

# Loop-Mediated Isothermal Amplification (LAMP) for Rapid Detection and Quantification of *Dehalococcoides* Biomarker Genes in Commercial Reductive Dechlorinating Cultures KB-1 and SDC-9

Yogendra H. Kanitkar,<sup>a</sup> Robert D. Stedtfield,<sup>a</sup> Robert J. Steffan,<sup>b</sup> Syed A. Hashsham,<sup>a,c</sup> Alison M. Cupples<sup>a</sup>

Department of Civil and Environmental Engineering, Michigan State University, East Lansing, Michigan, USA<sup>a</sup>; Biotechnology Development and Applications Group, CB&I Federal Services, LLC, Lawrenceville, New Jersey, USA<sup>b</sup>; Center for Microbial Ecology, Michigan State University, East Lansing, Michigan, USA<sup>c</sup>

Real-time quantitative PCR (qPCR) protocols specific to the reductive dehalogenase (RDase) genes *vcrA*, *bvcA*, and *tceA* are commonly used to quantify *Dehalococcoides* spp. in groundwater from chlorinated solvent-contaminated sites. In this study, loop-mediated isothermal amplification (LAMP) was developed as an alternative approach for the quantification of these genes. LAMP does not require a real-time thermal cycler (i.e., amplification is isothermal), allowing the method to be performed using less-expensive and potentially field-deployable detection devices. Six LAMP primers were designed for each of three RDase genes (*vcrA*, *bvcA*, and *tceA*) using Primer Explorer V4. The LAMP assays were compared to conventional qPCR approaches using plasmid standards, two commercially available bioaugmentation cultures, KB-1 and SDC-9 (both contain *Dehalococcoides* species). DNA was extracted over a growth cycle from KB-1 and SDC-9 cultures amended with trichloroethene and vinyl chloride, respectively. All three genes were quantified for KB-1, whereas only *vcrA* was quantified for SDC-9. A comparison of LAMP and qPCR using standard plasmids indicated that quantification results were similar over a large range of gene concentrations. In addition, the quantitative increase in gene concentrations over one growth cycle of KB-1 and SDC-9 using LAMP was comparable to that of qPCR. The developed LAMP assays for *vcrA* and *tceA* genes were validated by comparing quantification on the Gene-Z handheld platform and a real-time thermal cycler using DNA isolated from eight groundwater samples obtained from an SDC-9-bioaugmented site (Tulsa, OK). These assays will be particularly useful at sites subject to bioaugmentation with these two commonly used *Dehalococcoides* species-containing cultures.

Microbially mediated reductive dechlorination plays a vital role in the bioremediation of the chlorinated ethenes tetrachloroethene (PCE) and trichloroethene (TCE). Under the appropriate conditions, PCE and TCE undergo sequential reductive dechlorination via hydrogenolysis to *cis*-1,2-dichloroethene (cDCE) and vinyl chloride (VC), respectively, finally forming the nontoxic end product ethene (ETH) (1). When reductive dechlorination is linked to growth, it is called organohalide respiration, a metabolism commonly associated with microbial taxa, like *Dehalococcoides* and *Dehalobacter* (2–9). Commercially available reductive dechlorinating mixed cultures (e.g., KB-1 and SDC-9) containing such strains are frequently used for the bioaugmentation of contaminated groundwater aquifers (10–12). In fact, in 2009, it was estimated that bioaugmentation with *Dehalococcoides* spp. had been used at several hundred sites in the United States (13). The growth of these strains in the field and in the laboratory is commonly monitored using real-time quantitative PCR (qPCR) targeting the genes *vcrA*, *bvcA*, and *tceA*, which code for distinct reductive dehalogenases (RDases) implicated in organohalide respiration (14). To date, a number of qPCR protocols with DNA binding dyes or TaqMan probes to quantify *vcrA*, *bvcA*, and *tceA* genes have been developed (2, 15–17). Although qPCR has been successful for monitoring reductive dechlorination, alternative methods would be advantageous for laboratories or practitioners without access to a real-time thermal cycler. Also, any method that is more economical and faster than qPCR would be beneficial.

Loop-mediated isothermal amplification (LAMP) is a novel molecular method recently developed for the specific detection of nucleic acids (18). LAMP is a one-step amplification reaction that amplifies a target DNA sequence using four to six primers. The *Bst*

large-fragment DNA polymerase has strand displacement activity and helicase-like activity, allowing it to unwind and amplify DNA strands in a temperature range of 60 to 65°C (18). Because LAMP is rapid, sensitive, and specific and occurs isothermally, it has emerged as an alternative to PCR-based methods in a wide variety of applications. For example, many LAMP assays have been developed for testing foodborne bacterial pathogens and fungal contaminants (for a review, see reference 19). Recently, LAMP primer sets have been developed and tested for the detection of plasmids pXO1 and pXO2, which impart infectious properties to several strains of *Bacillus anthracis* (20–22). LAMP can also be used to detect RNA viruses. A reverse transcription step is used to convert the RNA from viruses, such as HIV-1 or the Ebola virus, to DNA (23, 24).

