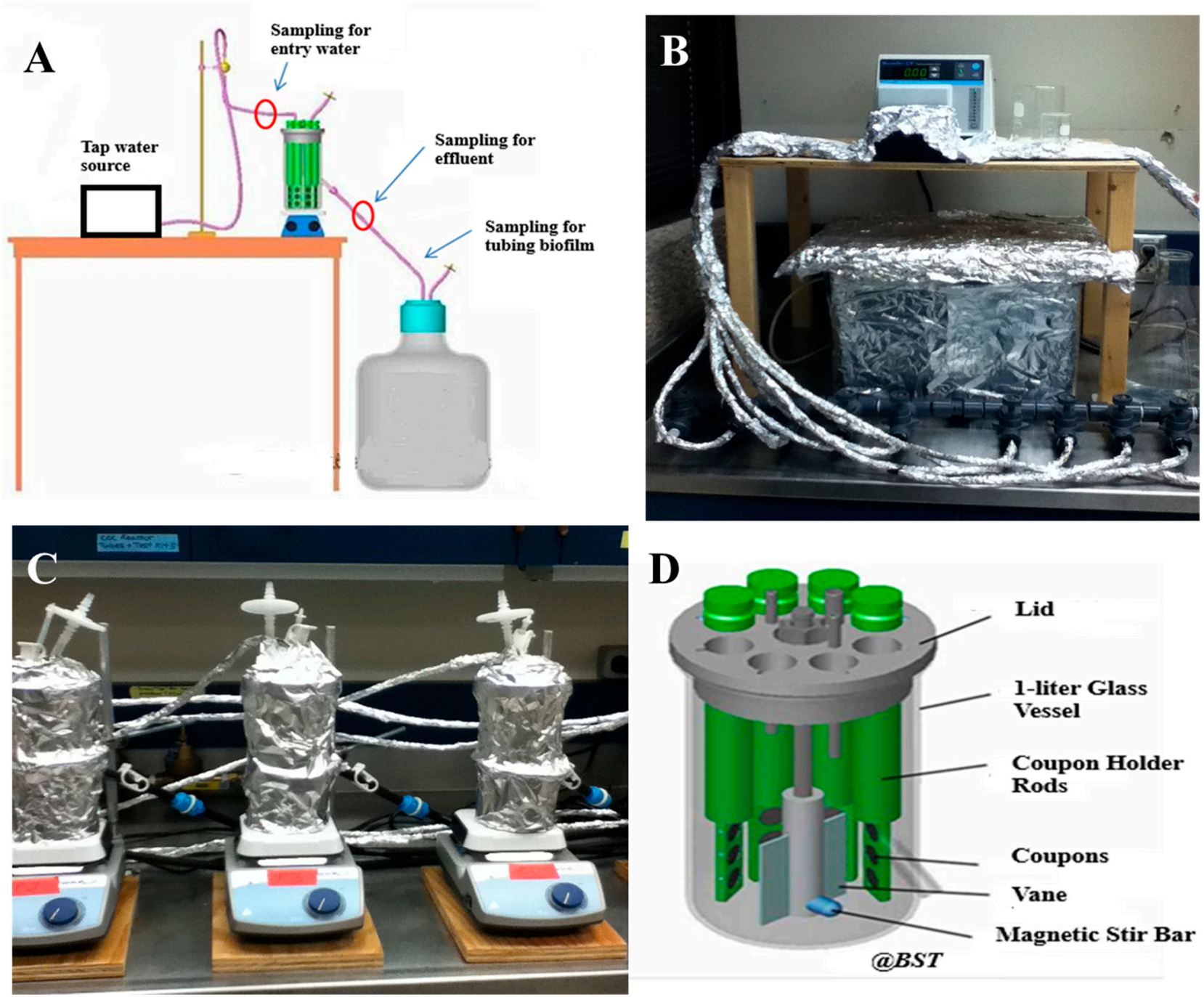

A
Sampling for entry water
Tap water source
Sampling for effluent
Sampling for tubing biofilm
B
C
D
Lid
1-liter Glass Vessel
Coupon Holder Rods
Coupons
Vane
Magnetic Stir Bar
@BST

Figure S1. The CDC reactor setup (A) consists of water tank (B), bioreactors and waste effluent line and container (C-D). The water tank (B) is fed with Cincinnati tap water at a flow rate of 11.67mL/min (residence time of 30 minutes) and a baffle rotation of up to 120 rpm for 1-2 months controlled using a peristaltic pump on the top of a shelf (B). Both the CDC reactors (C) and the water tank were covered with aluminum foil to prevent light. Each reactor contains 8 coupon holders, and each reactor vessel contains 24 coupons (D)

Table S1. Oligonucleotide qPCR primer sequences used to screen pathogens and sequence validation

