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Supplementary Information for

Avoiding Commercial Kit-Based DNA Isolation and Purification Steps: A Rapid Method for *Cryptosporidium* Oocyst Detection

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725

Species/Abbrv	
1. XM 662396.1(Ref seq)	CAGATTTGATGGACAAAGAAACTAGAATATTAATTAATCC
2. AY161084.1	CAGATTTGATGGACAAAGAAACTAGAATATTAATTAATCC
3. AB119648.1	CAGATTTGATAGACAAAGAAACTAGAATATTAATTAATCC
4. AB119646.1	CAGATTTGATGGACAAAGAAACTAGAATATTAATTAATCC

F3 F2(FIP)

Species/Abbrv	
1. XM 662396.1(Ref seq)	ATCTGGCAGATTTACAATTGGGGGGCCAGCAGCAGATGCT
2. AY161084.1	ATCTGGCAGGTTTACAATTGGTGGGCCAGCAGCAGATGCT
3. AB119648.1	ATCTGGCAGATTTACAATTGGGGGGCCAGCAGCGGATGCT
4. AB119646.1	ATCTGGCAGATTTACAATTGGGGGGCCAGCAGCAGATGCT

F2(FIP) LF F1c(FIP)

Species/Abbrv	
1. XM 662396.1(Ref seq)	GGATTAACAGGGCGCAAGATAATTGTAGATACATACGGAG
2. AY161084.1	GGATTAACAGGGCGCAAGATAATTGTAGATACATACGGAG
3. AB119648.1	GGATTAACAGGGCGCAAAATAATTGTAGATACATACGGAG
4. AB119646.1	GGATTAACAGGGCGCAAGATAATTGTAGATACATACGGAG

F1c(FIP) B1(BIP)

Species/Abbrv	
1. XM 662396.1(Ref seq)	GATGGGGTGCTCATGGGGGTGGTGCATTTAGCGGGAAAGA
2. AY161084.1	GATGGGGTGCTCATGGGGGTGGTGCATTTAGCGGGAAAGA
3. AB119648.1	GATGGGGTGCACATGGAGGTGGTGCATTTAGCGGGAAAGA
4. AB119646.1	GATGGGGTGCTCATGGGGGTGGTGCATTTAGCGGGAAAGA

B1(BIP) LB B2c(BIP)

Species/Abbrv	
1. XM 662396.1(Ref seq)	TGCAACTAAAGTAGATAGATCAGGTGCATATATGGCAAGA
2. AY161084.1	TGCAACTAAAGTAGATAGATCAGGTGCATATATGGCAAGG
3. AB119648.1	TGCAACTAAAGTAGATAGGTCAGGCGCATATATGGCAAGG
4. AB119646.1	TGCAACTAAAGTAGATAGATCAGGTGCATATATGGCAAGA

B2c(BIP) 940

Species/Abbrv	
1. XM 662396.1(Ref seq)	CTTGTTGCAAAGTCAATCGTCTTTTCTGGCTTGTGTAGCA
2. AY161084.1	CTTGTTGCAAAGTCAATCGTCTTTTCCGGCTTGTGTAGCA
3. AB119648.1	CTTGTTGCAAAGTCAATCGTCTTTTCTGGATTGTGTAGCA
4. AB119646.1	CTTGTTGCAAAGTCAATCGTCTTTTCTGGCTTGTGTAGCA

B3

Figure S1. *LAMP primer orientation in multiple sequence alignment of S-adenosyl-l-synthetase (SAM) gene of Cryptosporidium parvum (AB119646.1 and AY161084.1), C. hominis (XM_662396.1) and C. meleagridis (AB119648.1).*

