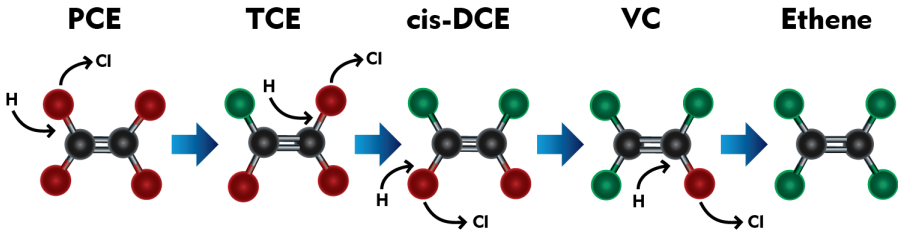
DHC Interpretation

Dehalococcoides 16S rRNA gene (qDHC)

Under anaerobic conditions, tetrachloroethene (PCE) and trichloroethene (TCE) can undergo sequential reductive dechlorination through the daughter products *cis*-dichloroethene (*cis*-DCE) and vinyl chloride to nontoxic ethene (1,2).



While a number of bacterial cultures capable of utilizing PCE and TCE as growth supporting electron acceptors have been isolated (3-7), *Dehalococcoides* spp. may be the most important because they are the only bacterial group that has been isolated to date which is capable of complete reductive dechlorination of PCE to ethene (8). In fact, the presence of *Dehalococcoides* spp. has been associated with complete dechlorination to ethene at sites across North America and Europe (9).

Status	*Dehalococcoides spp.*	Observation
	**≥ 10^4** (cells/mL)	Lu et al. proposed that a concentration of 1 x 10^4 DHC cells/mL could be used as a screening criterion to identify sites where reductive dechlorination will yield a generally useful biodegradation rate (10). Similarly, in an internal study conducted with nearly 1000 groundwater samples obtained from sites across the US, ethene production was observed in approximately 80% of samples in which CENSUS® qDHC results were greater than or equal to 10^4 DHC cells/mL.
	**10^1 to < 10^4** (cells/mL)	When vinyl chloride reductase genes (See DHC functional genes discussion below) are also detected, complete reductive dechlorination of PCE and TCE to ethene may still occur even with moderate DHC concentrations. When the DHC population is below the 10^4 cells/mL criterion proposed by Lu et al. (10), project managers should carefully consider other site-specific data to determine whether subsurface conditions may be limiting reductive dechlorination. For example, the addition of an electron donor may be able to stimulate DHC growth and enhance anaerobic bioremediation.
	**< 10^1** (cells/mL)	DHC concentrations are low suggesting that complete reductive dechlorination of PCE and TCE to ethene is unlikely to occur under existing conditions. Enhanced anaerobic bioremediation options (biostimulation or bioaugmentation) may need to be considered.

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DHC Functional Genes (*tce*A, *bvc*A, *vcr*A)

A "stall" where daughter products *cis*-DCE and vinyl chloride accumulate can occur at PCE- and TCE-impacted sites especially under MNA conditions. The accumulation of vinyl chloride, generally considered more carcinogenic than the parent compounds, is particularly problematic. Although elevated *Dehalococcoides* concentrations correspond to ethene production in numerous studies, the range of chlorinated ethenes metabolized and cometabolized varies among species and strains within the *Dehalococcoides* genus. For example, *Dehalococcoides ethenogenes* str. 195 metabolizes PCE, TCE, and *cis*-DCE and cometabolizes vinyl chloride (8) to produce ethene. Conversely, *Dehalococcoides* sp. CBDB1 utilizes PCE and TCE but does not cometabolize additional chloroethenes (11). Other *Dehalococcoides* strains, such as BAV1, GT and VS, are known to fully dechlorinate cis-DCE and VC to ethene (14,16,19). Quantification of reductive dehalogenase genes is used to more definitively confirm the potential for reductive dechlorination of TCE, cis-DCE, and vinyl chloride (12-15).

Functional Gene	Observation
TCE Reductase	
***tce*A gene**	The *tce*A gene encodes the enzyme responsible for reductive dechlorination of TCE to *cis*-DCE in some strains of *Dehalococcoides*. Absence of *tce*A does not preclude the potential for reductive dechlorination of TCE in the field since the *tce*A gene is not universally distributed among all DHC and is not present in other microorganisms capable of reductive dechlorination of TCE (e.g. *Dehalobacter*). Detection of the *tce*A gene provides an additional line of evidence indicating the potential for dechlorination of TCE.
Vinyl Chloride Reductase	
***bvc*A gene**	The *bvc*A gene encodes the vinyl chloride reductase enzyme responsible for reductive dechlorination of vinyl chloride to ethene by *Dehalococcoides* sp. str. BAV1 (16). Presence of *bvc*A gene indicates the potential for reductive dechlorination of VC to ethene. Absence of both *bvc*A and *vcr*A genes suggests VC may accumulate. An internal study with ~1,000 samples showed ethene production was observed in 80% of the samples that the DHC population was greater than or equal to 10^4 cells/mL. The *bvc*A gene was detected in over 50% of these samples. Van Der Zaan et al (17) noted that the bvcA gene was the only VC reductase gene detected at three of their sites. Alfred Spormann's laboratory at Stanford University (18) reported that the *bvc*A gene was the most abundant and active at the outflow of a PCE fed column study. This section of the column was in the DCE to VC stages of reductive dechlorination thus confirming the importance of the *bvc*A gene for complete reductive dechlorination.
***vcr*A gene**	The *vcr*A gene encodes the vinyl chloride reductase enzyme responsible for reductive dechlorination of *cis*-DCE and vinyl chloride by *Dehalococcoides* sp. strain VS (14). Presence of *vcr*A gene indicates the potential for reductive dechlorination of DCE and/or VC to ethene. Absence of both *bvc*A and *vcr*A genes suggest VC may accumulate. As with the *bvc*A gene, detection of the *vcr*A gene is associated with ethene production in internal studies (67%) and vinyl chloride reduction in independent studies (14, 17).

