Supplementary Material

Universal Enzyme-Based Field Workflow for Rapid and Sensitive Quantification of Water Pathogens

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Supplementary Table S1. List of primers used for quantification PCR against *C. jejuni*, *C. parvum*, *G. lamblia* and *E. coli.* Primer sets marked with asterisks (*) were selected for further optimisation and quantification.

Organism	Primer name	Target gene	Amplicon size (bp)	Sequence	Reference
Campylobacter jejuni	*CJ HipO-F *CJ HipO-R	*HipO*	100	5' TGCTAGTGAGGTTGCAAAAGAATT 3' 5' TCATTTCGCAAAAAAATCCAAA 3'	[1]
Cryptosporidium parvum	*COWP P702F	*COWP*	151	5′ CAAATTGATACCGTTTGTCCTTCTG 3′	[2]
	*COWP P702R			5′ GGCATGTCGATTCTAATTCAGCT 3′	
	CRULib13F CRULib13R	*Lib13*	166	5′ TCCTTGAAATGAATATTTGTGACTCG 3′ 5′ TTAATGTGGTAGTTGCGGTTGAAC 3′	[3]
Giardia lamblia	gdhF gdhR	*gdh*	261	5′ GGGCAAGTCCGACAACGA 3′ 5′ GCACATCTCCTCCAGGAAGTAGAC 3′	[4]
	*P241F *P241R	*β-Giardin*	75	5′CATCCGCGAGGAGGTCAA 3′ 5′GCAGCCATGGTGTCGATCT 3	[2]
Escherichia coli	401 F 611 R	*ybbW*	211	5′ TGATTGGCAAAATCTGGCCG 3′ 5′ GAAATCGCCCAAATCGCCAT 3′	[5]
	*uidA_F *uidA_R	*uidA*	70	5′ CGGAAGCAACGCGTAAACTC 3′ 5′ TGAGCGTCGCAGAACATTACA 3′	[5]

Supplementary Table S2 Optimal concentrations for primer combinations as determined by titration. Mean and STD Cp calculated from triplicate reactions. Highlighted cells indicate best performing primer set against each pathogen that were used for all subsequent experiments. Abbreviations: F/R – forward/reverse primers. 100-500 nm -final concentration of 100-500 nM per reaction. Highlighted cells – best performing primer concentrations.

	C. jejuni		*C. parvum*		*G. lamblia*		*E. coli*	
Primers used	HipO-F + HipO-R		COWP 702F + COWP 702R		P241F + P241R		uidA_F + uidA_R	
Primer concentrations	MeanCp	STD Cp	Mean Cp	STD Cp	MeanCp	STD Cp	MeanCp	STD Cp
F100 nm - R100 nm	27.53	0.06	24.78	0.03	29.33	0.26	27.48	0.19
F100 nm - R250 nm	27.47	0.02	24.84	0.03	29.59	0.27	27.01	0.12
F100 nm - R500 nm	27.48	0.03	24.77	0.10	29.56	0.25	26.06	0.10
F250 nm - R100 nm	26.90	0.03	23.89	0.02	27.91	0.19	26.34	0.04
F250 nm - R250 nm	27.12	0.05	24.08	0.02	28.35	0.09	25.89	0.02
F250 nm - R500 nm	27.16	0.04	24.03	0.04	28.54	0.15	25.93	0.04
F500 nm - R100 nm	26.88	0.04	23.81	0.02	27.18	0.06	26.06	0.13
F500 nm - R250 nm	27.07	0.06	24.00	0.03	27.65	0.11	25.85	0.03
F500 nm - R500 nm	26.98	0.04	24.03	0.03	27.81	0.30	25.89	0.02

Supplementary Table S3. Final concentration of primer pairs used for primer titration experiment.

