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Critical evaluation of current isolation, detection, and genotyping methods of *Cryptosporidium* species and future direction

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Table S1: Comparison of different filtration approaches

Filtration type/materials of membrane	Water type analysed	Volume of sample	Enrichment and detection	Average recovery	Observation/Comment	Ref
Filta Max by IDEXX	Seeded treated and raw water	10L 100L	• IMS • IFA	19.6 – 28.2%(1) 72.6%(2,3)	• Depending on the water quality recovery varied.	1–3
Envirochek HV	Seeded tap and surface water	10L – 100L	• IMS • IFA	51–70%	• Depending on the water quality recovery varied.	1,3,4
Size-selective continuous flow filtration Filter pairs used for filtration ((5µm, 3 µm), (8µm, 3 µm), (10µm, 3 µm))	*C. parvum* oocysts spiked into finished water	20L	• IMS • IFA	(5µm, 3 µm) pair = 67.3%, (8µm, 3 µm) pair = 77.2–85.0%, (10µm, 3 µm) pair = 76.9–82.2%	• Simple, rapid and cost-effective	5
Dead-end ultrafiltration (DEUF)	Seeded lake water	100L	• IMS • IFA	49 – 87%	• High turbidity decreases the recovery.	6,7
High-flux metallic micro/nano-filtration membrane	Spiked tap-water	10L	• IMS • IFA	85%,	• a long lifetime • Easily cleanable	8
Counter-Flow Micro-Refinery (CFMR) systems	Spiked surface water	10L – 100L	• IMS • IFA	81.3%,	• No significant clogging has been observed	9
Portable continuous flow centrifugation (PCFC)	Spiked water with various matrices	10L – 100L	• IMS • IFA	35% – 66.8%	• User-friendly, clogging -free concentration. • Recovery drops if when more water volume increases	10

Table S2: Comparison of different molecular detection techniques/studies reported for *Cryptosporidium* spp. detection.

Techniques/study	Gene target, primers, and probes (5′→3′)	Sample analyzed	LOD	Results/Comments	Ref
FISH	Target: 18S rRNA > CRY1 probe TRITC/CGGTTATCCATGTAAGTAAAG/CY3	Along with *C. baileyi* and *C. muris,* 19 different isolates of *C. parvum* were tested to check CRY1 probe specificity. 10 to 20 L samples concentrated by flocculation, 100µL of this concentrate was seeded with different numbers of oocysts.	NA	• No fluorescence was observed for *C. baileyi* and *C. muris.* • CRY1 probe is *C. parvum*-specific. • Viability assessment of 100 oocysts (6 replicates) by FISH and excystation corroborated each other. • Fluorescent was not bright enough in environmental concentrate, however, in combination with immunofluorescence staining FISH was able to detect oocysts.	11
FISH	Target: 18S rRNA > EUK probe[12] (targets 18S rRNA of Eukarya) FITC/ACCAGACTTGCCCTCC > ANTI-EUK probe (for measuring nonspecific fluorescence) GGAGGGCAAGTCTGGT	Used pre-confirmed *C. parvum* oocysts sample	NA	• Optimized a rapid (<1.0 hour) FISH protocol. • Kinetics of probe binding to rRNA • Calculated 18S rRNA molecules (3.5×10^5) per oocysts. • Concluded that fluorescent signal cannot be achieved beyond what was achieved by Vesey et al.(11)	13

