Supplemental materials

Supplemental Figure 1. SDS-polyacrylamide gel electrophoresis (PAGE) analysis of recombinant pLDH and human LDH proteins. To evaluate the protein purity, recombinant protein in processing and/or final sample was run on SDS-PAGE. The gel was stained with Coomassie blue G250. Migration of pre-stained molecular weight (MW) standard of proteins is shown in the first lane. Protein purity (expressed as a percentage) was estimated by band intensity analysis using Image Lab software (Bio-rad, Hercules, CA). A) *Pf*LDH, 97%; B) *Pm*LDH, 99%; C) *Po*LDH, > 99%; D) *Pv*LDH, 97%; and E) human LDH, 99%.



Supplemental Figure 2. The reactivity of recombinant and native pLDH proteins on PanLDH, *Pf*LDH, and *Pv*LDH spots of the reference assay. A) A serial dilution with recombinant pLDH proteins for four *Plasmodium* species including *P. falciparum*, *P. vivax*, *P. malariae*, and *P. ovale curtisi*, and recombinant human LDH protein as a control was evaluated with the 5-Plex, detecting the range between LDH concentrations of 250–40,000 pg/mL. B) Clinical blood samples with different parasite infection (*P. falciparum* [n =29], *P. vivax* [n = 60], *P. malariae* [n =15], and *P. ovale* [n =4] were tested on the 5-Plex. Parasitemia in samples was determined using microscopy or PCR methods. Plots show the log of the concentration of expected recombinant pLDH protein or parasitemia (x-axis) versus the log of the signal intensity from PanLDH, *Pf*LDH, and *Pv*LDH spots measured with neat samples (y-axis). The lines in (B) represent regression lines of best fit for each set of clinical samples.



Supplemental Table 1. Percentages of *P. knowlesi*–infected red blood cells at different stages over 30 hours of culture after synchronization.

Hour post		Intraerythrocytic stages					
synchronization	Parasitemia (%)	% ring	% schizont				
1	1.49	100	0	0			
2	1.46	100	0	0			
6	1.71	100	0	0			
9	1.48	47	50	3			
19	1.5	3	94	3			
21	0.94	0	9	91			
25	0.9	0	0	100			
27	1.7	30	0	70			
28	2.0	89	0	11			
29	2.34	93	0	7			
30	2.6	98	2	0			

Supplemental Figure 3. Native pLDH amount per parasite estimated by the 5-Plex. The measured PanLDH values were normalized against the parasite density examined by microscopy or qPCR. Any clinical samples which showed unquantifiable or infection-negative test results by the 5-Plex were excluded from analysis. Depicted are box plots of PanLDH amount produced by a parasite of *Plasmodium* species as indicated. The medians are shown by the horizontal lines inside the boxes, the 25th and 75th percentiles are shown as the bottoms and tops of the boxes, and data points outside the range represent outliers. One-way ANOVA plus Sidak multiple comparison test was used to determine the statistical significance relative to PanLDH values measured with *P. falciparum* samples. Significant differences (** p < 0.001). fg, femtogram; ns, not significant

Log₁₀ [PanLDH, fg/parasite]

Supplemental Figure 4. Alignment of pLDH sequences from various *Plasmodium* species. Clustal Omega multiple sequence alignment program (http://www.clustal.org/omega/) was used to align multiple pLDH amino acid sequences from strains of *Plasmodium* species: *P. cynomolgi* Strain B (PCYB), *P. cynomolgi* Strain M (PCYM), *P. knowlesi* strain H (PKNH), *P. knowlesi* Malayan (PKNOH), *P. vivax* P01 (PVP01), *P. vivax* Sal-1 (PVX), *P. falciparum* 3D7 (PF3D7), *P. falciparum* Dd2 (PFDD2), *P. ovale* GH01 (POCGH01), and *P. malariae* UG01 (PMUG01). A conserved region and several species-specific regions are indicated.^{1, 2, 3} The figure was created using MView

(https://www.ebi.ac.uk/Tools/msa/mview/). Coverage and percentage identity values are indicated as cov

and pid, respectively.

