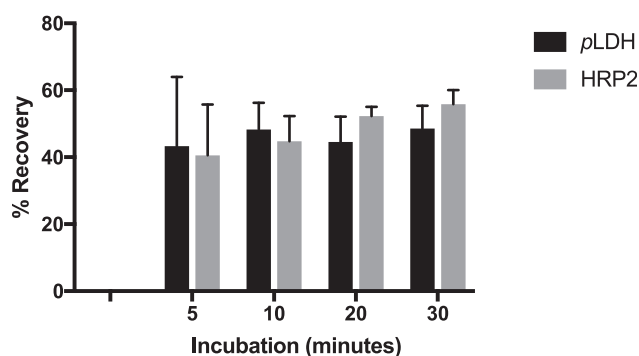
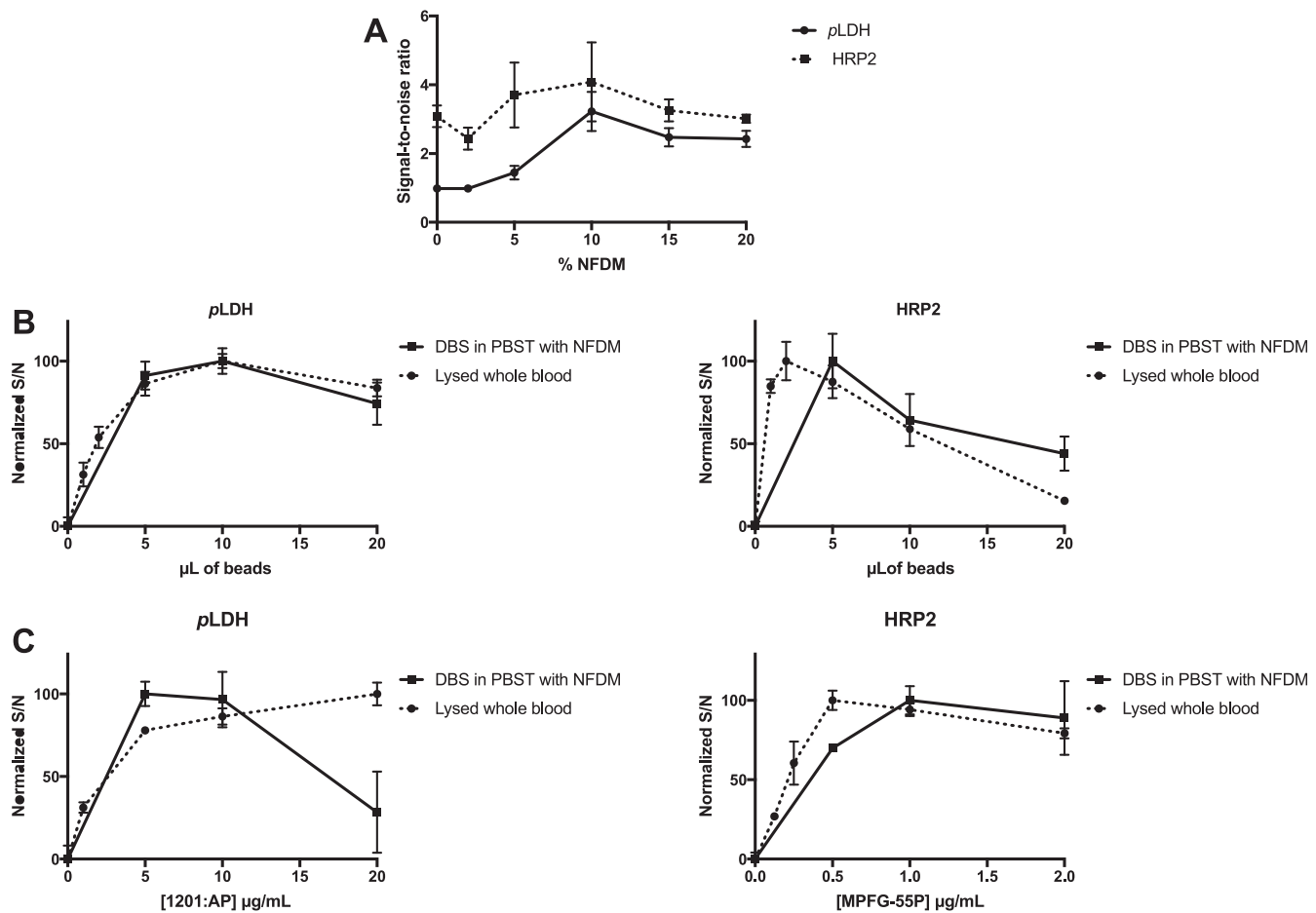