FISH	Target: 18S rRNA > CRY1 probe [11] CGGTTATCCATGTAAGTAAAG > EUK probe[12] ACCAGACTTGCCCTCC > ANTI-EUK probe (13) GGAGGGCAAGTCTGGT	Purified *C. parvum* oocysts	NA	• Probes were conjugated with a range of fluorophores (FITC, FAM, BD, OG, CY3) • Observed a five-fold increase in fluorescence when CRY1 probes carry FITC with an 18-carbon spacer containing six ethylene glycol moieties. • FISH-based flow cytometric detection.	14
FISH	Target: 18S rRNA > Cpar677 probe (specific for *C. parvum*) Cy3/TCATATACTAAAATATATAGTAATAT Sequence alignment of 18S rRNA genes from *Cryptosporidium* spp. for designing Cpar677 probe, which binds one of the 4 variable regions.	33 human fecal samples	NA	• 8 different *Cryptosporidium* species such as *C. parvum, C. hominis, C. andersoni, C. muris, C. meleagridis, C. felis, C. cervine genotype* and *C. rabbit genotype* oocysts were tested. • Only *C. parvum* showed positive fluorescence. • Feacal sample tested by FISH-Cpar677, detected *C. parvum*, validated by PCR-RFLP	15
FISH-based fluorescence microscopy	Target: 18S rRNA gene >Cpar677 probe (specific for *C. parvum*) (15) Cy3/TCATATACTAAAATATATAGTAATAT > Chom253 probe (specific for *C. hominis*) FITC/TCACATTAATTGTGATCC	Different ratio of *C. parvum* to *C. hominis* (10:90 to 90:10). Pre-confirmed 50 human fecal samples positive for *Cryptosporidium* spp.	10 oocysts	• Two color assays for simultaneous detection of *C. parvum* and *C. hominis* • Chrom253 was validated against *C. parvum, C. hominis, C. andersoni, C. muris, C. meleagridis, C. felis, C. cervine genotype and C. rabbit genotype.* • Chrom253 cross reacts with C. rabbit genotype due to being 100% sequence homology in target region. • FISH positive results validated by PCR-RFLP	16

RT-PCR for in vitro infectivity assay	Target: *hsp* 70 mRNA For all *Cryptosporidium* spp. > CpHSP2386F CTGTTGCTTATGGTGCTGCTG > CpHSP2672R CCTCTTGGTGCTGGTGGAATA For *C. parvum* specific > CpHSP2423F AAATGGTGAGCAATCCTCTG > CpHSP2764R CTTGCTGCTCTTACCAGTAC	Purified *C. parvum* oocysts 65 to 100 liters of concentrated environmental water samples seeded with 1-10 oocysts.	A single *C. parvum* oocyst	• mRNA isolated from sporozoite infected cacao-2 cell. • Infection takes almost 4 days, and so does analysis.	17
In vitro cell culturing and PCR (CC-PCR) based infectivity assay	Target: *hsp* 70 mRNA For *C. parvum* specific primers (17) > CpHSP2423F AAATGGTGAGCAATCCTCTG > CpHSP2764R CTTGCTGCTCTTACCAGTAC	122 raw water and 121 filter backwash water samples, 10L each. Seeded with viable *C. parvum* oocysts for recovery analysis by IMS-IFA and flotation-IFA	NA	• Human ileocecal adenocarcinoma (HCT-8, ATCC) cell line was used for CC-PCR assay. • Optimized oocysts dissociation after IMS (with 0.1N HCl) • Due to the small volume recovered after IMS compared to floatation, is generally amenable for CC-PCR. • CC-PCR results were comparable to IFA	18
Comparison of USEPA 1623 and CC-PCR	Target: *hsp 70 gene* For PCR > CPHSPT2F (forward primer) TCCTCTGCCGTACAGGATCTCTTA > CPHSPT2R (reverse primer) TGCTGCTCTTACCAGTACTCTTATCA	593 water samples were collected from flowing streams, reservoirs and lakes of different locations in the USA.	NA	• Comparison was performed to evaluate the performance of *Cryptosporidium* oocyst detection. • Recovery efficiencies were 58.5% and 72% for CC-PCR and method 1623, respectively. • 3.9% of samples were detected by CC-	19