In 2014, LAMP primer sets were developed for *vcrA* and the *Dehalococcoides* 16S rRNA gene (25). In that study, a field-deployable approach for harvesting biomass from samples of groundwater bioaugmented with SDC-9 was described. The direct amplifi-

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Address correspondence to Alison M. Cupples, cupplesa@egr.msu.edu.

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cation of templates with LAMP was performed using the handheld Gene-Z platform. Detection limits of  $<10^7$  gene copies/liter were reported (this is the generally accepted threshold for acceptable *in situ* dechlorination). However, larger volumes of groundwater were required when *Dehalococcoides* species numbers were  $<10^4$  gene copies/liter.

Here, the objective was to evaluate if LAMP can be used for the quantification of *Dehalococcoides mccartyi* RDase genes (*vcrA*, *bvcA*, and *tceA*) in two commonly used commercial bioaugmentation cultures, KB-1 (from SiREM) and SDC-9 (from CB&I). This study involved DNA templates rather than direct amplification of harvested biomass. Quantification results (i.e., gene copies per liter values) were compared between LAMP and qPCR during one growth cycle to evaluate the effectiveness of LAMP as a tool to monitor the growth of *Dehalococcoides* spp. in KB-1 and SDC-9. Further, we used DNA templates isolated from eight groundwater samples to validate the quantification of the developed assays with LAMP on the Gene-Z platform (25). The data generated from the groundwater samples were also compared to data obtained using a real-time thermal cycler.

## MATERIALS AND METHODS

**Cultures and growth conditions.** All experiments were carried out in triplicate serum bottles (160-ml nominal volume) containing 100 ml (final volume) of culture and sealed with gray butyl rubber septa. After the microcosms were transferred into an anaerobic chamber, the KB-1 or SDC-9 inoculum (10 ml) and sterile mineral medium (90 ml) were added to the serum bottles using aseptic techniques. The bottles were capped with gray butyl rubber septa, removed from the anaerobic chamber, and sparged with 30% CO<sub>2</sub>–70% N<sub>2</sub> to adjust the pH. During the growth cycle, the pH of each bottle was measured and adjusted to neutral using 1.0 M NaOH, as needed. The bottles were incubated quiescently, shielded from light, and kept at room temperature (~22 to 24°C), and the liquid was kept in contact with the septum to minimize the loss of volatile compounds. The concentration of chlorinated ethenes was monitored by gas chromatography with a flame ionization detector (GC-FID), as previously described (1). All KB-1 serum bottles were amended with 10 µl of feed solution (1:10 dilution of neat TCE in methanol) to yield final concentrations of ~23 µmol TCE and ~112 µmol methanol in each bottle. The bottles were also amended with ethanol (~44.0 mg/liter) each week if residual cDCE and VC were observed. An aliquot (1.0 ml) of culture fluid was removed on days 0, 7, 14, 21, 28, 35, 38, 41, and 44 for DNA extraction. DNA was isolated from 100-µl aliquots using the Mo Bio DNA isolation kit, per the manufacturer's protocol (Mo Bio Laboratories, Inc., Carlsbad, CA). All SDC-9 bottles were amended with ~20 µmol VC, along with a 0.1-ml spike of 100 mM sodium lactate. DNA was extracted from 30-µl aliquots of 3 ml of culture fluid on days 0, 6, 22, 27, 32, and 40.

**Groundwater samples.** Groundwater samples were obtained from a site in Tulsa, OK, which had been recently bioaugmented with SDC-9. Eight amber-colored glass bottles containing an ~1.0-liter groundwater sample representative of monitoring wells 1 to 4 (MW1 to MW4) and injection wells 1 to 4 (IW1 to IW4) were bubble-wrapped and shipped overnight in a cooler packed with ice packs. Upon receipt, the bottles were stored at 4.0°C in the absence of light for the duration of testing. Groundwater samples (100.0 ml each) were filtered through a 0.22-µm-pore-size filter (EMD Millipore Corp., Billerica, MA) using a vacuum pump. Membranes were cut into 0.5-mm strips inside a petri dish with a no. 15 blade using aseptic technique, and these were added to 15.0-ml bead tubes supplied with the Mo Bio UltraClean water kit. DNA (1.5 ml) was extracted from this solution, according to the manufacturer's protocol. The DNA was precipitated by adding 150.0 µl of 5 M NaCl and 3.0 ml of absolute ice-cold ethanol and incubating for 30 min at 4°C. Following centrifugation (14,000 × g for 20 min at room temperature), the DNA pellet was

rinsed with 70% ethanol, air dried, and suspended in 100 µl of distilled water (dH<sub>2</sub>O). The extracted DNA was immediately used for amplification or stored at –20°C for future use.