		Forward (F) primer		
		100 nM	250 nM	500 nM
Reverse (R)	100 nM	F100-R100	F250-R100	F500-R100
primer	250 nM	F100-R250	F250-R250	F500-R250
	500 nM	F100-R500	F250-R500	F500-R500

Supplementary Table S4. Sensitivity of qPCR against the four pathogens. Abbreviation 1E-1 to 1E-6 – serial dilutions of pathogen templates, NTC – No template control. CV%-coefficient of variation. LoQ – limit of quantification. LoD – limit of detection. NA – no amplification. UA – unspecific amplification based on melt curve analysis. Linear regression line established with series of six ten-fold dilutions. LgY- log of cell numbers/genome copies, lgX- log of mean Cp.

Samples	Cp (mean)	Cp (SD)	Estimated genome copies/cell count (mean)	Estimated genome copies/cell count (SD)	CV (%)	Sensitivity	Linear regression line
C. jejuni 1E-1	24.50	0.04	7107.12	209.51	3%		
C. jejuni 1E-2	27.58	0.05	766.25	25.46	3%		
C. jejuni 1E-3	30.67	0.11	103.43	6.87	7%		lgY=(lgX-1.5937)/-0.0531
C. jejuni 1E-4	33.45	0.32	20.17	3.30	16%		
C. jejuni 1E-5	37.84	0.87	1.98	0.69	35%	LoQ, LoD	
C. jejuni NTC	*NA*	-	-	-	-	-	
G. lamblia 1E-1	29.34	0.30	326.55	63.04	19%		
G. lamblia 1E-2	30.26	0.22	169.31	24.02	14%		
G. lamblia 1E-3	32.67	0.15	32.73	3.04	9%		lgY = (lgX-1.5849)/-0.0467
G. lamblia 1E-4	34.28	0.50	11.71	3.14	27%	LoQ	
G. lamblia 1E-5	35.67	1.86	4.99	3.31	66%	LoD	
G. lamblia NTC	*UA*	-	-	-	-	-	
C. parvum 1E-1	19.14	0.05	79780.35	3457.10	4%		
C. parvum 1E-2	21.98	0.01	6339.47	42.90	1%		
C. parvum 1E-3	25.00	0.01	603.88	2.08	1%		
C. parvum 1E-4	27.72	0.10	91.18	5.75	6%		lgY= (lgX-1.55)/-0.0547
C. parvum 1E-5	29.72	0.29	25.57	4.18	16%		
C. parvum 1E-6	32.95	0.70	3.88	1.24	32%	LoD, LoQ	
C. parvum NTC	*NA*	-	-	-	-	-	
E. coli 1E-4	30.80	0.05	95.64	3.12	3%		
E. coli 1E-5	33.60	0.41	18.59	3.78	20%	LoQ	lgY = (lgX-1.6836)/-0.0481
E. coli 1E-6	38.03	1.61	1.80	0.98	54%	LoD	
E. coli NTC	*NA*	-	-	-	-	-	

Supplementary Table S5.1 Results of water analysis – inorganics.

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Australian Government

National Measurement Institute

QUALITY ASSURANCE REPORT

Client: **MACQUARIE UNIVERSITY**

NMI QA Report No: MACQ20/201016 T1

Sample Matrix: **Water**

Analyte	Method	LOR	Blank	Duplicates			Recoveries	
				Sample	Duplicate	RPD	LCS	Matrix Spike
		ug/L	ug/L	ug/L	ug/L	%	%	%
Inorganics Section				N20/024380				N20/024380
Arsenic	NT2.47	1	<1	<1	<1	NA	90	102
Cadmium	NT2.47	0.1	<0.1	<0.1	<0.1	NA	94	96
Chromium	NT2.47	1	<1	<1	<1	NA	93	105
Copper	NT2.47	1	<1	160	160	2	96	97
Lead	NT2.47	1	<1	<1	<1	NA	101	98
Mercury	NT2.47	0.1	<0.1	<0.1	<0.1	NA	95	95
Nickel	NT2.47	1	<1	2.4	2.5	NA	96	104
Zinc	NT2.47	1	<1	32	33	0	85	98
Calcium Total- **ppm**	NT2.47	0.005	<0.005	7.2	7.3	1	98	100
Magnesium Total-**ppm**	NT2.47	0.005	<0.005	4.6	4.6	0	99	99
PotassiumTotal-**ppm**	NT2.47	0.05	<0.05	1.7	1.7	0	94	98
Sodium Total-**ppm**	NT2.47	0.05	<0.05	12	12	0	98	100