				PCR whereas 10.1% were by method 1623. • Method 1623 detects both viable and non-viable while CC-PCR detects viable one.	
Comparison of CC-IFA, CC-PCR, and CC-RT-PCR for in vitro infectivity assay	Target: hsp70 gene and hsp70 mRNA For PCR[19] > CPHSPT2F (forward primer) TCCTCTGCCGTACAGGATCTCTTA > CPHSPT2R (reverse primer) TGCTGCTCTTACCAGTACTCTTATCA For RT-PCR[17] For *C. parvum* specific > CpHSP2423F AAATGGTGAGCAATCCTCTG > CpHSP2764R CTTGCTGCTCTTACCAGTAC	*C. parvum* and *C. hominis* purified oocysts. Treated drinking water (10L) spiked with oocysts	A single oocyst by each method	• HCT-8 cell line was used for in-vitro infection for IFA, PCR, and RT-PCR • Sensitivity order: CC-PCR > CC-IFA > CC-RT-PCR • False-positive in mock infection: CC-PCR > CC-RT-PCR > CC-IFA (0 false-positive) • Spiked water was processed according to USEPA method 1623, and CC-IFA detected all samples tested. False-negative observed in the case of CC-PCR and CC-RT-PCR.	20
PCR and evaluation of six DNA isolation methods and effect of PCR inhibitor	Target: 18S rRNA gene Primers for *Cryptosporidium* spp.[21] > Forward primer AACCTGGTTGATCCTGCCAGTAGTC > Reverse primer Product length: ~ 820 bp	*Cryptosporidium* oocyst-seeded samples, DNA-spiked samples, and 55 wastewater samples.	5 oocysts (extraction of DNA by QIAamp DNA mini kit after oocyst isolation by IMS.)	• PCR inhibition removal can be done by adding 400 ng of BSA/µL or 25 ng of T4 gene 32 protein/µL of the PCR reaction. • PCR performance was similar for extracted DNA with FastDNA SPIN kit for soil without oocyst isolation QIAamp DNA mini kit after oocysts were purified by IMS	22

Nested-PCR	Target: 18S rRNA gene Inner primers[23] > Forward primer TTCTAGAGCTAATACATGCG > Reverse primer CCCTAATCCTTCGAAACAGGA Product length: ~826 bp	Wastewater from bus terminals, airports and wastewater treatment plants (WWTP)	NA	• Epidemiology study • Reported the presence of *C. cuniculus* in wastewater in São Paulo, Brazil, and Lima, Peru for the first time.	24
Nested-PCR assay and RT-PCR with internal positive control	Target: hsp70 gene and hsp70 mRNA Primers for RT-PCR internal positive control (IPC) [25] > IPCF ATGACAGCCACTCCT >IPCR ATGTCAGTTGTGACCACGAA Primers for nested-PCR internal positive control (IPC2) > chsp1ipc AGCAATCCTCTGCCGTACAGGATGACAGCC > chsp4ipc AAGAGCATCCTTGATCTTCTATGTCAGTTG Nested PCR primers Outer primers [26] > CHSP1 AGCAATCCTCTGCCGTACAGG >CHSP4 AAGAGCATCCTTGATCTTCT Product length: 590 bp Inner primers > CPHSP2511 ATGACCAAGCTTATTGAAC-3′)	Finished water and untreated surface water. 10^4 or 8 *C. parvum* oocysts spiked into 1mL reagent water containing 10L equivalent concentrate	8 oocysts by nested PCR. 5 oocysts by RT-PCR	• Nested-PCR an RT-PCR had similar sensitivity (8 versus 5 oocysts); however, nested-PCR was observed to be more reproducible. • Untreated water has inhibitory effect on when processed with flocculation whereas processing the samples with Envirochek filters effective to remove inhibitors. • This study counter verified the primers and results of Kaucner *et al.*[25]	27