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Reporting

Microbial Insights can provide a variety of data packages and reporting levels to suit the needs of any project. Data packages range from simple analytical reports with results only to more complex data packages that include a report narrative, analytical results, QC data, and supporting materials including all raw data and chain-of-custody documentation. The figure below shows our standard report and explains the way values are reported.

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CENSUS

Client: Company Name
Project: Your Project Name

MI Project Number: Unique Laboratory Identifier
Date Received: Date Samples Arrived

Sample Information

Client Sample ID:		Sample A	Sample B	Sample C
Sample Date:		00/00/0000	00/00/0000	00/00/0000
Units:		cells/mL	cells/mL	cells/mL
Analyst:		Intials	Intials	Intials
Dechlorinating Bacteria				
Dehalococcoides spp.	DHC	**1.84E+05**	**2.76E+02**	**2.28E+01 (J)**
Functional Genes				
tceA Reductase	TCE	**6.00E+01**	**3.23E+01**	<4.00E-01
bvcA Reductase	BVC	**1.17E+04**	**1.81E+01**	<4.00E-01
vcrA Reducatase	VCR	**8.42E+04**	**1.74E+02**	<4.00E-01

"J" value
Result is an estimated value. This data qualifier (flag) is used when the target gene is detected but at a concentration or abundance below the practical quantification limit (PQL).

Legend:
NA = Not Analyzed NS = Not Sampled J = Estimated gene copies below PQL but above LQL I = Inhibited
< = Result not detected

< value
The target gene was not detected at the limit of quantitation (LOQ) reported for that sample.

"I" value
QA Procedure indicated that the sample may have exhibited PCR inhibition. Although relatively rare, PCR inhibition can occur due to the presence of metals or humic acids at high concentrations in the sample.

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Quality Assurance

Microbial Insights' comprehensive Quality Assurance (QA) Program is the foundation of all laboratory analyses, ensuring that our clients receive high-quality analytical services that are timely, reliable, and meet their intended purpose in a cost effective manner. MI is committed to providing quality data that surpasses regulatory and industry standards, thus enabling the client to make well-informed decisions. MI maintains strict standard operating procedures and QA/QC measures throughout all of the analyses offered. The following Table details specific QA/QC procedures that are used for CENSUS.

QA/QC	Description
Date of Extraction	DNA and RNA extractions are performed the day the samples are received by MI to minimize the possibility of any changes to the microbial community prior to analysis.
Laboratory Method Blanks	An extraction blank (no sample added) is processed alongside each set of field samples from DNA extraction through CENSUS® analysis to ensure that cross contamination has not occurred. Although MI has never experienced this issue, the detection of the CENSUS® target (e.g. *Dehalococcoides*) in an extraction blank is direct evidence of cross contamination with a sample or contamination of a reagent and would invalidate the results. If this were to occur, MI would re-extract the sample. If not possible to re-extract, MI would contact the client immediately and notate it on the laboratory report.
Laboratory Control Samples (LCS)	A laboratory control sample (LCS) or positive control (target DNA) is included with each CENSUS® plate to confirm amplification and as a continuing calibration check.
Negative Controls	A negative control (no DNA) is included with each CENSUS plate to ensure that cross contamination has not occurred during amplification. As with the extraction blank, detection of CENSUS target (e.g. DHC) in a negative control is direct evidence of contamination and would invalidate the results. If this were to occur, MI would rerun the analysis.

References

1. Freedman, D. L. and J. M. Gossett. 1989. Biological reductive dechlorination of tetrachloroethylene and trichloroethylene to ethylene under methanogenic conditions. Applied and Environmental Microbiology 55(9): 2144-2151.

2. DiStefano, T. D., J.M. Gossett, and S.H. Zinder. 1991. Reductive dechlorination of high concentrations of tetrachlorethene to ethene by an anaerobic enrichment culture in the absence of methanogenesis. Applied and Environmental Microbiology 57(8): 2287-2292.

3. Gerritse, J., V. Renard, T. M. Pedro Gomes, P. A. Lawson, M. D. Collins, and J. C. Gottschal. 1996. *Desulfitobacterium* sp. Strain PCE1, an anaerobic bacterium that can grow by reductive dechlorination of tetrachloroethene or ortho-chlorinated phenols. Archives of Microbiology 165(2): 132-140.