SUPPLEMENTAL TEXT 1

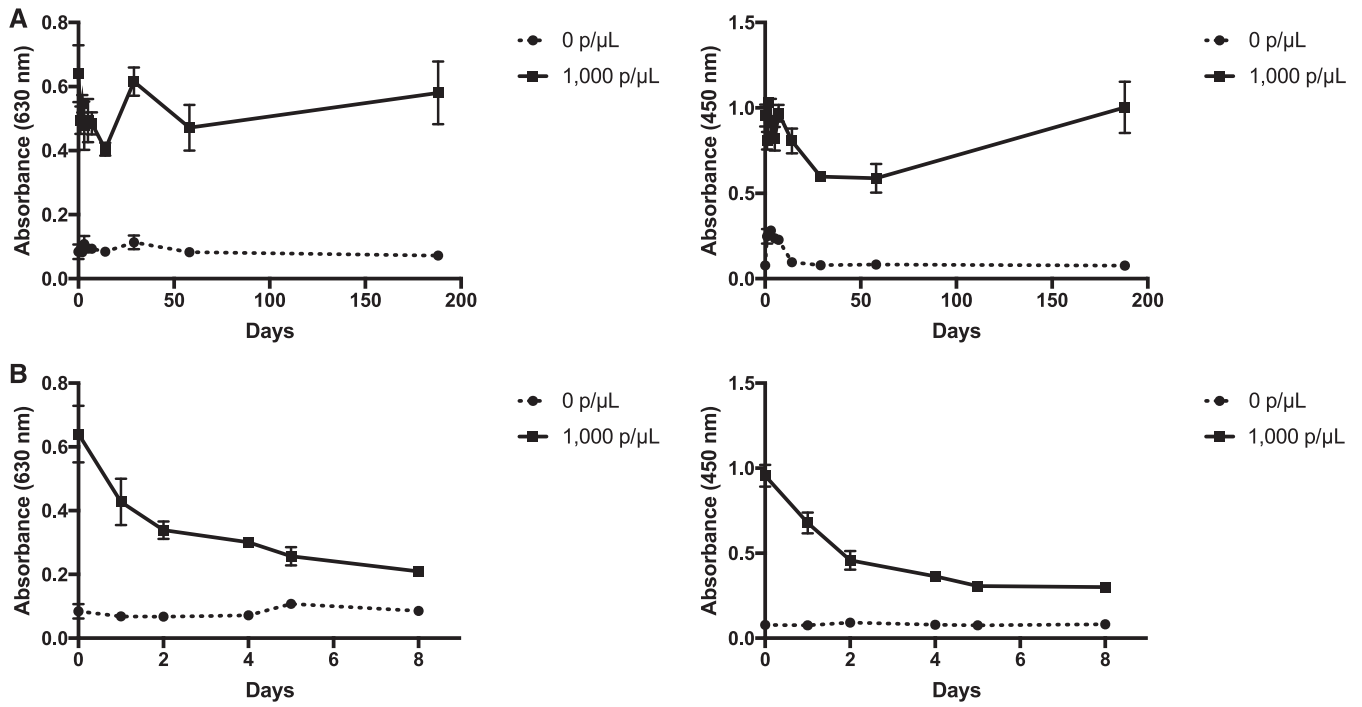
**Plate ELISA for *Plasmodium* lactate dehydrogenase standardization in in-house D6 culture.** First, 100  $\mu$ L of 2  $\mu$ g/mL 19g7 in PBS was placed in a 96-well plate and incubated for 1 hour. The plate was washed three times with 250  $\mu$ L of 1 $\times$  PBS with 0.1% Tween-20 (PBST). Next, 250  $\mu$ L of 15% nonfat dried milk in PBST was added to each well and the plate was incubated for 2 hours. After washing three times with PBST, 100  $\mu$ L of standard *rcPf*LDH and in-house culture diluted in PBST with 0.1% nonfat dried milk were placed on the plate and incubated for 2 hours. The plate was then washed five times with PBST, and 100  $\mu$ L of 2  $\mu$ g/mL 1201: HRPx in PBST with 0.5% nonfat dried milk was added to each well and the plate was incubated for 1 hour. After washing five times with PBST, 100  $\mu$ L of TMB One solution was added to the wells, the plate was incubated for 15 minutes, and the reaction was quenched with 2M H<sub>2</sub>SO<sub>4</sub>. Absorbance was measured at 450 nm.