							β	strand-turn-		
1 PCYB_123790 2 PCYM_1234100 3 PKNH_1203900 4 PKN0H_5110074400 5 PVP01_1229700 6 PVX_116630 7 PF3D7_1324900 8 PFDD2_130030800 9 POCGH01_12037700 10 PMUG01_12039800	COV 100.0% 100.0% 100.0% 100.0% 100.0% 100.0% 100.0% 100.0% 100.0%	pid 100.0% 97.5% 97.5% 97.2% 97.2% 89.6% 89.6% 88.6% 90.5%	1	[MAPKPKIVLVGSGMIGGVMAT MAPKPKIVLVGSGMIGGVMAT MAPKPKIVLVGSGMIGGVMAT MIPKPKIVLVGSGMIGGVMAT MIPKPKIVLVGSGMIGGVMAT MAPKAKIVLVGSGMIGGVMAT MAPKAKIVLVGSGMIGGVMAT MAPKAKIVLVGSGMIGGVMAT	LIVQKNLGDVVMFDV LIVQKNLGDVVMFDV LIVQKNLGDVVMFDV LIVQKNLGDVVMFDV LIVQKNLGDVVMFDV LIVQKNLGDVVFDI LIVQKNLGDVVFDI LIVQKNLGDVVMFDI	VKNMPQGKALDT VKNMPQGKALDT VKNMPQGKALDT VKNMPQGKALDT VKNMPQGKALDT VKNMPHGKALDT VKNMPHGKALDT VKNMPHGKALDT VKNMPHGKALDT	SHSNVMAYSNCK SHSNVMAYSNCK SHSNVMAYSNCK SHSNVMAYSNCK SHSNVMAYSNCK SHSNVMAYSNCK SHTNVMAYSNCK SHTNVMAYSNCK SHTNVMAYSNCK SHTNVMAYSNCK SHTNVMAYSNCK	c. helix VTGSNSYEDLKS VTGSNSYEDLES VTGSNSYEDLES VTGSNSYEDLES VTGSNSYDDLKS VSGSNTYDDLKS VSGSNTYDDLAS VSGSNTYDDLAS VSGSNTYDDLAS VTGSNSYEDLKS	ADLVIVTA ADLVIVTA ADVVIVTA ADVVIVTA ADVVIVTA ADVVIVTA ADVVIVTA ADVVIVTA ADVVIVTA ADVVIVTA	80
	cov	pid	81	loop 1			<i>Pf</i> -spe	cific 1	. 1	160
1 PCVB 123790	100.0%	100.0%		GETKAPGKSDKEWNRDDLLPL	NNKTMTETGGHTKNI					
2 PCVM 123/100	100.0%	100.0%			NNKTMTETGGHTKNI	CPNAETTVVTNP	VDVMVOL L EEHS		VIDTSRIK	
3 PKNH 1203900	100.0%	97.5%		GETKAPGK5DKEWNRDDLLPL	NNKTMTETGGHTKKI	CPNAFTTVVTNP	VDVMVOL L FEHS		VIDTSRIK	
A PKNOH \$110074400	100.0%	97.5%			NNKTMTETGGHTKKI	CPNAFTTVVTNP	VDVMVOL L FEHS		VIDTSRIK	
5 PVP01 1229700	100.0%	97.2%		GETKAPGK5DKEWNRDDLLPL	NNKIMIEIGGHIKNL	CPNAFIIVVTNP	VDVMVOLLFEHS	GVPKNKIIGLGG	VLDTSRLK	
6 PVX 116630	100.0%	97.2%		GETKAPGKEDKEWNRDDLLPL	NNKIMIEIGGHIKNL	CPNAFIIVVTNP	VDVMVOLLFEHS	GVPKNKIIGLGG	VLDTSRLK	
7 PF3D7 1324900	100.0%	89.6%		GFTKAPGK5DKEWNRDDLLPL	NNKIMIEIGGHIKKN	CPNAFIIVVTNP	VDVMVOLLHOHS	GVPKNKIIGLGG	VLDTSRLK	
8 PFDD2 130030800	100.0%	89.6%		GFTKAPGK5DKEWNRDDLLPL	NNKIMIEIGGHIKKN	CPNAFIIVVTNP	VDVMVOLLHOHS	GVPKNKIIGLGG	VLDTSRLK	