4. Gerritse, J., O. Drzyzga, G. Kloetstra, M. Keijmel, L. P. Wiersum, R. Hutson, M. D. Collins, and J. C. Gottschal. 1999. Influence of different electron donors and acceptors on dehalorespiration of tetrachloroethene by *Desulfitobacterium frappieri* TCE1. Applied and Environmental Microbiology 65(12): 5212-5221.

5. Holliger, C., G. Schraa, A.J.M. Stams, and A.J.B. Zehnder. 1993. A highly purified enrichment culture couples the reductive dechlorination of tetrachloroethene to growth. Applied and Environmental Microbiology 59 (9): 2991-2997.

6. Krumholz, L. R., R. Sharp, and S. S. Fishbain. 1996. A freshwater anaerobe coupling acetate oxidation to tetrachloroethylene dehalogenation. Applied and Environmental Microbiology 62(11): 4108-4113.

7. Löffler, F.E., R.A. Sanford, and J.M. Tiedje. 1996. Initial characterization of a reductive dehalogenase from *Desulfitobacterium chlororespirans* Co23. Applied and Environmental Microbiology 62(10): 3809–3813.

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8. Maymó-Gatell, X., T. Anguish, and S.H. Zinder. 1999. Reductive dechlorination of chlorinated ethenes and 1,2-dichloroethane by *Dehalococcoides ethenogenes* 195. Applied and Environmental Microbiology 65(7): 3108–3113.

9. Hendrickson, E.R., J. Payne, R.M. Young, M.G. Starr, M.P. Perry, S. Fahnestock, D.E. Ellis, and R.C. Eversole. 2002. Molecular analysis of *Dehalococcoides* 16S ribosomal DNA from chloroethene-contaminated sites throughout North America and Europe. Applied and Environmental Microbiology 68(2): 485-495.

10. Lu, X., J.T. Wilson, and D.H. Kampbell. 2006. Relationship between *Dehalococcoides* DNA in ground water and rates of reductive dechlorination at field scale. Water Research 40:3131-3140.

11. Adrian, L, U. Szewzyk, J. Wecke, and H. Görisch. 2000. Bacterial dehalorespiration with chlorinated benzenes. Nature 408(6812): 580-583.

12. Holmes, V.F., J. He, P.K.H. Lee, and L. Alvarez-Cohen. 2006. Discrimination of multiple *Dehalococcoides* strains in a trichlorethene enrichment by quantification of their reductive dehalogenase genes. Applied and Environmental Microbiology 72(9): 5877-5883.

13. Lee, P.K.H., D.R. Johnson, V.F. Holmes, J. He, and L. Alvarez-Cohen. 2006. Reductive dehalogenase gene expression as a biomarker for physiological activity of *Dehalococcoides* spp. Applied and Environmental Microbiology 72(9): 6161-6168.

14. Müller, J.A., B.M. Rosner, G. von Avendroth, G. Meshulam-Simon, P.L. McCarty, and A.M. Spormann. 2004. Molecular identification of the catabolic vinyl chloride reductase from *Dehalococcoides* sp. strain VS and its environmental distribution. Applied and Environmental Microbiology 70(8): 4880-4888.

15. Ritalahti, K.M., B.K. Amos, Y. Sung, Q. Wu, S.S. Koenigsberg, and F.E. Löffler. 2006. Quantitative PCR targeting 16S rRNA and reductive dehalogenase genes simultaneously monitors multiple *Dehalococcoides* strains. Applied and Environmental Microbiology 72(4): 2765-2774.

16. Krajmalnik-Brown, R., T. Hölscher, I. N. Thomson, F. M. Saunders, K. M. Ritalahti, and F. E. Löffler. 2004. Genetic identification of a putative vinyl chloride reductase in *Dehalococcoides* sp. strain BAV1. Applied and Environmental Microbiology 70:6347–6351.

17. van der Zaan, B., F. Hannes, N. Hoekstra, H. Rijnaarts, W.M. de Vos, H. Smidt, and J. Gerritse. 2010. Correlation of *Dehalococcoides* 16S rRNA and chlorethene-reductive dehalogenase genes with geochemical conditions in chloroethene-contaminated groundwater. Applied and Environmental Microbiology 76(3):843-850.

18. Behrens, S., M.F., Azizian, P.J. McMurdie, A. Sabalowsky, M.E. Dolan, L. Semprini, and A.M. Spormann. 2008. Monitoring abundance and expression of *Dehalococcoides* species chloroethene-reductive dehalogenases in a tetrachloroethene-dechlorinating flow column. Applied and Environmental Microbiology 74(18):5695-5703.

19. Sung, Y., K. M. Ritalahti, R. P. Apkarian, and F. E. Löffler. 2006. Quantitative PCR confirms purity of strain GT, a novel trichloroethene (TCE)-to-ethene respiring Dehalococcoides isolate. Appl. Environ. Microbiol. 72:1980-1987

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