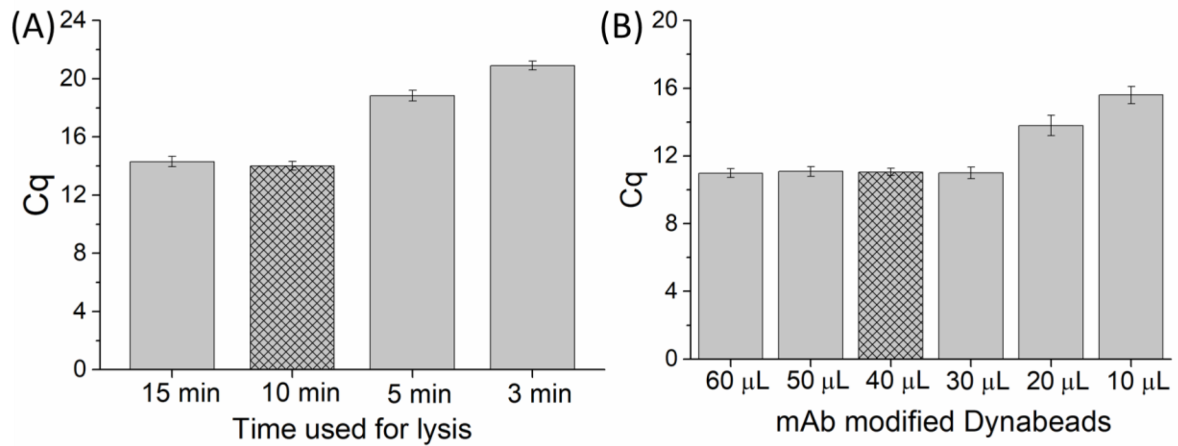

Figure S2. *Optimisation analytical parameters. (A) Optimisation of heat(95°C) lysis time (3, 5, 10, 15 min (B) the number of magnetic beads for immunomagnetic isolation (IMS). Each bar represents the mean Cq value, and the error bar denotes the standard deviation of four measurements.*

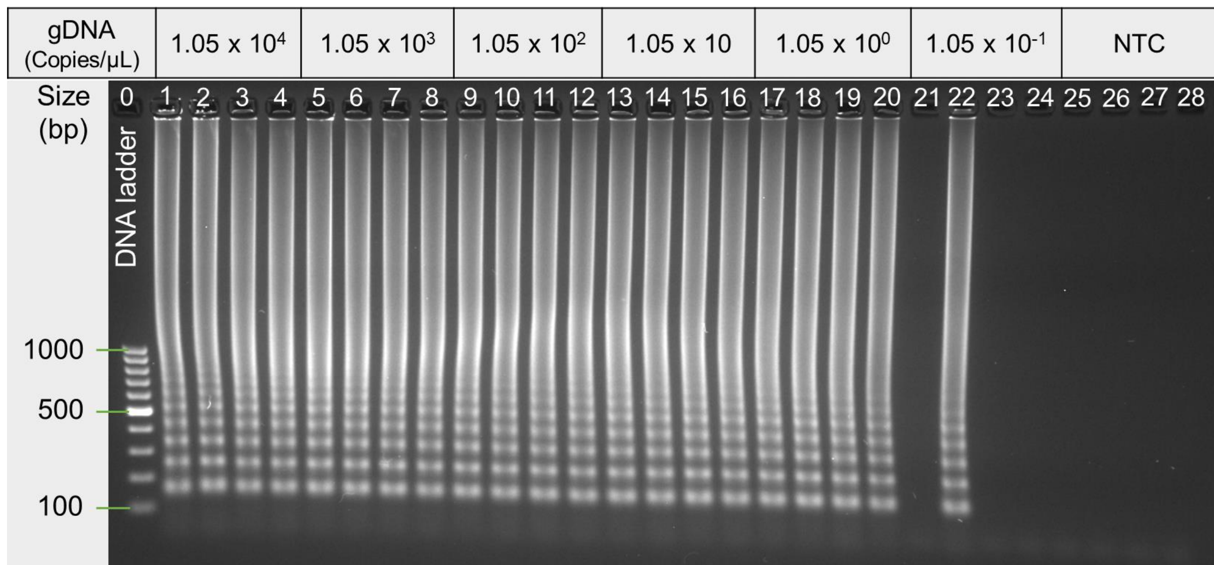

Figure S3. *Gel electrophoresis of colourimetric LAMP products of different concentrations of C. parvum genomic DNA (1.05×10^4-1.05×10^{-1} Copies/µL).*