9 POCGH01_12037700	100.0%	88.6%		GFTKAPGK5DKEWNRDDLLPL	NNKIMIEIGGHIKNY	CPNAFIIVVTNP	VDVMVÕLLHÕHS	GVSKNKIVGLGG	VLDTSRLK	
10 PMUG01_12039800	100.0%	90.5%		GFTKVPGK5DKEWNRDDLLPL	NNKIMIEIGGHVKNY	CPNAFIIVVTNP	VDVMVQLLHKHS	GVPKNKIVGLGG	VLDTSRLK	
				Pan-specific ²						
	cov	pid	161	. Pan-specific	· ·	2 0	t helix and loop		. :	240
1 PCYB 123790	100.0%	100.0%		YYLSQKLNVCPRDVNALIVGA	HGNKMVLLKRYITVG	GIPLQEFINNKK	ITDEEVEAIFNR	TINTALEIVNLL	ASPYVAPA	
2 PCYM 1234100	100.0%	100.0%		YYLSQKLNVCPRDVNALIVGA	HGNKMVLLKRYITVG	GIPLQEFINNKK	ITDEEVEAIFNR	TINTALEIVNLL	ASPYVAPA	
3 PKNH 1203900	100.0%	97.5%		YYLSQKLNVCPRDVNALIVGA	HGNKMVLLKRYITVG	GIPLQEFINNKK	ITDEEVEAIFDR	TVNTALEIVNLL	ASPYVAPA	
4 PKNOH S110074400	100.0%	97.5%		YYLSQKLNVCPRDVNALIVGA	HGNKMVLLKRYITVG	GIPLQEFINNKK	ITDEEVEAIFDR	TVNTALEIVNLL	ASPYVAPA	
5 PVP01_1229700	100.0%	97.2%		YYISQKLNVCPRDVNALIVGA	HGNKMVLLKRYITVG	GIPLQEFINNKK	ITDEEVEGIFDR	TVNTALEIVNLL	ASPYVAPA	
6 PVX_116630	100.0%	97.2%		YYISQKLNVCPRDVNALIVGA	HGNKMVLLKRYITVG	GIPLQEFINNKK	ITDEEVEGIFDR	TVNTALEIVNLL	ASPYVAPA	
7 PF3D7_1324900	100.0%	89.6%		YYISQKLNVCPRDVNAHIVGA	HGNKMVLLKRYITVG	GIPLQEFINNKL	ISDAELEAIFDR	TVNTALEIVNLH	ASPYVAPA	
8 PFDD2_130030800	100.0%	89.6%		YYISQKLNVCPRDVNAHIVGA	HGNKMVLLKRYITVG	GIPLQEFINNKL	ISDAELEAIFDR	TVNTALEIVNLH	ASPYVAPA	
9 POCGH01_12037700	100.0%	88.6%		YYISQKLNVCPRDVNAHIVGA	HGNKMVVLKRYITVG	GIPLQEFINNKK	ITDAELDAIFDR	TVNTALEIVNYH	ASPYVAPA	
10 PMUG01_12039800	100.0%	90.5%		YYISQKLNVCPRDVNALIVAA	HGNKMVPLKRYITVG	GIPLQEFINNKK	ITDAELDAIFDR	TVNTALEIVNLH	ASPYVAPA	
						P	v or <i>Pf</i> -specific ²	Charles.		
	cov	p1d	241	:		•		Chelix .	J 316	
1 PCYB_123790	100.0%	100.0%		AATTEMAESYLKDIKKVLVCS	TLLEGQYGHSNIFGG	TPLVIGGIGVEQ	VIELQLNAEEKA	KFDEAVAETKRM	KALV	
2 PCYM_1234100	100.0%	100.0%		AATTEMAESYLKDIKKVLVCS	TLLEGQYGHSNIFGG	TPLVIGGIGVEQ	VIELQLNAEEKA	KFDEAVAETKRM	KALV	
3 PKNH_1203900	100.0%	97.5%		AATTEMAESYLKDIKKVLVCS	TLLEGQYGHKNIFGG	TPLVIGGIGVEQ	VIELQLIAEEKA	KFDEAVAETKRM	KALI	
4 PKNOH_\$110074400	100.0%	97.5%		AATTEMAESYLKUTKKVLVCS	TILEGUYGHKNIFGG	TRUVIGGIGVEQ	VIELQUIALEKA	KEDEAVAETKRM	KALI	