	> CPHSP2769 GTGATCTTGCTGCTCTTACCA Product length: 280 bp RT-PCR primers CHSP1/CHSP4 used for RT-PCR.				
TaqMan MGB-qPCR assay	Target: 18S rRNA gene Generic TaqMan assay for *Cryptosporidium* spp. > CcF18S (forward primer) GTTTTCATTAATCAAGAACGAAAGTTAGG > CcR18S (reverse primer) GAGTAAGGAACAACCTCCAATCTCTAG > Csp18S (generic probe) FAM/TCAGATACCGTCGTAGTCTTAACCATAAACTATGCC/TAMRA Product length: ~107bp. Specific TaqMan MGB assays > ChvF18S (forward primer) CAATAGCGTATATTAAAGTTGTTGCAGTT > ChvR18S (reverse primer) CTGCTTTAAGCACTCTAATTTTCTCAAA > Cp/Ch18S (probe for *C. parvum*/*C.* hominis) FAM/GTTAATAATTTATATAAAATATTTTGATG/NFQ-MGB > Ca18S (probe for *C. andersoni*) FAM/CCAAGGTAATTATTATATTATC	Four surface water samples (40 L) from a dam and spiked *C. parvum* oocysts at a concentration of 1, 5 and 25 oocysts L^{-1} Samples were processed with Envirochek HV	1 oocyst per reaction Detected 5 oocysts L^{-1} concentration from spiked sample.	• Multiple sequence alignment was done for the 18S rRNA gene from 21 *Cryptosporidium* spp. to design a generic assay. Specific probes were designed targeting the hypervariable region from the same alignment. • External amplification control to monitor amplification false negative due to inhibition. • Specificity was tested against 11 Cryptosporidium species. • Specific assay probed to be unaffected by high amount of non-target DNA. • Collected samples had naturally occurring C. *andersoni which was detected along with spiked C. parvum oocysts by specific assay.*	28

	/NFQ-MGB > Cb18S (probe for *C. bovis*) NED/AAAAGCTCGTAGTTAATCTTCTGTTA/NFQ-MGB Product length: ~170bp				
TaqMan probe-based qPCR	Target: COWP gene >Forward primer CAAATTGATACCGTTTGTCCTTCTG >Reverse primer GGCATGTCGATTCTAATTCAGCT > Probe HEX/TGCCATACATTGTTGTCCTGACAAATTGAAT/BHQ-1 Product length: 151 bp	Water sample from pond, river, lake. Samples(2L) were vacuum filtered with cellulose nitrate filters (pore size: 3-µm, diameter: 47-mm) Raw sewage water(1L), processed through centrifugation at 30 min at 3,000 × g	4 copies of the COWP gene (equivalent to 1 oocyst)	• 26 precursor COWP gene sequence from different strains of *Cryptosporidium* was aligned to design primers for *C. parvum* genotypes 1(anthroponotic) and genotype 2(zoonotic) • DNeasy (Qiagen) DNA extraction modified with 3 cycles freeze-thaw and sonication increased DNA yield by two- to fivefold • PCR inhibition was observed in sewage sample. • Addition of 20% Chelex 100 along with 2% PVP 360 during DNA extraction successfully removed inhibition.	29
TaqMan MGB-qPCR assay	Target: 18S rRNA gene For *Cryptosporidium* spp. > CRU18SFc (forward primer) GAGGTAGTGACAAGAAATAACAATACAGG >CRU18SRc (reverse primer) CTGCTTTAAGCACTCTAATTTTCTCAAAG > CRU18STM(probe)	Human fecal samples	2 oocysts per PCR reaction (calculated from the number of oocysts seeded/gram of stool and DNA	• 136 positive stool sample were typed by this assay and PCR-RFLP for COWP for *C. hominis* and *C. parvum.* • Both the techniques type almost same number of *C. parvum* or *C. hominis*, 129 and 128 for TaqMan and COWP-RFLP assay. • Given that different gene and primer sets were used by COWP-PCR and TaqMan assay, TaqMan assay happened to be	30