**Preparation of plasmid standards.** Genomic DNA was extracted from 5 ml of KB-1 (SiREM, Guelph, ON, Canada) using the Mo Bio DNA isolation kit, per the manufacturer's protocol. The *vcrA* and the *bvcA* genes were amplified using PCR with previously described primers (17). The amplified templates were cloned into *Escherichia coli* DH5α using the pCR2.1 TOPO TA cloning vector (Invitrogen) to generate plasmid inserts. *E. coli* cultures were grown overnight in LB medium amended with 50 mg/ml ampicillin and 7.0% glycerol at 37°C. Plasmid standards for *tceA* were provided by Frank Löffler (University of Tennessee, Knoxville, TN). Plasmid inserts were extracted using 5 ml of *E. coli* culture and the Qiagen plasmid extraction kit. Gene copies were calculated as previously described (17). Serial dilutions of plasmid inserts from  $3.16 \times 10^8$  plasmids/µl to ~316 plasmids/µl for *vcrA*,  $2.65 \times 10^9$  plasmids/µl to ~265 plasmids/µl for *bvcA*, and  $1.41 \times 10^{10}$  plasmids/µl to 141 plasmids/µl for *tceA* were used as standards for the amplifications. By plotting the log of the calculated copy number against the cycle threshold (for qPCR) or threshold time (for LAMP) at which fluorescence for that sample crosses the threshold value, standard curves were obtained.

**Design of LAMP primers.** The LAMP primer sets used for this study are listed in Table 1. FASTA files for the functional RDase genes *vcrA* (GenBank accession no. NC\_013552.1, region 1187298 to 1188857), *bvcA* (GenBank accession no. NC\_009455.1, region 834959 to 836509), and *tceA* (GenBank accession no. AY165309.1) of *Dehalococcoides* spp. were downloaded and aligned with the relevant environmental sequences in the NCBI nucleotide database to identify the conserved regions. Next, LAMP primer sets were designed for those regions using Primer Explorer V4 (<https://primerexplorer.jp/e/>). For the *vcrA* gene, two new LAMP primer sets, *vcrA* set A and *vcrA* set C, targeting the bp 857-to-1072 region, were designed and used along with *vcrA* set B, which was designed and tested previously (25). One primer set, *bvcA* set A, targeting the bp 895-to-1139 region, was designed for the *bvcA* gene. Similarly, *tceA* set A was designed to target the bp 882-to-1156 region of the *tceA* gene. Finally, NCBI nucleotide BLAST was used to determine the fidelity of the primer sets to the target sequences in environmental submissions to the database by setting the default expect value as  $1 \times 10^{-5}$ .

**qPCR and LAMP amplification.** Each 20-µl LAMP reaction mixture contained 1× isothermal amplification buffer (catalog no. B0537S; NEB), 1.4 mM deoxynucleoside triphosphates (dNTPs), 0.8 mM betaine, 6.0 mM MgSO<sub>4</sub>, 1.6 U of *Bst* 2.0 WarmStart (NEB), 0.8 µl of Syto82 orange fluorescent dye (Life Technologies, Inc., Grand Island, NY), 0.8 µl of Pluronic (Life Technologies, Inc.), 0.8 µl of bovine serum albumin, a 0.25 µM concentration of 10× primer mix, and balance water to make up 18 µl. The reaction mixtures were incubated at 63°C for 60 min for amplification. All TaqMan assays were set up as 20-µl reaction mixtures. Each 20-µl reaction mixture contained 10 µl of iTaq Universal super mix supplied by Bio-Rad, 1.2 µl of TaqMan probe, described previously (15, 17, 26, 27), and balance water to make up 18 µl. PCR amplifications were performed using cycling conditions of 95°C for 15 s, 60°C for 1 min, a slow ramp of 1% to 95°C for 15 s, and 60°C for 15 s. DNA templates and plasmid standards were added to each LAMP and qPCR mixtures as 2-µl aliquots. All qPCR primers and probes used in this study are listed in Table 2. All qPCR experiments were performed in the commercially available Chromo 4 PCR real-time thermal cycler. For KB-1 and SDC-9 templates, amplification with LAMP was carried out in the real-time thermal cycler, while amplification with groundwater templates was performed in both the Gene-Z platform (described below) and the real-time thermal cycler. Triplicate reactions for each test and positive and no-template controls were used for all experiments.

