

Supplementary Materials: Smilansky *et al.*

Table S2. Cq values for the Perkinsea PPC and NAG01a-c uniplex and duplex qPCR assays. Cq values per template dilution used to determine the efficiency and sensitivity of the NAG01 Perkinsea strain-specific qPCR assays in both uniplex and duplex conditions. Also presented are Cq values for PPC (KNA_DNA) and NAG01a+ (G2.13 and G8.1) DNA samples, extracted from tadpole livers. Grey highlights correspond to the linear dynamic ranges calculated for each assay. Dark grey highlights correspond to the upper and lower limits of detection calculated for each assay. “NA” in the Replicate columns indicates that the BioRad software was unable to calculate a Cq. An asterisk (*) in the ‘Replicate’ columns indicates that the regression method (as opposed to the default single threshold method) was used to determine Cq due to a high level of background noise. The regression method uses multiple data points to determine Cq and is therefore less sensitive to noise.

PPC uniplex assay

	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Average Cq	SD	ΔCq
1*10 ⁻¹⁰ ng	36.74	No amp.	36.95	No amp.	36.845	0.148492	NA
1*10 ⁻⁹ ng	36.05	38.25	No amp.	37.63	37.31	1.134372	-0.465
1*10 ⁻⁸ ng	36.01	No amp.	38.03	36.42	36.82	1.067755	0.49
1*10 ⁻⁷ ng	No amp.	37.01	37.63	37.4	37.3467	0.313422	-0.52667
1*10 ⁻⁶ ng	33.12	33.29	33.38	33.52	33.3275	0.167606	4.019167
1*10 ⁻⁵ ng	30.42	30.31	30.25	30.04	30.255	0.159687	3.0725
1*10 ⁻⁴ ng	26.64	26.73	26.66	26.83	26.715	0.085829	3.54
1*10 ⁻³ ng	23.17	23.3	23.13	23.17	23.1925	0.074106	3.5225
0.01 ng	19.05	19.16	19.04	18.99	19.06	0.071647	4.1325
0.1 ng	15.35	15.59	15.48	15.42	15.46	0.101653	3.6
1 ng	12.24	12.11	12.16	12.13	12.16	0.057155	3.3
NTC	No amp.	No amp.	No amp.	No amp.	NA	NA	NA
KNA_DNA	20.19	20.27			20.23	0.056569	

NAG01a-c uniplex assay

	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Average Cq	SD	ΔCq
1*10 ⁻¹⁰ ng	No amp.	No amp.	No amp.	No amp.	NA	NA	NA
1*10 ⁻⁹ ng	No amp.	NA	No amp.	No amp.	NA	NA	NA
1*10 ⁻⁸ ng	No amp.	No amp.	No amp.	No amp.	NA	NA	NA
1*10 ⁻⁷ ng	No amp.	No amp.	No amp.	No amp.	NA	NA	NA
1*10 ⁻⁶ ng	35.68	36.13	NA	NA	35.905	0.318198	NA
1*10 ⁻⁵ ng	31.42	31.39	NA	32.75	31.405	0.021213	4.5
1*10 ⁻⁴ ng	30.33	27.48	26.05	27.67	26.765	1.011163	4.64
1*10 ⁻³ ng	25.39	23.99	25.8	24.68	25.06	0.949052	1.705
0.01 ng	21.95	21.42	22.03	20.17	21.8	0.331512	3.26
0.1 ng	18.41	17.86	NA	18.02	18.135	0.388909	3.665
1 ng	15.74	16.04	16.71	15.09	15.89	0.212132	2.245
NTC	No amp.	No amp.	No amp.	No amp.	NA	NA	NA
G2.13	NA	No amp.	No amp.		NA	NA	
G8.1	33.97	NA	33.81		33.89	0.113137	

PPC duplex assay

	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Average Cq	SD	ΔCq
1*10 ⁻⁷ ng	36.5	37.16	33.97	35.49	35.78	1.388404	NA
1*10 ⁻⁶ ng	31.29	30.73	33.73	32.51	32.065	1.335802	3.715
1*10 ⁻⁵ ng	28.6	28.44	28.58	28.53	28.5375	0.071356	3.5275
1*10 ⁻⁴ ng	25.35	25.42	25.23	25.5	25.375	0.114455	3.1625
1*10 ⁻³ ng	22.13	23.11	22.94	22.91	22.7725	0.437293	2.6025
0.01 ng	19.21	18.15	18.43	18.26	18.5125	0.479053	4.26
0.1 ng	17.5	16.05	17.32	15.16	16.5075	1.106085	2.005
1 ng	11.4	12.15	13.55	12.08	12.295	0.90246	4.2125
NTC	No amp.	No amp.	No amp.	No amp.	NA	NA	NA

KNA_DNA	21.24	21.13	21.87		21.4133	0.399291	
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NAG01a-c duplex assay

	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Average Cq	SD	ΔCq
1*10 ⁻⁷ ng	37.43	32.33	37.23	33.9	35.2225	2.517848	NA
1*10 ⁻⁶ ng	NA	28.58	NA	33.11	30.845	3.203194	4.3775
1*10 ⁻⁵ ng	28.01	28.13	29.25	28.44	28.4575	0.558532	2.3875
1*10 ⁻⁴ ng	27.62*	25.13	26.08	23.25*	25.52	1.828351	2.9375
1*10 ⁻³ ng	22.06	24.1	22.13	23.52	22.9525	1.018475	2.5675
0.01 ng	18.78	15.57	16.36	17.21	16.98	1.374215	5.9725
0.1 ng	13.95*	16.74	12.16*	13.43	14.07	1.93227	2.91
1 ng	10.46*	8.82*	7.37*	12.46	9.7775	2.188948	4.2925
NTC	No amp.	No amp.	No amp.	No amp.	NA	NA	NA

G8.1	33.01	32.77	NA		32.89	0.169706	
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