081	1	Darien	M	nd	nd	1.2	0.35	30	28	21
61	19082	0	Darien	F	19	192	6.3	0.11	34	36	33
62	19083	0	Darien	F	49	192	4.5	1.39	31	34	32
63	19084	1	Darien	M	69	192	6	0.21	32	35	40
Median					26	7					
Min					0.5	0					
Max					76	192					

Ct values



15

40

Table G. Field validation of a qRT-PCR assay for the detection of *Plasmodium vivax* gene transcripts in smear positive and negative field samples preserved at ambient temperature in RNAprotect compared against microscopy.

Evaluation												
Pv18SrRNA					Pvs25				PvLAP5			
Microscopy	All stages	Positive	Negative	Total	Gametocytes	Positive	Negative	Total	Gametocytes	Positive	Negative	Total
	Positive	59	9	68	Positive	35	31	66	Positive	33	30	63
	Negative	4	7	11	Negative	0	13	13	Negative	2	14	16
Total		63	16	79	Total	35	44	79	Total	35	44	79
		%	95 % CI				%	95 % CI			%	95 % CI
Sensitivity		93.65	84.53, 98.24				100.00	90.00, 100.00			94.29	80.84, 99.30
Specificity		43.75	19.75, 70.12				29.55	16.76, 45.20			31.82	18.61, 47.58
PLR		1.66	1.08, 2.58				1.42	1.17, 1.72			1.38	1.11, 1.72
NLR		0.15	0.05, 0.44				0.00	-			0.18	0.04, 0.74
PPV		86.76	80.90, 91.03				53.03	48.25, 57.75			52.38	46.94, 56.76
NPV		63.64	36.83, 84.01				100.00	-			87.50	63.00, 96.64

PLR = Positive Likelihood Ratio
NLR = Negative Likelihood Ratio
PPV = Positive Predictive Value
NPV = Negative Predictive Value