**Gene-Z analysis of groundwater samples.** Inside the Gene-Z device, an array of 64 light-emitting diodes (LEDs), a bundle of optical fibers, and a single photodiode were used to measure fluorescence in real time (28). An iPod Touch (gen 5) was used to control the reaction temper-

TABLE 1 LAMP primer sets designed and used in this study

Target gene	Primer set	Primer name	Sequence (5'→3')	GenBank accession no., region (bp)
<i>vcrA</i>	vcrA set A	F3	GTAAGTTTTACGCGAGATGG	NC_013552.1, 1187298–1188857 (857–1072)
		B3	GTCATCGGCTGAGCTTTC	
		FIP	ACCCTCCCATTTTGGTACGCTTGTATGGTCCGCCACAT	
		BIP	AAGACAATTTTCTAATGCTGAGGGCATTGGGATCTGCCAGGT	
		LF	CATCAGGTGGCGCTGAATC	
	vcrA set B (25)	LB	AGCTGCAAAATATTTTGGTGCTGG	NC_013552.1, 1187298–1188857 (652–886)
		F3	ACTAATATATAAGAAAGCTCAGCC	
		B3	TCTTATTGAGTCTTGTGGTTG	
		FIP	GGTCAGGAACCTTGGGATAAATTTTGTACTCTAGGAAAAGGAACA	
		BIP	AACTTTAAGGAAGCGGATTATAGCTATGGATTACACTTTGTTGG	
	vcrA set C	LF	CCTGGTCCACCTAATTCAGTGA	NC_013552.1, 1187298–1188857 (857–1072)
		LB	ACTACAATGATGCAGAGTGGGTTA	
		F3	GTAAGTTTTACGCGAGATGG	
		B3	GTCATCGGCTGAGCTTTC	
		FIP	ACCCTCCCATTTTGGTACGCTTGTATGGTCCGCCACAT	
<i>bvcA</i>	bvcA set A	BIP	AAGACAATTTTCTAATGCTGAGGGCATTGGGATCTGCCAGGT	NC_009455.1, 834959 to 836509 (895–1139)
		LF	CCATCAGGTGGCGCTGAA	
		LB	TGGTGCTGGTGGCGTT	
		F3	ACAATGCCTTTACCAGAAGA	
		B3	ACCGTATTTGGGGCTGAT	
<i>tceA</i>	tceA set A	FIP	TCGGCCTCCATTAAGCCATTCTCTAGGGTGGTCATGT	AY165309.1 (882–1156)
		BIP	ATCAAGGACTTGGTGGCGACCTTGTTCGGAAAGACTCA	
		LF	AGGCAATCATACTTGAAGCGTC	
		LB	TGTGGGGACCTGGTGGT	
		F3	GCCGTTTATCCATTTCATGG	
		B3	GCATAGACTGGATGAAGGAA	
		FIP	ACATAATTGCTGGGAGAACCCG-TCGCATAGAGAGATAAGGCC	
		BIP	GCCATTCTGGCGGCATATAT-CAGATTATGACCCTGGTGAA	
		LF	CTTTATGGACGCTATGAAGTTCTA	
		LB	TCTTCCCTGCGGTCGCCATA	

ature and time, start the device, stream data via Bluetooth connectivity, and sort, plot, store, and transmit results. Disposable chips were made by etching channels and wells into black acrylic (1.58 mm thick) via a 40-W CO<sub>2</sub> laser (Full Spectrum). The etched chips were cleaned and prepared, as previously described (29). Briefly, the chips were cleaned with distilled water, soaked in 70% ethanol for 10 min, and dried for 10 min at 70°C. Once dry, primers were dispensed and dried in wells at 70°C for 5 min. The wells were enclosed with optical adhesive film (MicroAmp; Applied Biosystems), and the chips were stored at –20°C until use. The chips were cut into 8 reaction wells per sample, with 4 samples per chip (i.e., 32 reaction wells per chip with 20-μl reaction volume). Six chips were used to test groundwater samples,

and two additional chips were used to test plasmid dilution standards. *vcrA* and *tceA* primers were each dispensed into three separate reaction wells per sample lane.

## RESULTS

**Amplification with LAMP primers and their application.** As stated previously, *vcrA* set B was previously designed and tested using templates obtained from groundwater spiked with SDC-9 (25). In that study, larger volumes of groundwater samples (1 to 4 liters) were required when the *vcrA* gene copies were <10<sup>4</sup> gene copies/liter. In this study, we developed two new LAMP primer

TABLE 2 qPCR primers used in this study

Target gene	Primer	Sequence <sup>a</sup>	Reference
<i>vcrA</i>	vcrA1022F	CGGGCGGATGCACTATTTT	17
	vcrA1093R	GAATAGTCCGTGCCCTTCCTC	17
	vcrA1042Probe	FAM-CGCAGTAACTCAACCATTTCCTGGTAGTGG-TAMRA	17
<i>bvcA</i>	bvcA925F	AAAAGCACTTGGCTATCAAGGAC	17
	bvcA1017R	CCAAAAGCACCACCAGGTC	17
	bvcA977Probe	FAM-TGGTGGCGACGTGGCTATGTGG-TAMRA	17
<i>tceA</i>	tceA1270F	ATCCAGATTATGACCCTGGTGAA	26, 27
	tceA1336R	GCGGCATATATTAGGCATCTT	26, 27

<sup>a</sup> FAM, 6-carboxyfluorescein; TAMRA, 6-carboxytetramethylrhodamine.