	FAM/TACGAGCTTTTTAACTGCAACAA/MGB-NFQ Product length: ~300 bp For *C. parvum* > CRULib13F (forward primer) TCCTTGAAATGAATATTTGTGACTCG > CRULib13RCp (reverse primer) TTAATGTGGTAGTTGCGGTTGAAC > CRULib13TMCp(probe) VIC/TATCTCTTCGTAGCGGCGTA/MGB-NFQ Product length: 166 bp For *C. hominis* >CRULib13F (forward primer) (as above) > CRULib13RCh (reverse primer) AAATGTGGTAGTTGCGGTTGAAA >CRULib13TMCh(probe) VIC/CTTACTTCGTGGCGGCGT/MGB-NFQ Product length: 169 bp		final extraction volume)	more sensitive and specific.	
FRET probe-based qPCR assay	For details about primers and probes, refer to the reference number. Primers were designed for 3 assays. Two based on 18S rRNA gene (18S-LC1 and 18S-LC2 assays) and 3rd one targeted hsp90.	Analytical performances were determined by *C. parvum* seeded human fecal specimens (as low as 10 oocysts) and 10L lake water	0.2-2 oocysts/PCR and 0.1-1 oocysts/PCR in spiked feces and water	• All three assays produced distinct melt curves depending on the probes, product length and polymorphism present in the targeted region of 18S rRNA and *hsp* 90 genes. • For sensitive FRET analysis Mg^{2+} concentration was crucial. • Successfully differentiated *C. parvum, C.*	31

		concentrates (as low as 10 oocysts) Source water.	samples respectively depending on the primers and probes.	*hominis, C. andersoni, C. ubiquitum, C. cuniculus, C.* deer mouse genotype III*, C.* muskrat genotype I*, C. skunk genotype*	
Comparison between droplet digital PCR (ddPCR) and qPCR	Target: 18S rRNA gene and actin gene Primers for 18S rRNA gene[32] >18SiF (forward primer) AGTGACAAGAAATAACAATACAGG >18SiR (reverse primer) CCTGCTTTAAGCACTCTAATTTTC TaqMan probe[33] FAM/AAGTCTGGTGCCAGCAGCCGC/BHQ1 Product length: ~298 bp	Purified *C. parvum* oocysts Flow cytometry counted oocysts. 18 animal and human fecal samples	2 and 2.5 oocyst equivalents for both ddPCR and qPCR (18S and actin locus) on haemocytometer and flow-cytometry counted oocysts	• ddPCR is more precise and sensitive than qPCR and offers absolute quantification. • ddPCR is not affected by the PCR inhibitor present in the fecal sample. • While both offer similar sensitivity, however, qPCR data need to be corrected as general spectroscopic techniques overestimate the DNA standard. • Relative cost of 96 well plate ddPCR analysis is more than twice that of qPCR.	34
Loop-mediated isothermal amplification (LAMP)	Target: gp60 gene Primers targeted the 189 bp segment. > F3(forward primer) TCGCACCAGCAAATAAGGC > B3 (backward primer) GCCGCATTCTTCTTTTGGAG > FIP (forward inner primer) ACCCTGGCTACCAGAAGCTTCAGAA CTGGAGACGCAGAA	Purified *C. parvum* oocysts. Pre-confirmed (IFA positive) fecal and water samples for validation.	0.1 Oocysts (determined from the response of 7 fold serial dilution of DNA	• First report of application of LAMP in Cryptosporidium detection. • LAMP detected Cryptosporidium from all fecal and environmental samples.	35