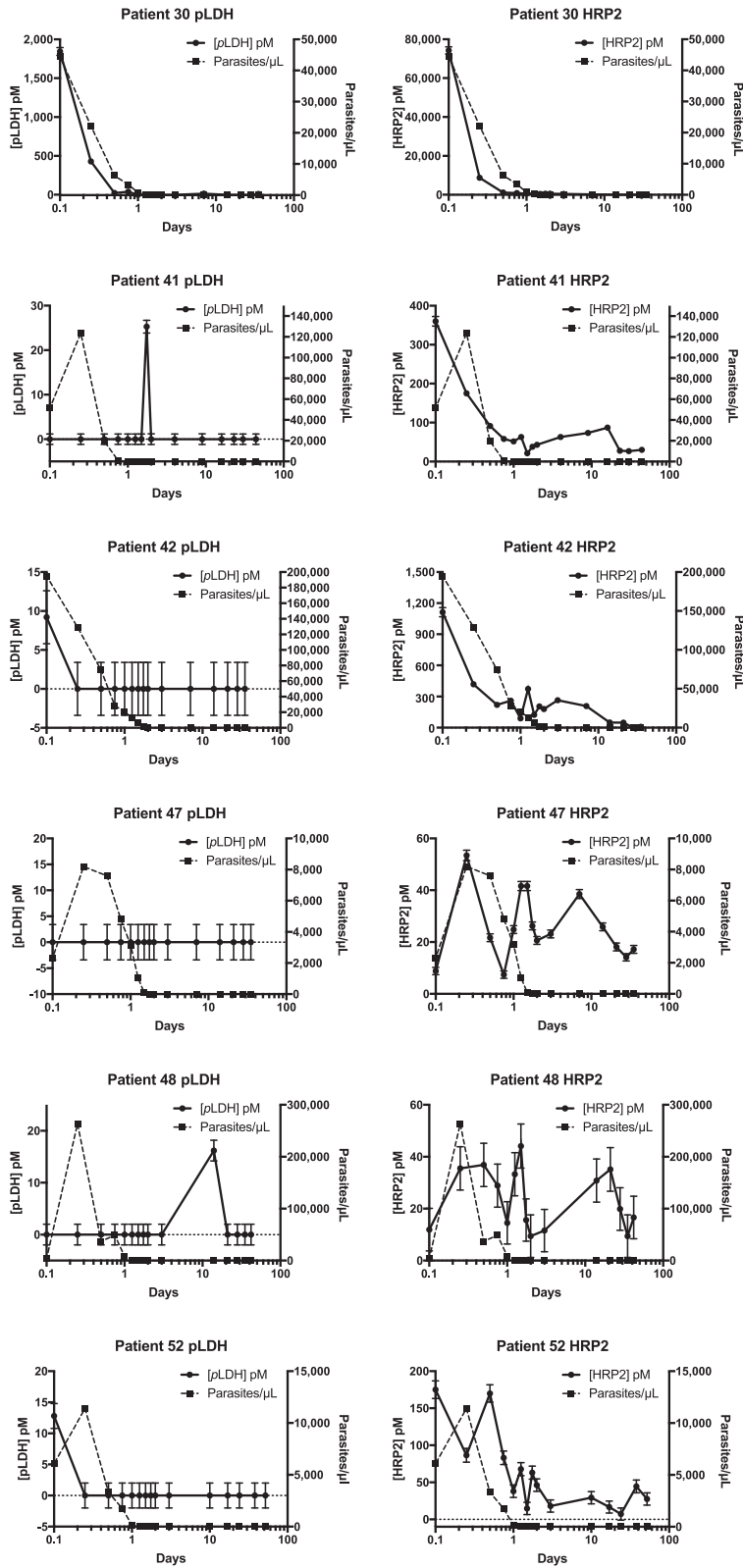
SUPPLEMENTAL FIGURE 1. Recovery of *Plasmodium* lactate dehydrogenase (pLDH) and histidine-rich protein 2 (HRP2) as a function of dried blood spot incubation time in PBS with 0.1% Tween-20. No significant differences were found between extraction efficiencies of pLDH and HRP2, and 10 minutes was chosen as the optimum incubation time.



