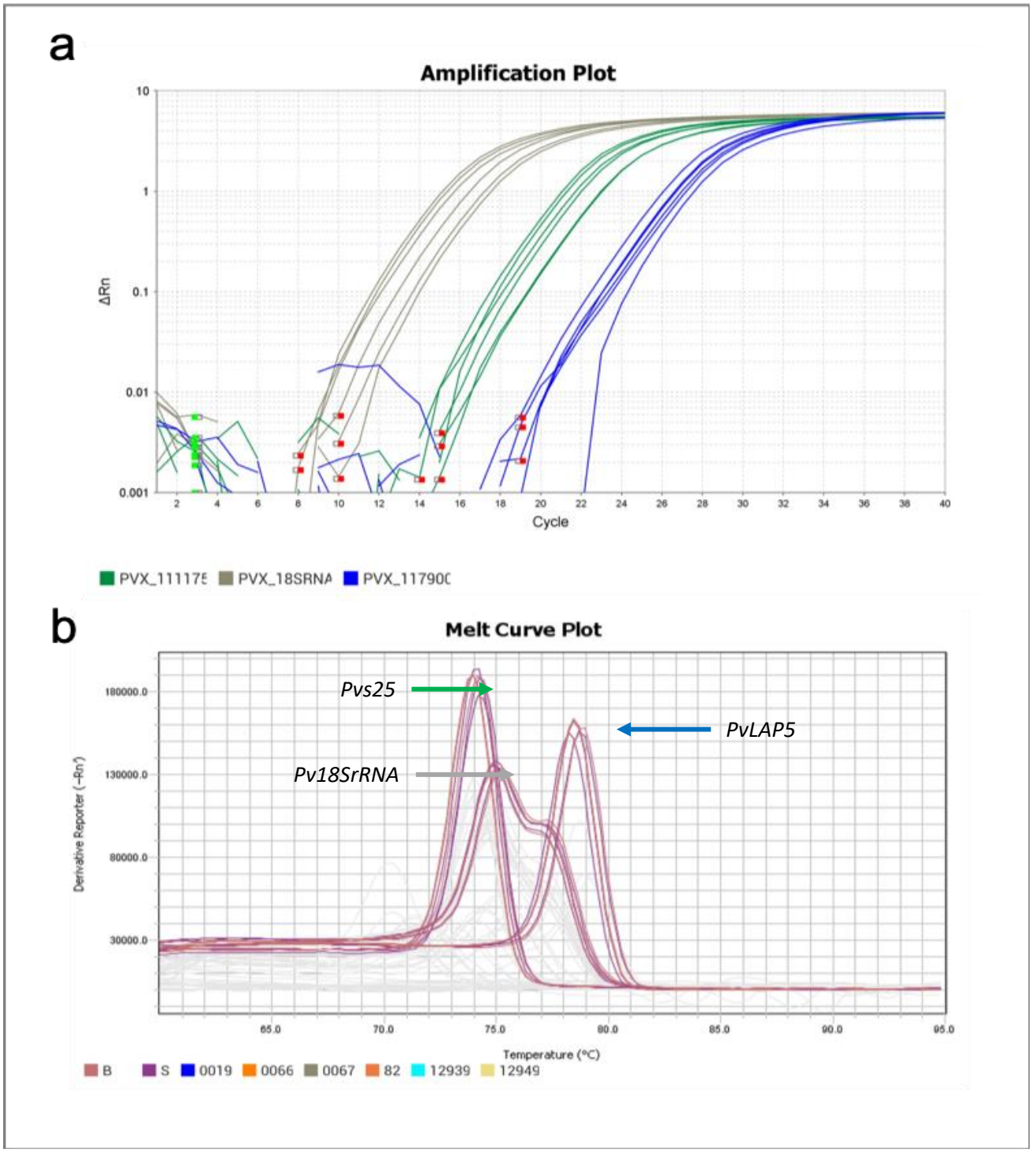

Supplementary Fig. S1. Typical amplification and melt curve plots of a qRT-PCR assay for the detection of gametocyte stage-specific markers PVX_111175 (*Pvs25*), PVX117900 (*PvLAP5*) and constitutive gene *Pv18SrRNA*. a) Amplification plot of *P. vivax* positive human control S; b) Melt curves plot of positive *P. vivax* human control S.