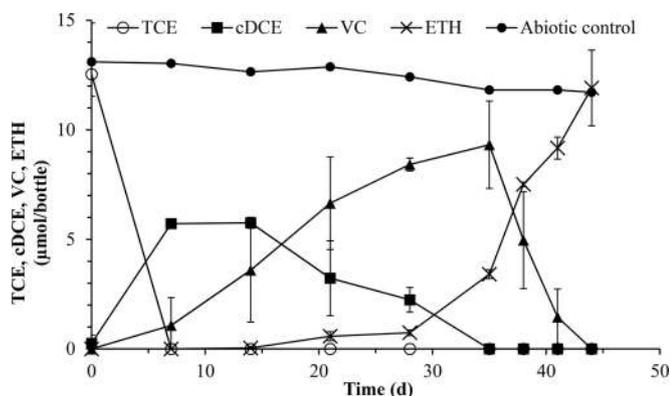


FIG 1 Mean masses of TCE, cDCE, VC, and ETH in triplicate KB-1 cultures and an abiotic control. The data are presented as the standard means and standard deviations.

sets (*vcrA* set A and *vcrA* set C) that exhibited faster LAMP threshold times than that of *vcrA* set B. For example, with  $10^{3.5}$  gene copy templates, the threshold times were  $23.9 \pm 0.4$  min for *vcrA* set A,  $21.2 \pm 0.2$  min for *vcrA* set C, and  $28.3 \pm 0.3$  min for *vcrA* set B. Moreover, the new primer sets had an equivalent or better detection limit than that of *vcrA* set B ( $10^{3.5}$  gene copies/reaction for *vcrA* set A,  $10^{2.5}$  gene copies/reaction for *vcrA* set C, and  $10^{3.5}$  gene copies/reaction for *vcrA* set B).

Here, the first set of experiments targeted *vcrA* in SDC-9 using both *vcrA* set A and *vcrA* set B. The second set of experiments involved *vcrA* set C, the most refined LAMP primer set for *vcrA*, with KB-1 templates.

Additionally, new primer sets were developed for the *bvcA* and *tceA* genes, and these were tested with a KB-1 template. One aim was to evaluate if the new LAMP primer sets could be used to track the growth of *Dehalococcoides* spp. in actively dechlorinating KB-1 and SDC-9 cultures over one growth cycle. As SDC-9 does not contain *bvcA*, this assay was not tested with this culture. qPCR was used as a control assay for all experiments. While the specificities of the new primer sets were not evaluated experimentally, LAMP reactions require six different primers for amplification and are thus unlikely to produce false positives.

**Monitoring *Dehalococcoides* species growth in KB-1 and SDC-9 cultures.** The mean masses of TCE, cDCE, VC, and ETH in triplicate KB-1 cultures and an abiotic control are shown in Fig. 1. As expected, TCE was reduced to cDCE and VC to ETH. cDCE accumulated and peaked at 7 days after inoculation, while VC peaked at ~35 days before being rapidly degraded to ETH. Stoichiometric amounts of ETH accumulated at the end of the growth cycle. TCE, cDCE, and VC were not detected at the end of the 48-day incubation period. At each time point, the concentrations of two *Dehalococcoides* strains, VS and BAV1, were investigated using qPCR and LAMP targeting the *vcrA* and *bvcA* genes in DNA extracted from the KB-1 cultures. Figure 2 illustrates the gene copies of *vcrA*, *bvcA*, and *tceA* per liter in triplicate cultures of KB-1 while growing on TCE. We observed a comparable steady increase in the number of *vcrA* gene copies, from  $\sim 5.8 \times 10^6$  gene copies/liter on day 7 to  $\sim 6.4 \times 10^9$  gene copies/liter on day 38 using both LAMP and qPCR. This was followed by a more rapid increase between days 38 and 44, from  $\sim 6.4 \times 10^9$  gene copies/liter to  $\sim 1.1 \times 10^{11}$  gene copies/liter, coupled with a significant reduction