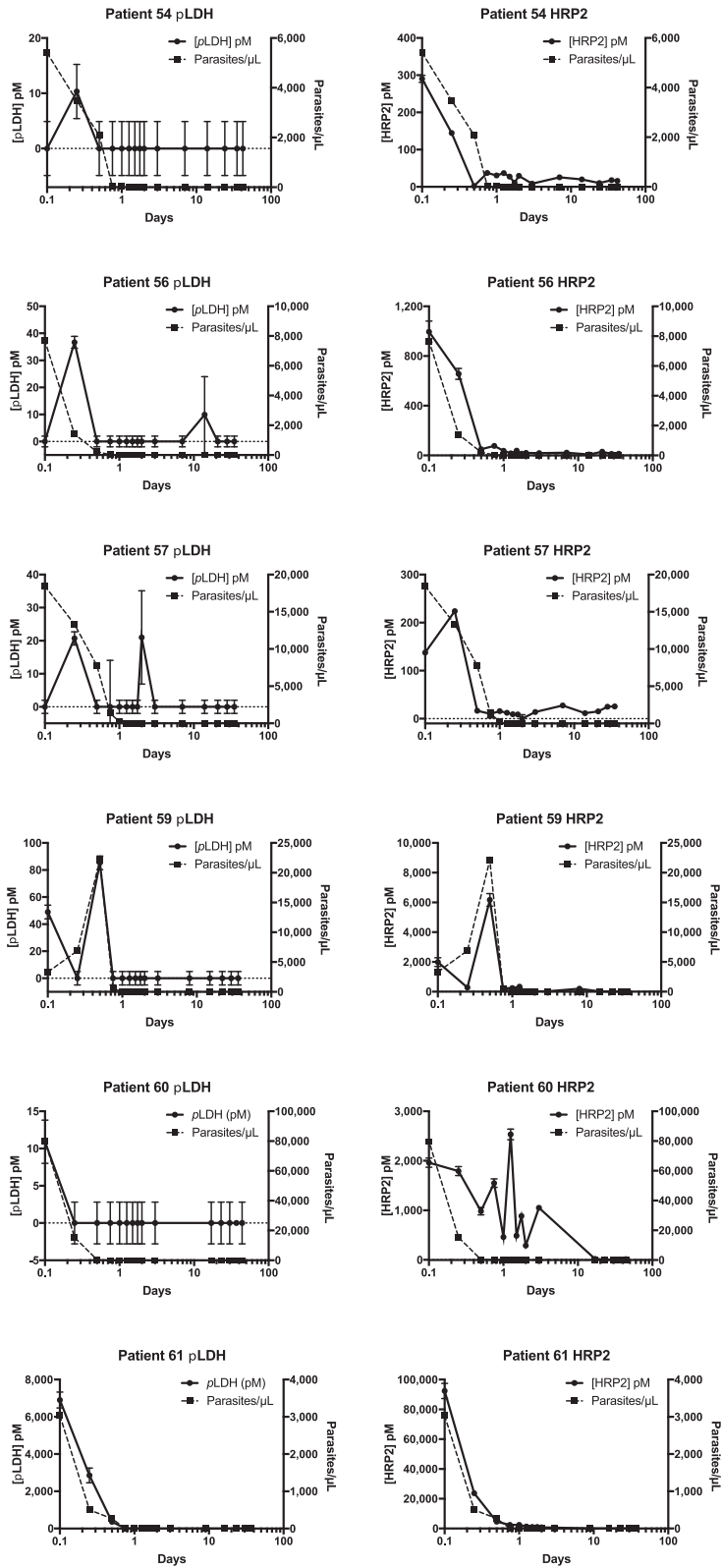
SUPPLEMENTAL FIGURE 2. Optimization of (A) nonfat dried milk concentration in blocking solution, (B) antibody-functionalized magnetic bead volume for *Plasmodium* lactate dehydrogenase (pLDH) (left) and histidine-rich protein 2 (HRP2) (right), and (C) detection antibody concentration for LDH (left) and HRP2 (right).