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Legend:
Acceptable recovery is 75-120%.
Acceptable RPDs on duplicates is 44% at concentrations >5 times LOR. Greater RPD may be expected at <5 times LOR.
LOR = Limit Of Reporting ND = Not Determined
RPD = Relative Percent Difference NA = Not Applicable
LCS = Laboratory Control Sample.
#: Spike level is less than 50% of the sample's concentration, hence the recovery data cannot be reported.

Comments:
Results greater than ten times LOR have been rounded to two significant figures.
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Supplementary Table S5.2 Results of water analysis – chlorine, fluoride and CaCO₃.

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Australian Government

National Measurement Institute

QUALITY ASSURANCE REPORT

Client: Macquarie University Environment & Geography

NMI QA Report No: MACQ20/201016 QA   **Sample Matrix:**   water

Analyte	Method	LOR	Blank	Duplicates			Recoveries	
				Sample	Duplicate	RPD	Matrix spk	LCS
		mg/L	mg/L	mg/L	mg/L	%	%	%
Waters Section				N20/024379			N20/024379	
Chlorine - Total	NW_S16	0.05	<0.05	<0.05	NA	NA	NA	105
Bicarbonate as CaCO3	NW_B1	5	<5	<5	NA	NA	NA	NA
Carbonate as CaCO3	NW_B1	5	<5	22	NA	NA	NA	NA
Hydroxide as CaCO3	NW_B1	5	<5	<5	NA	NA	NA	NA
Alkalinity - Total as CaCO3	NW_B1	5	<5	20	NA	NA	NA	80
Fluoride	NW_B3_B14	0.1	<0.1	1.1	NA	NA	NA	111

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Legend

Acceptable recovery is 80-120%.

Acceptable RPDs on duplicates is 30% at > 5 times LOR. Greater RPD may be expected at < 5 LOR.

LOR = Limit Of Reporting   ND = Not Determined

RPD = Relative Percent Difference   NA = Not Applicable

LCS = Laboratory Control Sample.

Comments

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Results greater than ten times LOR have been rounded to two significant figures.

Supplementary Table S6. Overall quantification or recovery efficiency of molecular-based pathogen quantification workflows.

Methods	Target organisms	Quantification/Recovery efficiency		References
Filtration and qPCR	*Giardia spp.* and *Cryptosporidium spp.*	*Cryptosporidium* 70%	*Giardia* 84.9%	Al-Sabi *et al.* [6]
Immuno-separation (IMS)+ Fluorescent antibody (FA) + Filtration	*Giardia spp.* and *Cryptosporidium spp.*	*Cryptosporidium* FA 95% IMS+FA 92% Filtration+IMS+FA 18%	*Giardia* FA 94% IMS+FA 89% Filtration+IMS+FA 77%	Hu *et al.*[7]
Flow-cytometry, field deployment adaptations	*Giardia spp.* and *Cryptosporidium spp.*	*Cryptosporidium* 13%	*Giardia* 30%	Keserue *et al.* [8]
qPCR after DNA extraction	*Giardia spp.* and *Cryptosporidium spp.*	DNA extraction efficiency affected by inhibitors		Guy *et al.*[2]
Microfluidics and qPCR	Multiple Food- and Waterborne Pathogens	qPCR efficiency 90-110% (30 pathogens) Recovery efficiency 22-27% (EHEC only)		Ishii *et al.*[9]
Digital PCR and q PCR	*Campylobacter jejuni*	Incomplete recovery		Papić *et al.* [10]
Direct qPCR, Integrated Cell Concentration and DNA Purification	*Campylobacter jejuni*	Subject to higher cell number, requires centrifugation		Rudi *et al.* [11]

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