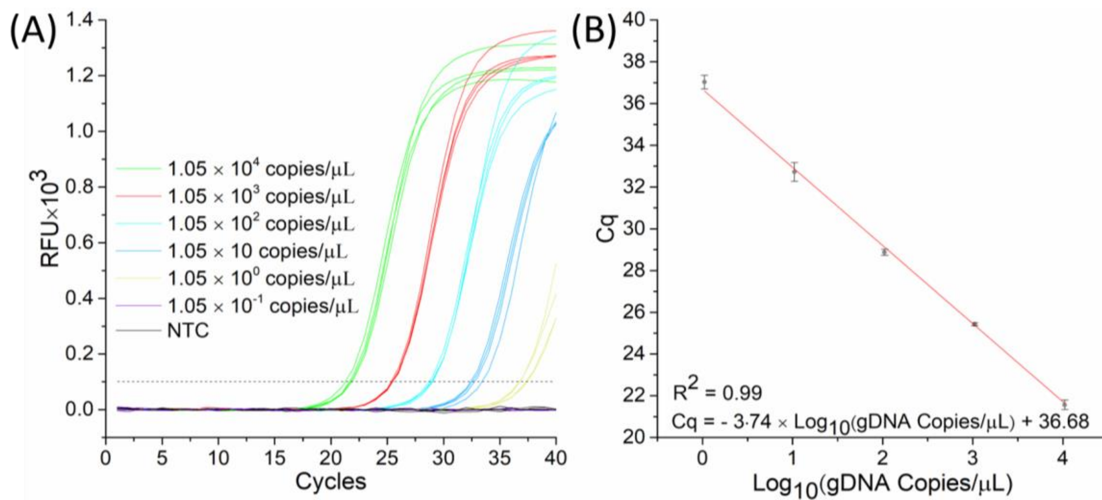

Figure S4. *(A) qPCR of Cryptosporidium genomic DNA with a 10-fold serial dilution from 1.05×10^4 copies/μL to 1.05×10^{-1} copies/μL, and (C) the corresponding calibration curve. Each data point in the calibration curve represents the mean value of four measurements. The error bar on each data point represents the standard deviation.*

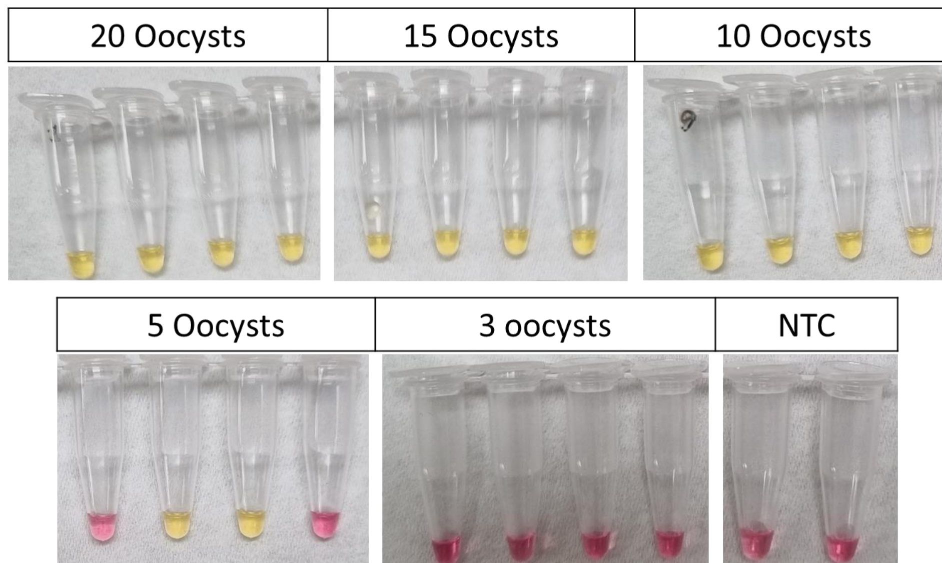

Figure S5. *cLAMP reactions of different oocysts (3 to 20 oocysts). Oocysts and mud spiked in 10 mL of tap water. Samples were subjected to IMS and heat lysis, followed by cLAMP.*

Table S1: Primer set for LAMP assay to amplify SAM gene of *Cryptosporidium* species.

Primer type	Sequence 5′—> 3′	Primer length	Amplicon size (bp)	Target
F3	ATTTGATRGACAAAGAAACTAG	22	216	S-adenosyl methionine synthetase (SAM) gene of *C. parvum*, *C. hominis* and *C. meleagridis*
B3	CGATTGACTTTGCAACAAG	19		
FIP (F1c–F2)	TTGCGCCCTGTTAATCCAGCAT-TAATTAATCCATCTGGCAGRTTT	45		
BIP (B1–B2c)	TTGTAGATACATACGGAGGATGGG-TCTACTTTAGTTGCATCTTTCC	46		
LF	CTGCTGGCCCMCCAATTG	18		
LB	CATGGRGGTGGTGCATTTAG	20		

*Degenerate nucleotides for LAMP primers are underlined.