Target	Oligo name	Sequences (5'-3')	Tm (°C)	Detection limit (CE per reaction)	Reference
Campylobacter spp. (qPCR)	CampF2	CACGTGCTACAATGGCATAT	58	1	Lund et al. 2004
	CampR2	GGCTTCATGCTCTCGAGTT			
	CampP2	FAM-CAGAGAACAATCCGAACTGGGACA-TAMRA			
Campylobacter spp. (PCR)	CampF	GGA TGA CAC TTT TCG GAG C	60	2	Linton, 1996
	CampR	CAT TGT AGC ACG TGT GTC			
Legionella spp. (qPCR)	Leg F1c	TAGTGGAATTTCCGGTGTA	50	2	This study
	Leg R1c	CCAACAGCTAGTTGACATC			
	Leg-probe	6Fam-CGGCTACCTGGCCTAATACTGA-Tamra			
Salmonella spp. (qPCR)	invA_176F	CAACGTTTCCTGCGGTACTGT	60	4	Gonzalez-Escalona, N., et al. 2009
	invA-Tx_208	FAM-CTCTTTCGTCTGGCATTATCGATCAGTACCA-TAMRA			
	invA_291R	CCCGAACGTGGCGATAATT			
Mycobacterium spp. (qPCR)	23SmycoF	GGG GTGTGGTGTTTGAG	62	1	Bruijnesteijn van Coppenraet, 2004
	23SmycoR	CTCCCACGTCCTTCATC			
	23SmycoProbe	6-carboxyfluorescein-TGGATAGTGGTTGCGAGCATC-BHQ1			
Trk system potassium uptake protein CeoB [*M. abscessus*, NC_010397]	MA-ceoBf1	GGCGATCATCGACAAGGAA	62	NA	This study
	MA-ceoBr1	GTAAGAGAACGTCGCGATCAA			
	MA-ceoBf2	GTGCGAGTAGTGGTGATGG			
	MA-ceoBr2	CGGCTTCCTTGTCGATGAT			
mycolic acid synthesis: STEAROYL-ACP DESATURASE	MA-acp1 F1	TCG AAG AGG TCA CCG GTA T			
	MA-acp1 R1	ATC TTC ACG CCG TAC TTG TC			
Potassium homeostasis antigen 85A and 85C *[M. abscessus]*	MA-85A F5	TGG TCA GTC CAG CTT CTA CA			
	MA-85A R5	CCC TTG TTC GCA GCA AGA TA			
	MA-85C F3	TCA ACG GCT GGG ATA TCA AC			
	MA-85C R3	CGA ATA CCA GTC GGT GTA GAA G			
K^+ transporter	Lp-kupBf	TCGTGCTGATAATGATGG	60		
	Lp-kupBr	GCCTCACCACCAGTCA			

Acanthamoeba spp. (qPCR)	TaqAcF1	CGACCAGCGATTAGGAGACG	60	2	Riviere et al. 2006
	TaqAcR1	CCGACGCCAAGGACGAC			
	TaqAcP1	FAM-TGAATACAAAACACCACCATCGGCGC-TAMRA			
Vermamoeba vermiformis (qPCR)	Hv1227F	TTA CGA GGT CAG GAC ACT GT	56	2	Kuiper et al. 2006
	Hv1728R	GAC CAT CCG GAG TTC TCG			
Acanthamoeba spp. (PCR)	JDP1	GGCCCAGATCGTTTACCGTGA A	60	2	Schroeder et al. 2001
	JDP2	TCTCACAAGCTGCTAGGGAGTCA			
Cryptosporidium spp. (qPCR)	CRU18Sf	GAG GTA GTG ACA AGA AAT AAC AAT ACA GG	60	2	Hadfield et al 2011
	r	CTG CTT TAA GCA CTC TAA TTT TCT CAA AG			
	P	6FAM-TAC GAG CTT TTT AAC TGC AAC AA-BHQ			
	JVA18S f	ATG ACG GGT AAC GGG GAA T	60	2	Hill et al. 2007
	r	CCA ATT ACA AAA CCA AAA AGT CC			
	p	CGC GCC TGC TGC CTT CCT TAG ATG			
Giardia spp. qPCR	β-Giardin P241 F	CATCCGCGAGGAGGTCAA	60	2	Guy, 2003
	R	GCAGCCATGGTGTCGATCT			
	P	FAM/AAGTCCGCCGACAACATGTACCTAACGA/BHQ-1			
Naegleria fowleri	NaegIF192	GTG CTG AAA CCT AGC TAT TGT AAC TCA GT	63	1	Qvarnstrom et al. 2006
	NaegIR344	CAC TAG AAA AAG CAA ACC TGA AAG G			
	NfowlP	HEX-AT AGC AAT ATA TTC AGG GGA GCT GGG C-BHQ1			

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