5 PVP01_1229/00	100.0%	97.2%		AATTEMAESTERUTKKVEVCS	TILECOVCHENTECC	TPLVIGGTGVEQ	VIELQUNAEEKI	CEDEAVAETKRM	KALI T	
6 PVX_116630	100.0%	90.6%		AATTEMAESVI KDI KKVLVCS	TLLEGOVCHEDIECC	TRIVICANCIED	VIELOLNSEEKA		KALA	
/ PF3D/_1324900	100.0%	80.6%				TDV/LGANGVEQ	VTELOLINSEEKA		KALA	
0 PCCH01 12027200	100.0%	88 64			TILEGOVGHTGVEGG	TPLVLGCNGVEQ	VEELOLNAEEVVI		KALA	
10 PMUC01 1203000	100.0%	90.5%				TPI VI GANGVEQ	VTELOLNSEEVVI		KALV	
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over the erythrocytic cycle of the P. knowlesi culture. **RBC** pellet Supernatant Hour post PanLDH **PvLDH** PanLDH **PvLDH** Pan/Pv Pan/Pv synchronization (pg/mL) (pg/mL) (pg/mL) (pg/mL) 73,974.3 31,775.3 823.1 296.1 1 2.33 2.78 2 75,550.5 32,739.1 2.31 638.7 293.4 2.18 135,437.4 58,408.3 2.32 1,001.6 447.5 2.24 6 224,167.8 98,373.0 1,378.5 2.34 9 2.28 589.3 461,451.6 258,914.0 1.78 1,905.4 2.63 19 5,018.9 523,228.5 305,626.5 21 1.71 5,096.4 2,001.0 2.55 25 439,765.7 244,306.3 1.80 18,473.4 8,559.6 2.16 454,845.4 221,902.3 27 2.05 22,611.3 9,234.2 2.45 28 260,037.4 114,659.1 2.27 34,634.2 16,968.0 2.04 29 242,654.4 107,746.1 2.25 46,014.4 21,959.7 2.10 30 301,172.8 135,057.2 2.23 73,191.8 29,993.0 2.44 2.35 2.11 Mean SD 0.24 0.24

Supplemental Table 2. PanLDH/PvLDH ratio measured with the RBC pellet and supernatant materials

SD: standard deviation, CV: coefficient of variation, Pan/Pv: Pan LDH/PvLDH

References

- 1. Verma P, Biswas S, Mohan T, Ali S, Rao DN, 2013. Detection of histidine rich protein & lactate dehydrogenase of Plasmodium falciparum in malaria patients by sandwich ELISA using in-house reagents. Indian J Med Res 138: 977-987.
- 2. Hurdayal R, Achilonu I, Choveaux D, Coetzer THT, Goldring JPD, 2010. Anti-peptide antibodies differentiate between plasmodial lactate dehydrogenases. Peptide 31: 523-532.
- 3. Tomar D, Biswas S, Tripathi V, Rao DN, 2006. Development of diagnostic reagents: raising antibodies against synthetic peptides of PfHRP-2 and LDH using microsphere delivery. Immunobiol 211: 797-805.