	> BIP (backward inner primer) GGCCAAACTAGTGCTGCTTCCCGTT TCGGTAGTTGCGCCTT		isolated from 10^6) oocysts		
Loop-mediated isothermal amplification. (LAMP)	Target: SAM-1, gp60, *hsp* 70 gene For the detailed list of primers, refer to the reference. SAM and HSP LAMP primers included degenerate base at the position if multiple sequence alignment showed SNP at the primer binding region.	270 fecal samples from cattle, sheep and horses	NA	• Specificity: SAM LAMP for C. parvum, C. hominis and *C. meleagridis* gp60 LAMP for *C. parvum* hsp70 LAMP for *C. andersoni* • Nested-PCR negative (18S rRNA gene) was amplified by LAMP and validated by cloning and sequencing of LAMP products, probably due to DNA concentration was below the detection limit or PCR was inhibited.	36
Comparison of y IFA, nested-PCR and LAMP in surface water	Target: SAM-1 gene (LAMP), 18S rRNA gene (nested PCR) SAM-1 LAMP used the primers from Bakheit *et al*.[36] For nested PCR, primers were selected from Xiao *et al.*[21,37]	Surface water from the river. At least 10L water was used for each data point. filtrated by 142 mm membrane filters used for filtration.	NA	• According to this study sensitivity order in detecting oocysts from 20 environmental samples is: LAMP (45%) > IFA (30%) > nested-PCR (5%)	38
Comparison of y IFA, nested-PCR	Target: SAM-1 gene (LAMP), 18S rRNA gene (nested PCR) SAM-1 LAMP used the primers from Bakheit *et al*.[36]	70 water samples, including tap, river, fountain and well water.	NA	• According to this study sensitivity order in detecting oocysts from 70 water samples is LAMP (27.1%) > IFA (25.7%) > nested-PCR (0)	39

and LAMP in surface water	For nested-PCR, primers were selected from Xiao *et al.*[21,37]	16 river and tap water pellets were spiked with 10 oocysts.		• 100 percent spiked samples were positive for LAMP assay whereas 43.75% of samples were positive by nested PCR. As nested PCR has sensitivity down to single copy, in this case, suggesting the presence of inhibitor affected nested-PCR but not the LAMP assay.	
Comparison of y IFA, nested-PCR and LAMP in surface water	Target: SAM-1 gene (LAMP), 18S rRNA gene For the detailed list of primers, refer to the reference.	Effluent, influent from WWTPs processed by flocculation and sucrose centrifugation. Surface water, drinking water and tap water are processed by microfiber filtration.	NA	• According to this study overall sensitivity order in detecting oocysts from 227 samples is LAMP (43.6%) > PCR (41.9%) > IFA (30.4) • However, depending on the water sources, variation is observed. Such as, nested PCR produced a more positive response (45.5%) than LAMP (35.3%). • Irrespective of the sample type, detection performance was consistently lower than the other two.	40
Real-time nucleic acid sequence-based amplification (NASBA)	Target: MIC1 mRNA Primers > C.par MIC1 P2 TCATATAAACCAGAATCAGTAGGA > C.par MIC1 P1 AATTCTAATACGACTCACTATAGGG AGGAAGCCAAGATGACCATT > C.hom MIC1 P2 GATGTGGAATCTACAGGATA > C.hom MIC1 P1 AATTCTAATACGACTCACTATAGGG CTATGACAAGGATAATATGGT Probes > C.par MIC1 MB	Serial dilution purified *C. parvum* and *C. hominis* oocysts to test the method's sensitivity and applicability for viability analysis.	5 oocysts	• Originally assay was designed to distinguish oocysts and *C. parvum* and *C. hominis* and intent to have the viability information. • The assay detected and differentiated both oocysts. It happened to be positive for both live and dead oocysts. Hence, hypothesized that mRNA remained protected even if in dead oocysts.	41