Table S1. 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)	GGCAAAGTCCCCAATCCAGA GCCTTCCATACACTGGCACT	97.99%
Pv18SrRNA			-	GCTTTGTAATTGGAATGATGGGAAT ATGCGCACAAAGTCGATACGAAG	113.60%

Table S2. 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.	40	40	40	0
2	C18002	M	56	Cocle	0	Neg.	40	40	40	0
3	C18003	M	38	Guna Yala	0	Neg.	40	40	40	0
4	C18004	M	63	Veraguas	0	Neg.	40	37	40	0
5	C18005	M	59	Panama	1	Neg.	40	40	40	0
6	C19006	M	nd	Darien	1	Neg.	40	40	40	0
7	C19007	M	52	Panama	1	Neg.	40	40	40	0
8	C19008	M	65	Panama	1	Neg.	40	40	40	0
9	C19009	F	nd	Panama	1	Neg.	40	40	40	0
10	C19010	F	nd	Panama	1	Neg.	40	40	40	0
11	C19011	F	24	Panama	1	Neg.	40	40	40	0
12	C19012	M	65	Darien	14	Neg.	40	40	40	0
13	C19013	F	27	Darien	11	Neg.	40	40	40	0
14	C19014	F	15	Darien	3	Neg.	40	40	40	0
15	C19015	M	47	Darien	3	Neg.	40	40	40	0
16	C19016	F	46	Darien	3	Neg.	40	40	40	0
Median (range)			52 (24, 65)		3 (0, 14)	Mean (stdv)	40 (0)	40 (1)	40 (0)	

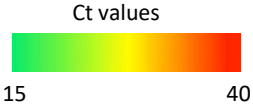

Table S3. *Plasmodium vivax* qRT-PCR data Ct values for constitutive marker Pv18SrRNA and gametocyte specific genes PVX_111175 (*Pvs25*) and PVX_117900 (*PvLAP5*) from malaria naive Aotus monkeys

Id	Monkey	Sex	Parasitemia %	*PvLAP5*	*Pvs25*	*Pv18SrRNA*	Result
1	24004	F	Neg.	40	40	40	0
2	27035	M	Neg.	40	40	40	0
3	28030	M	Neg.	40	40	40	0
4	29002	M	Neg.	40	40	40	0
5	29010	F	Neg.	40	40	40	0
6	30027	F	Neg.	40	40	40	0
7	31012	F	Neg.	40	40	40	0
8	31037	M	Neg.	40	40	40	0
9	33032	M	Neg.	40	40	40	0
10	33036	M	Neg.	40	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			40	40	40	

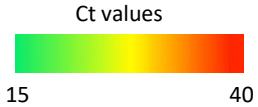

Table S4. Characteristics of *Plasmodium vivax* selected smear positive study participants stratified by place of residency, gender and age

Place of Residency			
Province	District	Frequency (%)	
Darién	Cemaco	8	(18)
Panamá	Arraijan	1	(2)
	Chepo	17	(37)
	La Chorrera	1	(2)
	Panamá	1	(2)
Guna Yala	Puerto Obaldía	4	(9)
	Tubualá	13	(29)
Total		45	(100)

Age Intervals	Male	Female	
<5	4	6	10 (22)
6-15	3	0	3 (7)
16-25	6	1	7 (15)
26-35	4	1	5 (11)
36-45	2	1	3 (7)
46-55	1	1	2 (4)
>55	3	0	3 (7)
nr	6	6	12 (27)
Total (%)	29 (64)	16 (36)	45 (100)
Mean age years +/- stdv	32 +/- 23	15 +/- 20	

nr = non-responders

Table S5. Demographic and socioeconomic characteristics of study participants for the validation of a qRT-PCR assay for the detection of *Plasmodium vivax* gametocytes in field isolates collected from Panama during 2017-2019

	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
Place of residence		
Cocle	1	1.4
Darien	19	26
Guna Yala	20	27.4
Panama	29	39.7
Veraguas	2	2.7
nr	2	2.7
Employment		%
Yes	8	11
No	19	26
nr	46	63
Literacy		
Yes	30	41
No	10	14
nr	33	45
House Type		
1	2	3
2	16	22
3	16	22
4	1	1
5	1	1
nr	37	51

n = 73 survey participants

nr = non-responders

Table S6. 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 2017-2019.