Table S2: LAMP primer mix (1.0 mL) preparation and final concentration of each primer.

Primer type and components	Volume used to prepare 10X primer mix	Volume of 10X primer mix used per 12.5 µL LAMP reaction	Final concentration for each primer
FIP (100µM)	160 µL	1.25 µL	1.6 µM
BIP (100µM)	160 µL		1.6 µM
F3(100µM)	20 µL		0.2 µM
B3(100µM)	20 µL		0.2 µM
LF (100µM)	40 µL		0.4 µM
LB (100µM)	40 µL		0.4 µM
Nuclease-free H_2O	560 µL		

Table S3: qPCR, RT-qPCR and LAMP reaction calculations

Components	Volume	Final concentration
qPCR reaction(10µL)		
SensiFAST SYBR® No-ROX Mix(2X)	5.0 µL	1X
10 µM forward primer (F3)	0.4 µL	400 nM
10 µM reverse primer (B3)	0.4 µL	400 nM
Target	2.5 µL	
Nuclease-free H_2O	1.7 µL	
RT-qPCR reaction (10 µL)		
Luna Universal One-Step Reaction Mix (2X)	5.0 µL	1X
Luna WarmStart® RT Enzyme Mix (20X)	0.5 µL	1X
10 µM forward primer (F3)	0.4 µL	400 nM
10 µM reverse primer (B3)	0.4 µL	400 nM
Target	2.5 µL	
Nuclease-free H_2O	1.2 µL	
Colorimetric LAMP reaction (12.5 µL)		
WarmStart Colorimetric LAMP Master Mix (2X)	6.25 µL	1X
LAMP Primer Mix (10X)	1.25 µL	1X
Target	2.5 µL	
Nuclease-free H_2O	2.5 µL	
Fluorescent LAMP reaction (12.5 µL)		
WarmStart Fluorescent LAMP Master Mix (2X)	6.25 µL	1X
Fluorescent dye (50X)	0.25	1X
LAMP Primer Mix (10X)	1.25 µL	1X
Target	2.5 µL	
Nuclease-free H_2O	2.5 µL	

Table S4: qPCR, RT-qPCR, and LAMP temperature cycling conditions

Cycle step	Temperature	Time	Cycles
qPCR thermal cycling condition			
Polymerase activation	95 °C	2 min	1
Denaturation	95 °C	5 sec	40
Annealing	56°C	10 sec	
Extension	72°C	15 sec (+ reading)	
RT-qPCR thermal cycling condition			
Reverse Transcription	55°C	10 min	1
Initial Denaturation	95°C	1 min	1
Denaturation	95°C	10 sec	45
Extension	60°C	30 sec (+ reading)	
LAMP thermal cycling condition			
Amplification	65°C	50 s*	50
Enzyme inactivation	75°C	5 min	1

*Fluorescence reading was taken if it was fLAMP, whereas, for cLAMP, a colour change was observed at the endpoint.

Table S5: Comparison of commercial kit-based nucleic acid extraction methods with direct lysis for cLAMP assay.

Nucleic Acid Extraction method	Number of oocysts											Matrices
	1000	500	100	50	30	20	15	10	5	3	2	
DNeasy Blood & Tissue Kits	100%	100%	100%	100%	100%	75%	-	Neg	Neg	-	-	NA
FastDNA™ SPIN Kit for Soil	100%	100%	100%	100%	100%	25%	-	Neg	Neg	-	-	NA
This assay	100%	100%	100%	100%	-	-	-	100%	100%	-	Neg	Tap water
This assay	-	-	-	-	-	100%	100%	100%	50%	Neg	-	0.1 mL packed pellet per 10 mL of tap water