	FAM/CGCGATAGCAGGAGTGTATTC AACTACAATCGCG/BHQ-1 > C.hom MIC1 MB CAL_Fluor_610/CGCGATTGATGGATC TTGACTTGGTAGTTATCGCG/BHQ2				
Comparison of Sanger sequencing (SgS) and NGS	Target gene for sequencing: 18S rRNA gene and gp60 Nested-PCR was performed according to Xiao *et al.*[21] except CCCATTTCCTTCGAAACAGGA was used as a reverse primer in primary PCR. Nested-PCR for gp60 was performed according to Sulaiman *et al.*[42]	101 *Cryptosporidium*-positive human fecal samples linked to swimming pool-associated cryptosporidiosis outbreaks in Western Australia in 2019 and 2020	NA	• Co-infections/mixed infections were present in multiple samples, which was overlooked by SgS, however, identified by NGS.	43
NGS for wastewater sample	Hypervariable V9 region of eukaryotic 18S(product length ~107bp) and a Cryptosporidium-specific 18S (product length ~ 298bp) were amplified by following primers: For *Cryptosporidium* spp.[32] > 18S iF AGTGACAAGAAATAACAATACAGG > 18S iR CCTGCTTTAAGCACTCTAATTTTC For Eukaryotes > Euk1391F GTACACACCGCCCGTC	Wastewater sample WWTP	NA	• Eukaryotic 18S was not sensitive to *Cryptosporidium* spp. detection by NGS. • Suggested *Cryptosporidium*-specific NGS.	44

	> EukBr TGATCCTTCTGCAGGTTCACCTAC > Mammalian blocking primer CCCGTCGCTACTACCGATTGG44444T TAGTGAGGCC3				

Waste Water Treatment Plant (WWTP), minor groove binder (MGB), black hole quencher 1 (BHQ1), non-fluorescent quencher (NFQ), molecular beacon (MB) fluorescein amidites (FAM), carboxytetramethylrhodamine (TAMRA)

Table 3: Comparison of different biosensor-based approaches for *Cryptosporidium spp.* detection.

Techniques/study	Sensor probes/target biomarkers	Sample analysed	Signal recorded/detection mode	LOD (Normalised LOD/µL)	Results/Comments	Ref
Electrochemical capitative biosensor	Interdigitated gold electrode modified with anti-*Cryptosporidium* monoclonal antibodies (IgG3)	Serial dilution of 250, 200, 150, 100, 50, and 0 *C. parvum* oocysts in 5 µL buffer	Relative capacitance	40 oocysts/5 µL (10 oocyst/µL)	• label-free biosensor. • Different water concentrates need to be analysed to understand the applicability.	45
Electrochemical immune sensor	Indium tin oxide (ITO) electrode functionalized with antibody	Serial dilution of *C. parvum* oocysts.	Differential pulse voltammetry (DPV)	3 oocysts/mL (0.003oocysts/ µL)	• Dual labelled gold nanoparticle (antibody and alkaline phosphatase) catalase the substrate p-nitro phenol for DPV signal generation.	46
Electrochemical ELISA-type screen-printed electrode	Antibody functionalised screen-printed electrode	Serial dilution of *C. parvum* oocysts.	Electrode potential	500 oocysts/mL (0.5 oocysts/ µL)	• HRP conjugated primary antibody used for detection. • HRP oxidases OPD in the presence of H_2O_2 and increases the electrode potential.	47

Electrochemical impedance spectroscopy in for viability analysis	Interdigitated microelectrode array	Serial dilution of purified *C. parvum* oocysts	Impedance measurement of suspension of oocysts	10 oocysts/ µL	• Label-free detection. • Buffer conductance is crucial. Low conductance buffer is suitable for oocyst's viability measurement. • 15% lower impedance had been observed for dead oocysts compared to live one. • There is no selectivity test done when other microbes present on the	48
Electrochemical impedimetric biosensor for infectivity analysis	PDMS well with interdigitated microelectrode arrays.	Purified *C. parvum* oocysts infection to HCT-8 cells	Impedance measurements	1 sporozoite or 1 oocyst	• Faster analysis (within 10h) compared to other CC-IFA, CC-PCR or CC-RT-PCR described by di Giovanni *et al.*[18] and Johnson *et al.*[20] • Need to evaluate the applicability in environmental samples.	49