No.	Code	Gametocytes	Province	Gender	Age	Samples days in transit	RNA ng/uL	Parasitemia`%	*PvLAP5*	*Pvs25*	*Pv18SrRNA*	Result
1	17001	1	Panama	F	3	5	1	0.26	34	32	33	1
2	18002	0	Panama	M	5	7	35.4	0.25	32	32	38	1
3	18003	1	Panama	F	3	7	17.8	0.1	40	33	32	1
4	18004	1	Panama	F	2	7	16.5	0.34	35	31	34	1
5	18005	1	Panama	F	0.5	2	32.7	0.05	33	31	36	1
6	18006	0	Panama	M	2	10	1.3	0.11	34	33	33	1
7	18007	0	Panama	M	nd	2	1.4	0.25	38	40	35	1
8	18008	0	Guna Yala	M	18	2	25.9	0.16	33	40	35	1
9	18009	1	Panama	M	nd	1	10.1	0.37	30	26	18	1
10	19010	0	Panama	F	nd	1	7.2	0.2	31	26	19	1
11	19012	0	Guna Yala	F	nd	40	31.9	0.23	36	36	32	1
12	19013	0	Guna Yala	M	nd	38	8.6	0.24	35	36	27	1
13	19014	1	Guna Yala	F	nd	38	59.4	0.12	37	37	30	1
14	19015	0	Guna Yala	M	nd	37	-5.9	0	36	35	32	1
15	19016	0	Guna Yala	M	nd	36	6.3	0.05	38	34	26	1
16	19017	1	Guna Yala	M	19	31	18.6	0.15	32	31	25	1
17	19020	1	Panama	M	34	7	5.9	0.66	33	30	22	1
18	19021	0	Panama	M	33	8	-0.9	0.14	32	29	20	1
19	19022	1	Panama	F	nd	1	1.6	0.13	32	29	28	1
20	19025	0	Panama	M	14	4	49.9	0.88	35	34	28	1
21	19027	1	Panama	M	nd	0	16.1	0.48	33	33	30	1
22	19028	0	Panama	F	nd	0	15	0.18	37	37	34	1
23	19029	0	Panama	F	nd	3	7.2	0.15	33	30	22	1
24	19030	0	Guna Yala	F	29	2	34.1	0.42	40	38	37	1
25	19031	1	Panama	M	24	3	6.5	0.09	28	26	18	1
26	19032	1	Panama	F	2	5	3.4	0.35	30	28	20	1
27	19033	0	Panama	F	53	14	3.9	0.25	40	33	28	1
28	19034	1	Panama	M	nd	1	13.6	0.38	36	31	22	1
29	19036	1	Darien	M	15	4	11.1	0.62	31	27	16	1
30	19037	0	Darien	F	17	4	2	0.002	31	27	23	1
31	19040	1	Guna Yala	M	5	4	17.3	0.82	33	31	29	1
32	19041	1	Darien	M	27	4	-0.6	0.09	36	33	22	1
33	19042	0	Darien	F	44	4	4	0.16	37	40	37	1
34	19043	1	Darien	M	42	1	8.9	0.001	29	25	15	1
35	19044	1	Darien	M	76	1	20.8	1.35	26	25	21	1
36	19045	1	Panama	M	71	11	7.6	0.4	35	31	21	1
37	19046	0	Panama	M	19	11	19.6	0.16	40	33	24	1
38	19047	1	Guna Yala	M	38	7	4.9	0.28	32	28	20	1
39	19048	0	Darien	F	0.6	14	2.8	0.08	37	40	38	1
40	19050	1	Guna Yala	M	7	4	6.4	0.95	33	30	26	1
41	19051	1	Guna Yala	M	16	8	2.3	0.38	35	36	28	1
42	19052	1	Guna Yala	M	28	4	3.2	0.32	32	29	25	1
43	19058	1	Panama	M	50	0	6.4	0.47	31	30	26	1
44	19059	1	Darien	M	2	7	0	1.04	29	27	21	1
45	19063	1	Darien	M	58	7	2.8	0.13	31	36	40	0
Mean (stdv)					24 (21)	9 (12)	12.1 (13.7)	0.32 (0.30)				
Median (min, max))					19 (1, 76)	4 (0, 40)	7.2 (-5.9, 59.4)	0.24 (0, 1.35)				

nd = not determine

1 = positive

0 = negative

Ct values

15 40

Table S7. Field validation of an ultrasensitive qRT-PCR assay for the detection of *Plasmodium vivax* gene transcripts in smear positive and negative field samples preserved at ambient temperature in RNAprotect®