SUPPLEMENTAL FIGURE 3. Dried blood spot (DBS) storage time at (A) -20°C and (B) room temperature vs. DBS simultaneous capture and sequential detection ELISA signal for *Plasmodium* lactate dehydrogenase (left) and histidine-rich protein 2 (right).



SUPPLEMENTAL FIGURE 4. Biomarker clearance patterns for individual patients from rural Zambia.



SUPPLEMENTAL FIGURE 4. (Continued)

SUPPLEMENTAL TABLE 1  
Patient biomarker concentrations over time

Patient ID	Time (days)	Parasites/ $\mu$ L	$\rho$ LDH (pM)		HRP2 (pM)	
			Average	Error	Average	Error
Patient 29	0	82,880	2,350	70	16,200	900
	0.25	150,189	1,980	70	14,700	900
	0.5	57,258	520	13	9,800	600
	0.75	1,680	49.6	2.0	9,500	600
	1	400	42.0	1.9	10,400	600
	1.25	112	26.8	1.7	4,600	300
	1.5	0	50.0	2.0	5,600	300
	1.75	0	35.9	1.8	10,900	600
	2	0	22.8	1.6	8,688.4	500
	3	0	13	1.6	12,200	709.4
	14	0	0	1.5	0	8
	21	0	0	1.5	0	8
	28	0	16.3	1.6	0	8
	35	0	0	1.5	0	8
42	0	0	11.6	1.6	0	8
Patient 30	0	44,400	1,840	50	74,000	2,000
	0.25	22,194	428	14	8,800	200
	0.5	6,280	22.3	1.4	1,180	40
	0.75	3,400	38.8	1.8	890	30
	1	800	0	1.2	564	19
	1.25	240	0	1.2	700	20
	1.5	112	0	1.2	446	15
	1.75	0	0	1.2	562	19
	2	0	0	1.2	534	18
	3	0	0	1.2	442	15
	7	0	14.7	1.3	134	5
	14	0	0	1.2	14.9	2.0
	21	0	0	1.2	0	2.3
	28	0	0	1.2	0	2.3
35	0	10.1	1.2	22.9	2.4	
Patient 41	0	51,480	0	1.2	360	12
	0.25	123,579	0	1.2	175	6
	0.5	19,440	0	1.2	92	4
	0.75	960	0	1.2	58	3
	1	112	0	1.2	52	3
	1.25	0	0	1.2	63	3
	1.5	0	0	1.2	21.6	2.4
	1.75	0	25.3	1.4	38	3
	2	0	0	1.2	43	3
	4	0	0	1.2	63	3
	9	0	0	1.2	73	3
	16	0	0	1.2	87	4
	23	0	0	1.2	27	3
	30	0	0	1.2	26	3
44	0	0	1.2	30	3	
Patient 42	0	194,840	9	3	1,110	40
	0.25	129,298	0	3	417	11
	0.5	74,267	0	3	220	6
	0.75	28,480	0	3	260	7
	1	20,840	0	3	89	3
	1.25	12,960	0	3	373	10
	1.5	6,800	0	3	123	4
	1.75	1,720	0	3	204	6
	2	720	0	3	180	5
	3	0	0	3	264	7
	7	0	0	3	209	6
	14	0	0	3	49.2	1.9
	21	0	0	3	47.8	1.9
	28	0	0	3	0	1.4
35	0	0	3	7.7	1.4	
Patient 47	0	2,320	0	3	8.8	1.4
	0.25	8,200	0	3	53.4	2.0
	0.5	7,600	0	3	21.7	1.5
	0.75	4,800	0	3	7.4	1.4
	1	3,160	0	3	24.8	1.5
	1.25	1,040	0	3	41.6	1.8
	1.5	80	0	3	41.6	1.8
1.75	0	0	3	26.2	1.5	