Aptamer-based electrochemical detection of oocysts	Gold nanoparticle-modified screen-printed carbon electrode modified with aptamer for capturing oocyst on the electrode surface. Aptamer: 5′-/5ThioMC6-D/GGCTTCTGGACTACCTATGC-3′	Purified *C. parvum* oocysts And spiked fruit sample	Square wave voltammetry (SWV) of $[Fe(CN)6]^{4-}$ /$[Fe(CN)6]^{3-}$	100 oocysts/30µL (3.33 oocysts/ µL)	• Aptamer was selected experimentally by SELEX. • Signal on electrode, means signal increases after oocysts binding.	50
Immuno-dot blot assay	Gold nanoparticle modified *Cryptosporidium* specific antibody and alkaline phosphatase (ALP)	Purified *C. parvum* oocysts 5 water sample (10L each) Filtrated through 142 mm dia Whatman 42 filter paper.	Color change of chromogenic substance	10 oocysts/mL (0.01oocysts/ µL)	• Gold nanoparticle harbors enormous ALP molecules which accelerate the catalysis of a chromogenic substance compared to conventional ALP-conjugated secondary antibody. • Results for water samples were validated by the 18S rRNA gene.	51
CRISPR/Cas12a-powered immunosensor	Antibody-DNA conjugates (texas red labelled) with CRISPR/Cas12a recognition site.	*C. parvum* oocysts Mud samples from the water treatment plant spiked with 5 oocysts	Fluorescence at 96 well plate fluorescent reader	1 oocyst per reaction	• Signal was amplified as each antibody harbors a lot of streptavidin sites where biotinylated DNA probes were attached.	52

CRISPR/Cas12a-based lateral flow strip (LFS)	RPA of the gp60 gene followed by CRISPR/Cas12a trans cleavage.	Purified *C. parvum* IIdA19G1	Strong Fluorescence readouts observed naked eye under blue light	10 oocysts/mL (0.01oocysts/ µL) This has been calculated from the response of the signal of serially diluted DNA	• Amplification through RPA before CRISPR/Cas12a cleavage activity has made this assay highly sensitive.	53
Microfluidic device with SUS micromesh	100(10x10) microcavities (diameter 2.7µm) on a stainless-steel plate to capture *Cryptosporidium* oocysts, followed by staining with fluorescent antibody.	Purified *C. parvum* oocysts spiked in tap water	Fluorescent microscopy	10 oocysts/mL (0.01 oocysts/ µL)	• 93.1% recovery efficiency with 60 minutes detection time, could be a promising platform for post-concentration analysis of water samples when considering other types of water samples with matrices.	54
Microfluidic-based multi-angle laser scattering (MALS) system	Distinctive scattering	*C.parvum, G.lamblia, E.coli* Polystyrene microsphere mixture(total 200 organisms with PS microsphere) in reagent water.	Support-vector-machine (SVM) algorithm	NA	• Rapid and label-free detection. • 98% percent accuracy in detecting *C. parvum.*	55

Microfluidic diffraction phase microscopy	Quantitative pixel-wise phase maps of individual oocyst	*Cryptosporidium* oocysts suspension in PBS	Imaging microscopy	NA	• Rapid and label-free detection	56
Optical microfluidic biosensors	Immunoagglutinated microbeads with COWP protein	*C. parvum* oocysts suspension in PBS Spiked sump water sample	Mie scattering intensity	1–10 oocysts/mL (0.001-0.01/ μL)	• Very rapid (10 minutes analysis time)	57
mRNA isolation and amplification by on-chip microfluidic device followed by LFA for detection	Microfluidic channel PAMAM dendrimers, which increase the binding of oligo$(dT)_{25}$ to capture mRNA. Then on-chip NASBA was performed for *hsp* 70 mRNA.	Purified *C. parvum* oocysts.	After hybridisation with NASBA product Reporter probe-tagged liposomes produce color on the LFA test line	30 oocysts per reaction	• On-chip mRNA isolation increased the mRNA capture, which in turn sensitized the NASBA.	58

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