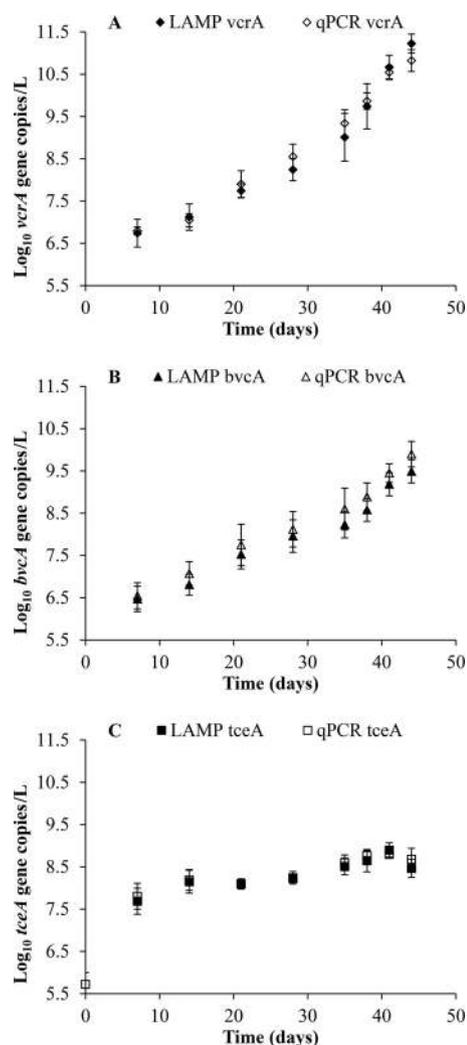


FIG 2 Gene copies of *vcrA* (A), *bvcA* (B), and *tceA* (C) per liter in triplicate cultures of KB-1 while growing on TCE. LAMP *vcrA* set C was used to target *vcrA*. The data are presented as the standard means and standard deviations.

of VC to ETH. A similar trend was observed with the *bvcA* gene. Gene copy numbers steadily increased from  $3.2 \times 10^6$  gene copies/liter at day 7 to  $5.7 \times 10^8$  gene copies/liter at day 38, followed by a more rapid increase to  $5.49 \times 10^9$  gene copies/liter at day 44. Gene copies of *tceA* increased to  $\sim 1.4 \times 10^8$  gene copies/liter from day 0 to day 14, coupled to the reduction of TCE to cDCE and VC, which was then followed by a slight increase to  $\sim 3.8 \times 10^8$  gene copies/liter on day 44.

The growth of *Dehalococcoides* spp. in SDC-9 culture was investigated using the *vcrA* gene. The mean masses of VC and ETH in triplicate SDC-9 cultures and an abiotic control are shown in Fig. 3. Rapid reductive dechlorination of  $\sim 24$   $\mu$ mol VC from days 20 to 40 was coupled to the stoichiometric accumulation of ETH. Figure 4 illustrates the *vcrA* gene copies per liter measured via two LAMP primer sets (*vcrA* set A and *vcrA* set B) and qPCR in the triplicate cultures. The *vcrA* gene copies steadily increased to  $\sim 9.0 \times 10^7$  gene copies/liter. As VC rapidly dechlorinated to ETH, we observed that the *vcrA* gene copies increased from  $\sim 9.0 \times 10^7$  gene copies/liter to  $\sim 1.1 \times 10^9$  gene copies/liter.

The mean gene copies of *vcrA*, *bvcA*, and *tceA* per liter in trip-

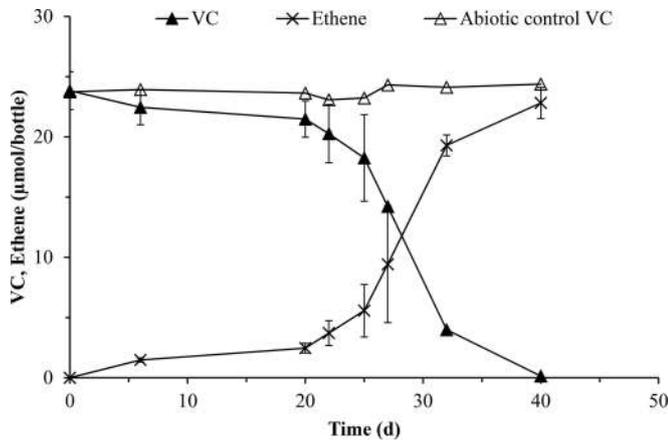


FIG 3 Mean masses of VC and ETH in triplicate SDC-9 cultures and an abiotic control. The data are presented as the standard means and standard deviations.

licate cultures of KB-1 while growing on TCE are shown in Fig. 5. Note that the  $y$  axis in Fig. 5 is a log scale, which does not start at zero, to illustrate the differences between *vcrA*, *bvcA*, and *tceA* concentrations. To elucidate the potential of LAMP as an alternate method to monitor *Dehalococcoides* spp. in commercial reductive dechlorinating cultures, the absolute quantification of each gene in a KB-1 template was determined using the two methods. Figure 6 shows a comparison of the mean gene copies (per liter) in *vcrA*, *bvcA*, and *tceA* in triplicate cultures of KB-1 while growing on TCE.

**Validation of new LAMP assays with the Gene-Z platform using groundwater templates.** Nucleic acids extracted from groundwater from a previously bioaugmented and chlorinated solvent-contaminated site were used to validate the novel LAMP assays with the Gene-Z handheld device. The data obtained using the new LAMP assays on the Gene-Z device were compared to those obtained using qPCR on a real-time thermal cycler. Specifically, *vcrA* and *tceA* gene copies (per liter) from four monitoring wells and four injection wells (in triplicate) were compared using the new LAMP assays and qPCR (Fig. 7).