(continued)

SUPPLEMENTAL TABLE 1  
Continued

Patient ID	Time (days)	Parasites/ $\mu$ L	$\rho$ LDH (pM)		HRP2 (pM)		
			Average	Error	Average	Error	
Patient 48	2	0	0	3	20.6	1.5	
	3	0	0	3	23.1	1.5	
	7	0	0	3	38.5	1.7	
	14	0	0	3	25.9	1.5	
	21	0	0	3	18.1	1.5	
	28	0	0	3	14.1	1.4	
	35	0	0	3	17.2	1.5	
	0	4,480	0	2.0	12	8	
	0.25	263,530	0	2.0	36	8	
	0.5	37,212	0	2.0	37	8	
	0.75	48,989	0	2.0	29	8	
	1	8,080	0	2.0	15	8	
	1.25	800	0	2.0	33	8	
	1.5	0	0	2.0	44	8	
	1.75	0	0	2.0	16	8	
	2	0	0	2.0	9	8	
	3	0	0	2.0	12	8	
14	0	16.2	2.0	31	8		
21	0	0	2.0	35	8		
28	0	0	2.0	20	8		
35	0	0	2.0	9	8		
42	0	0	2.0	17	8		
Patient 52	0	6,080	12.8	2.0	175	12	
	0.25	11,400	0	2.0	87	9	
	0.5	3,320	0	2.0	170	13	
	0.75	1,760	0	2.0	83	9	
	1	160	0	2.0	38	8	
	1.25	0	0	2.0	68	8	
	1.5	0	0	2.0	15	8	
	1.75	0	0	2.0	63	8	
	2	0	0	2.0	46	8	
	3	0	0	2.0	18	8	
	10	0	0	2.0	29	8	
	17	0	0	2.0	17	8	
	24	0	0	2.0	7	8	
	38	0	0	2.0	45	8	
	52	0	0	2.0	28	8	
	Patient 54	0	5,400	0	5	290	9
		0.25	3,480	10	5	145	5
0.5		2,080	0	5	0	0.9	
0.75		32	0	5	37.3	1.5	
1		32	0	5	30.6	1.3	
1.25		0	0	5	36.9	1.5	
1.5		0	0	5	27.5	1.3	
1.75		0	0	5	10.8	1.0	
2		0	0	5	29.5	1.3	
3		0	0	5	8.4	0.9	
7		0	0	5	25.7	1.2	
14		0	0	5	19.7	1.1	
24		0	0	5	10.2	1.0	
35		0	0	5	17.9	1.1	
42		0	0	5	15.9	1.0	
Patient 55		0	15,040	13	5	790	40
		0.25	11,160	72	6	2,130	90
	0.5	9,960	72	6	1,710	80	
	0.75	800	0	5	237	7	
	1	0	0	5	107	4	
	1.25	0	38	5	162	5	
	1.5	0	0	5	171	5	
	1.75	0	0	5	12.8	1.0	
	2	0	0	5	28.2	1.3	
	3	0	0	5	24.0	1.2	
	7	0	0	5	20.9	1.1	
	14	0	0	5	15.7	1.0	
	21	0	0	5	11.9	1.0	
	28	0	0	5	23.7	1.2	
	35	0	0	5	11.0	1.0	
	Patient 56	0	7,680	0	2.0	1,000	90

(continued)