Table S5: Comparison of different assays for *Cryptosporidium* oocyst detection

Assay	Sensor probes/target biomarkers	Signal recorded /Detection mode	LOD (Normalised LOD)	Comments and DNA/RNA extraction procedure	Ref
Electrochemical capitative biosensor	Interdigitated gold electrode modified with anti-*Cryptosporidium* monoclonal antibodies (IgG3)	Relative capacitance	40 oocysts/5 µL (10 oocyst/µL)	label-free biosensor	S1
Electrochemical ELISA-type screen-printed electrode	Antibody functionalized screen-printed electrode captures the oocysts. Detection is achieved due to the oxidation of OPD in the presence of H_2O_2 by HRP-conjugated primary antibody.	Electrode potential	500 oocysts/mL (0.5 oocysts/ µL)	Potential increases linearly as the number of captured oocysts increases	S2
Electrochemical apta-sensor for oocyst detection	Aptamer attached to gold nanoparticle-modified carbon electrode captures oocyst to the electrode surface. Aptamer: 5´-/5ThioMC6-D/GGCTTCTGGACTAC CTATGC-3´	Square wave voltammetry (SWV) of ferri/ferrocyani de	100 oocysts/30µL (3.33 oocysts/ µL)	High specificity and selectivity, signal-on sensor	S3
Microfluidics with micromesh	SUS micromesh having 100(10x10) microcavities (diameter 2.7µm). After capturing the oocysts to	Fluorescent microscopy	10 oocysts/mL (0.01 oocysts/µL)	Comparatively rapid (60 minutes)	S4

	microcavities, stained with fluorescent antibody.				
Optical microfluidic biosensors	Immunoagglutinated microbeads with COWP protein	Mie scattering intensity	1–10 oocysts/mL (0.001-0.01/ µL)	Very Rapid (10 minutes analysis time)	S5
CRISPR/Cas12a-powered immunosensor	Antibody-DNA conjugates (Texas red labeled) with CRISPR/Cas12a recognition site. The signal amplification happened due to multi-streptavidin sites on antibodies which allow a lot of biotinylated DNA probes to be attached.	96 well plate fluorescent reading	1 oocyst per reaction	Robust and rapid	S6
CRISPR/Cas12a-powered lateral flow strip (LFS)	RPA of the gp60 gene followed by CRISPR/Cas12a trans cleavage. Amplification through RPA before CRISPR/Cas12a cleavage activity has made this assay highly sensitive.	Fluorescence readout, naked eye observation under blue light	10 oocysts/mL (0.01oocysts/ µL)	DNA was extracted by boiling the sample with 10% N-lauroylsarcosine sodium salt (LSS) and purified with a homemade column.	S7
GP60 gene LAMP assay for *Cryptosporidium* detection.	gp60 gene	Gel electrophoresis	1 oocyst per reaction	First report on LAMP assay for *Cryptosporidium* oocyst detection	S8
Reverse transcription LAMP	18S rRNA	Fluorescent LAMP	6×10^{-3} oocysts per reaction	Highly sensitive due to the plenty of 18S rRNA transcripts	S9

			(calculated from the serial dilution of RNA)	Combination of Five freeze (-80°C) and thaw (37°C) cycles, heating, and proteinase K treatment.	
Uracil DNA glycosylase-supplemented loop-mediated isothermal amplification (UDG-LAMP)	SAM gene	Fluorescence	10 oocysts	More resistant to contaminant Stool DNA Isolation Mini-Kit (YT9032, Yekta Tajhiz Co, Iran)	S10
Detection of *Cryptosporidium* spp. from environmental samples using LAMP	SAM gene	Fluorescence and colorimetric	9 Copies per reaction		S11
Comparison of three LAMP assays to analyse feacal samples	SAM gene, *hsp* 70 and *gp* 60 gene SAM LAMP assay was more sensitive than the other two.	Fluorescence		270 fecal samples were analyzed by nested PCR. Nested PCR-negative samples were found to be positive by LAMP.	S12
Accelerated LAMP assay with *Cryptosporidium* oocyst's lysate	SAM gene and transcript	Colorimetric and fluorescent LAMP	1 copy genomic DNA per reaction 5-10 oocyst per reaction	Simple heat lysis was used for DNA extraction	This work
S-adenosyl methionine-L synthetase (SAM), Square wave voltammetry (SWV), glycoprotein 60 (gp60), heat shock protein 70 (hsp70). All the LAMP assays mentioned here targeted the SAM gene used primers published by Bakheit *et al.*[S12]					

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