## DISCUSSION

To date, LAMP primer sets have been developed for phylogenetic or functional genes of prokaryotes, eukaryotes, and viruses to de-

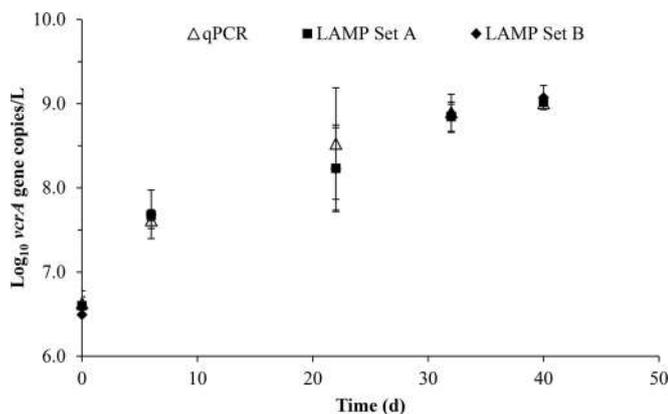


FIG 4 *vcrA* gene copies per liter measured via qPCR and two LAMP assays (*vcrA* set A and *vcrA* set B) in triplicate cultures of SDC-9 during growth on VC. The data are presented as the standard means and standard deviations.

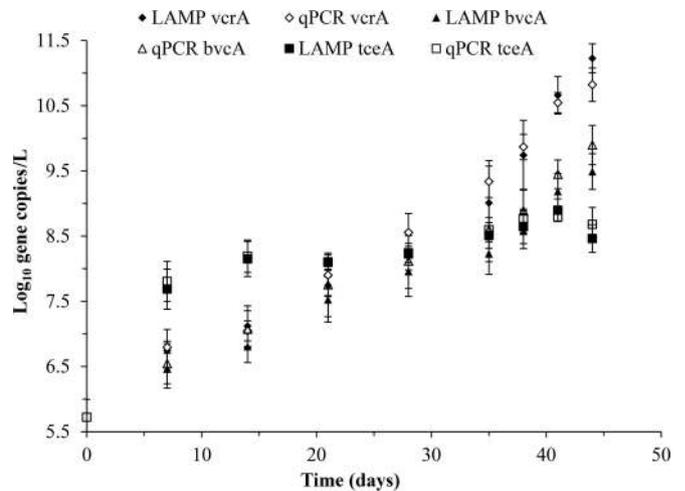


FIG 5 Mean gene copies of *vcrA*, *bvcA*, and *tceA* per liter in triplicate cultures of KB-1 while growing on TCE. The data are presented as the standard means and standard deviations. Note that the  $y$  axis is a log scale and does not start at zero.

tect target sequences in templates extracted from a variety of environmental matrices, such as air, water, soil, fecal matter, and blood. For example, LAMP can be used for detection of the *invA* gene in all known 89 strains of *Salmonella*, a foodborne bacterial pathogen causing salmonellosis (30, 31). LAMP was also applied to detect *Cryptosporidium* oocysts, which cause cryptosporidiosis, using the functional gene *gp60* (32). Similarly, LAMP primer sets for the detection of viral pathogens, such as HIV-1 and Ebola virus, have also been described (23, 24). Another application of LAMP has been the identification of organisms in beef contaminated with ostrich meat. The LAMP assay successfully identified ostrich meat contamination of up to 0.01% using direct-cell amplification from swabs (33). These examples demonstrate the versatility of LAMP in terms of its application to human health, environmental, and food microbiology. In this study, LAMP was applied for the rapid quantification of the key biomarker RDase genes *vcrA*, *bvcA*, and *tceA*. These biomarker genes are important for monitoring the activity of *Dehalococcoides* spp. in groundwater during natural attenuation, biostimulation, and bioaugmentation at chlorinated solvent-contaminated sites.

The growth patterns observed in this study are characteristic of cultures, such as KB-1 and SDC-9, when amended with TCE and VC, respectively. KB-1 is highly enriched in a few unique *D. mccartyi* strains, which are capable of catabolic growth using cDCE and VC as electron acceptors for reductive dechlorination (34). Typically, the individual cells of such strains carry one copy of the *vcrA* or *bvcA* gene coding for the two distinct vinyl chloride reductases (35–37). However, neither vinyl chloride reductase is capable of growth-linked metabolic reduction of TCE to cDCE or VC. When amended with TCE, initial growth is cometabolic and often slower (4, 6, 9). As a result, the increase in observed *vcrA* and *bvcA* gene copies is faster and more discernible when cDCE and VC are being dechlorinated. In contrast, the *tceA* gene codes for trichloroethene reductive dehalogenase, which is responsible for the reductive dechlorination of TCE to cDCE and VC (38). An initial increase in the *tceA* gene copies coupled to rapid reduction of TCE to cDCE and VC was observed in all KB-1 microcosms, but as TCE