SUPPLEMENTAL TABLE 1

Continued

Patient ID	Time (days)	Parasites/ $\mu$ L	$\rho$ LDH (pM)		HRP2 (pM)		
			Average	Error	Average	Error	
Patient 57	0.25	1,400	36.7	2.2	660	40	
	0.5	224	0	2.0	49.0	1.3	
	0.75	32	0	2.0	76.5	2.0	
	1	0	0	2.0	37.9	1.0	
	1.25	0	0	2.0	16.6	0.5	
	1.5	0	0	2.0	38.1	1.0	
	1.75	0	0	2.0	13.7	0.4	
	2	0	0	2.0	20.6	0.6	
	3	0	0	2.0	18.8	0.5	
	7	0	0	2.0	22.6	0.6	
	14	0	10	14	8	8	
	21	0	0	2.0	29.5	0.8	
	28	0	0	2.0	12.5	0.4	
	35	0	0	2.0	13.5	0.4	
	0	18,440	0	2.0	138	4	
	0.25	13,320	20.7	2.0	224	6	
	0.5	7,760	0	2.0	16.6	0.5	
0.75	1,360	0	14	10	8		
1	240	0	2.0	15.7	0.4		
1.25	0	0	2.0	12.3	0.4		
1.5	0	0	2.0	9.2	0.3		
1.75	0	0	2.0	9	0.3		
2	0	21	14	0	8		
3	0	0	2.0	13.9	0.4		
7	0	0	2.0	27.7	0.8		
14	0	0	2.0	11.7	0.3		
21	0	0	2.0	15.1	0.4		
28	0	0	2.0	24.9	0.7		
35	0	0	2.0	25.5	0.7		
Patient 58	0	32,360	9	5	3,200	300	
	0.25	21,720	30	5	2,900	300	
	0.5	4,520	0	5	266	4	
	0.75	3,920	10	5	3,500	300	
	1	480	0	5	3,900	300	
	1.25	112	10	5	3,900	300	
	1.5	0	0	5	182	3	
	1.75	0	0	5	77	3	
	2	0	0	5	166	3	
	3	0	0	5	201	3	
	8	0	0	5	910	70	
	15	0	0	5	18	3	
	22	0	0	5	23	2.0	
	29	0	0	5	33	2.0	
	36	0	0	5	54	2.0	
	Patient 59	0	3,320	49	5	1,100	300
		0.25	6,880	0	5	283	4
0.5		22,120	85	5	5,300	400	
0.75		560	0	5	230	4	
1		0	0	5	250	4	
1.25		0	0	5	339	4	
1.5		0	0	5	16	3	
1.75		0	0	5	25	3	
2		0	0	5	31	3	
3		0	0	5	27	3	
8		0	0	5	177	3	
15		0	0	5	22	3	
22		0	0	5	0	3	
29		0	0	5	14	3	
36		0	0	5	16	3	
Patient 60		0	79,840	11	3	1,960	90
		0.25	15,320	0	3	1,790	90
	0.5	400	0	3	980	80	
	0.75	0	0	3	1,550	90	
	1	0	0	3	460	40	
	1.25	0	0	3	2,530	110	
	1.5	0	0	3	490	40	
	1.75	0	0	3	880	50	
	2	0	0	3	290	40	

(continued)



SUPPLEMENTAL TABLE 1  
Continued

Patient ID	Time (days)	Parasites/ $\mu$ L	pLDH (pM)		HRP2 (pM)	
			Average	Error	Average	Error
Patient 61	3	0	0	3	1,050	50
	17	0	0	3	19	7
	23	0	0	3	12	7
	30	0	0	3	0	7
	37	0	0	3	0	7
	44	0	0	3	15	7
	0	3,035	6,400	300	89,000	5,000
	0.25	503	1,510	100	23,700	1,300
	0.5	269	360	60	4,600	200
	0.75	6	11	3	2,400	100
	1	0	0	3	2,420	90
	1.25	0	0	3	1,390	60
	1.5	0	0	3	1,010	50
	1.75	0	0	3	1,050	50
	2	0	0	3	750	40
	3	0	0	3	590	40
	9	0	0	3	18	7
	16	0	0	3	0	7
	23	0	0	3	0	7
	30	0	0	3	0	7
37	0	0	3	0	7	

HRP2 = histidine-rich protein 2; pLDH = *Plasmodium* lactate dehydrogenase.