Evaluation											
Disease											
Pv18srRNA	Present	Absent	Total	Pvs25	Present	Absent	Total	PvLAP5	Present	Absent	Total
Positive	44	0	44	Positive	41	1	42	Positive	41	0	41
Negative	1	16	17	Negative	4	15	19	Negative	4	16	20
Total	45	16	61	Total	45	16	61	Total	45	16	61
	%	95 % CI			%	95 % CI			%	95 % CI	
Sensitivity	98	88, 100			91	79, 98			91	79, 98	
Specificity	100	70, 100			94	70, 100			100	79, 100	
PLR	–	–			15	2, 97			–	–	
NLR	0.02	0.00, 0.15			0.09	0.04, 0.24			0.09	0.03, 0.23	
Prevalence	74	61, 84			74	61, 84			74	61, 84	
PPV	100	–			98	86, 100			100	–	
NPV	94	70, 99			79	59, 91			80	61, 91	

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

Table S8. Field validation of an ultrasensitive qRT-PCR assay for the detection of *Plasmodium vivax* gene transcripts in gametocyte positive and negative smears field samples preserved at ambient temperature in RNAprotect®

Evaluation											
Gametocytes											
Pv18srRNA	Present	Absent	Total	Pvs25	Present	Absent	Total	PvLAP5	Present	Absent	Total
Positive	25	0	25	Positive	26	4	38	Positive	25	3	28
Negative	1	19	20	Negative	0	15	7	Negative	1	16	17
Total	26	19	45	Total	26	19	45	Total	26	19	45
	%	95 % CI			%	95 % CI			%	95 % CI	
Sensitivity	96	81, 100			100	87, 100			96	81, 100	
Specificity	100	82, 100			79	54, 94			84	60, 97	
PLR	–	–			5	2, 11			6	2.17	
NLR	0.04	0.01, 0.25			-	-			0.05	0.01, 0.32	
Prevalence	59	42, 73			58	42, 72			58	42, 72	
PPV	100	–			87	73, 94			89	75, 96	
NPV	98	88, 100			100	-			94	70, 99	

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

Table S9. Field validation of an ultrasensitive qRT-PCR assay for the detection of *Plasmodium vivax* gene transcripts in gametocyte positive smears field samples and negative Aotus blood samples preserved at ambient temperature in RNAprotect®

Evaluation											
Gametocytes											
Pv18srRNA	Present	Absent	Total	Pvs25	Present	Absent	Total	PvLAP5	Present	Absent	Total
Positive	25	0	25	Positive	26	0	26	Positive	25	0	25
Negative	1	15	16	Negative	0	15	15	Negative	1	15	16
Total	26	15	41	Total	26	15	41	Total	26	15	41
	%	95 % CI			%	95 % CI			%	95 % CI	
Sensitivity	96	81, 100			100	87, 100			96	81, 100	
Specificity	100	78, 100			100	78, 100			100	78, 100	
PLR	–	–			-	-			-	-	
NLR	0.04	0.01, 0.25			-	-			0.04	0.01, 0.25	
Prevalence	63	47, 99			63.1	47, 99			63	47, 99	
PPV	100	–			100	-			100	-	
NPV	94	69, 99			100	-			94	69, 99	

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

Table S10. Reported malaria cases by methods of confirmation in Panama during 2010-2019

Year	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019
Suspected cases	141038	116588	107711	93624	80701	64511	50772	38270	23383	22171
Presumed and confirmed	418	354	844	705	874	562	811	689	715	1597
Microscopy examined	141038	116588	107711	93624	80701	64511	50772	76540	46766	18217
Microscopy positive	418	354	844	705	874	562	811	1378	1430	1209
Microscopy positivity %	0.3	0.3	0.8	0.8	1.1	0.9	1.6	3.6	6.1	5.5
RDT examined	-	0	0	0	0	0	0	829	1141	3954
RDT positive	-	0	0	0	0	3	5	5	427	388
RDT positivity %	-	-	-	-	-	-	-	0.6	37.4	9.8
Imported cases	-	-		9	10	16	42	40	31	43

Data source with modifications: WHO Malaria World Report, 2020 (1)