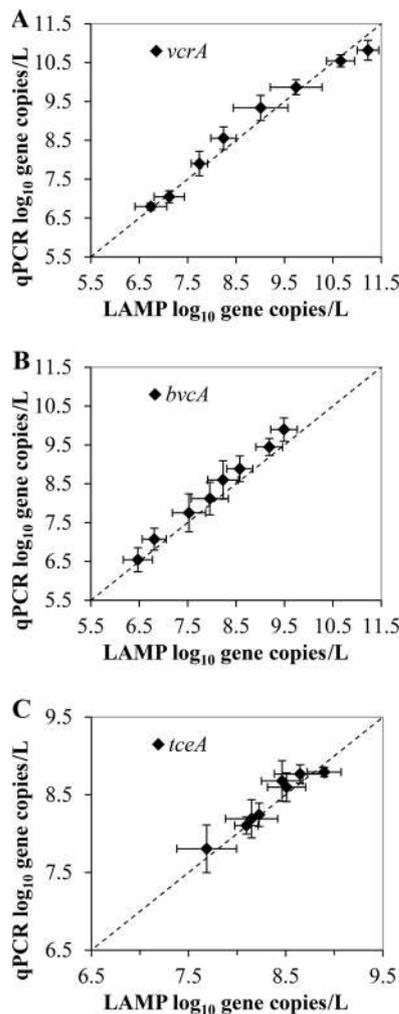


FIG 6 Comparison of *vcrA* (A), *bvcA* (B), and *tceA* (C) mean gene copies per liter in triplicate cultures of KB-1 while growing on TCE. The data are presented as the standard means and standard deviations (horizontal and vertical bars). The dashed line represents a 1:1 slope.

was depleted, the increase in *tceA* gene copies was substantially less. This is indicative of growth of *D. mccartyi* strains with the *tceA* gene. However, the abundance of the *tceA* gene within the *Dehalococcoides* genus is more widespread than that of the *vcrA* and *bvcA* genes, and strains that carry the *tceA* gene may also carry the *vcrA* or *bvcA* gene (6, 8, 17). Additionally, TCE dechlorination may also be driven by *Geobacter* strains in KB-1, along with *Dehalococcoides* spp. (39), which may explain the less-discernible growth pattern.

In this research, the novel LAMP assays for *vcrA* and *tceA* were validated using DNA extracted from groundwater samples. We compared the quantification results, i.e., gene copies per liter values, for both genes obtained using qPCR on a real-time thermal cycler with LAMP on the Gene-Z platform.

Overall, similar values were obtained for each gene on the two platforms, indicating that quantification with LAMP on the Gene-Z platform is a viable alternative to qPCR. For some data points, the Gene-Z platform yielded slightly higher values than those of qPCR, which may be attributed to the difference in the fluorescence-sensing mechanisms of the two platforms. This issue

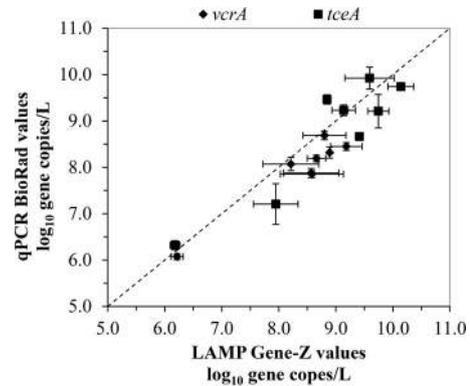


FIG 7 Comparison of *vcrA* and *tceA* mean gene copies per liter in triplicate cultures or eight different groundwater DNA templates observed using qPCR on a real-time thermal cycler and the Gene-Z platform. The dashed line represents a 1:1 slope.

will be examined in more detail in future studies. The new LAMP primer sets were able to detect quantities of  $<10^7$  gene copies/liter, the accepted limit for natural attenuation.

LAMP offers two key advantages over qPCR. First, the LAMP primer sets described here may be used with a variety of several less-expensive platforms (with diverse types of detection mechanisms), which include real-time turbidimeters, microfluidic chips (e.g., Gene-Z), and electrochemical or ultrasonic sensors (25, 40–43). Besides being cheaper, these platforms are more-accessible alternatives to qPCR thermal cyclers. Second, these platforms use different reaction chemistries (e.g., they produce significant visible fluorescence or postreaction electrochemical changes) to detect amplified target sequences and thus can be more economical than qPCR. In time-limited studies, another potential advantage is that amplification during LAMP is faster than that with qPCR. With the primer sets and reaction chemistries described in this study, all LAMP reactions were complete in  $<1$  h, which is significantly shorter than a typical qPCR run ( $>1.5$  h). In summary, the development of LAMP assays for the detection of the RDase genes *vcrA*, *bvcA*, and *tceA* using alternative and potentially field-deployable platforms, such as the Gene-Z platform (25), will enable the rapid detection and quantification of *Dehalococcoides* gene biomarkers in groundwater from solvent-contaminated sites. Further, the development of LAMP assays specific to two commonly used commercially available cultures will facilitate the specific detection of these RDase genes at sites subject to bioaugmentation.

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