Rødekro 2015. Vurdering af udviklingen i den naturlige nedbrydning i nedstrømsforureningsfane efter kildeoprensning

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SiREM Technical Note 1.5:

Guidelines for Interpretation of Gene-Trac® Test Results

This document provides technical background information and guidelines for interpreting the results for the following Gene-Trac® assays:

(1) Gene-Trac® Dhc
(2) Gene-Trac® VC
(3) Gene-Trac® Dhb

SiREM Technical Note 1.4 - *Quantitative Gene-Trac® Assay Test Procedure and Reporting Overview* provides detailed information on Gene-Trac® test procedures and reporting. Explanation of data qualifiers and commonly used notes is provided as Appendix A. Table 1 provides a brief interpretation for some common scenarios, more detailed interpretation information is provided in the following sections.

Table 1: Common Gene-Trac® Test Result Scenarios and Interpretation

Gene-Trac® Dhc (*Dehalococcoides*)	**Gene-Trac® VC (*vcrA*)**	**Gene-Trac® Dhb (*Dehalobacter*)**	**Interpretation**
>1 x10^7/L	**>1 x10^7/L**	Not Analyzed	Complete dechlorination to ethene likely as Dhc high and *vcrA* high
1 x10^7/L	**Not Detected**	Not Analyzed	VC accumulation possible as *vcrA* negative
Not Detected	**Not Detected**	Not Analyzed	Dhc negative/ lack of dechlorination or *cis*-DCE accumulation likely
Not Analyzed	Not Analyzed	**1 x10^6/L**	Dhb positive,potential for biodegradation of 1,1,1-TCA, 1,2-DCA, carbon tetrachloride and chloroform, PCE and TCE to *cis*-DCE
Not Analyzed	Not Analyzed	**Not Detected**	Biodegradation of 1,1,1-TCA, carbon tetrachloride and chloroform not expected as Dhb negative

Gene-Trac® Dhc -Total *Dehalococcoides* Test

Background:

Gene-Trac® Dhc is a quantitative PCR (qPCR) test for total *Dehalococcoides* (Dhc) microbes that targets Dhc specific sequences of the 16S ribosomal ribonucleic acid (rRNA) gene, a gene commonly used to indentify microbes. Dhc are the only known microorganisms capable of complete dechlorination of chloroethenes (i.e., tetrachloroethene, trichloroethene, cis-1,2-dichloroethene [cis-DCE] and vinyl chloride) to non-toxic ethene. Gene-Trac® Dhc may also be used to assess the in situ growth of Dhc containing bioaugmentation cultures such as KB-1®.

Negative Gene-Trac® Dhc Test Results (U qualified)

A non-detect in the Gene-Trac® Dhc assay (e.g., 4,000U) indicates that Dhc were not detected in the sample. The absence of Dhc is frequently associated with a lack of complete dechlorination or incomplete dechlorination of chlorinated ethenes. Where Dhc are absent the accumulation of cis-DCE is commonly observed, particularly after addition of electron donors. Bioaugmentation with Dhc containing cultures, such as KB-1®, is commonly used to improve bioremediation performance at sites that lack an indigenous Dhc population.

Positive Gene-Trac® Dhc Test Results

The detection of Dhc has been correlated with the complete biological dechlorination of chlorinated ethenes to ethene at contaminated sites (Hendrickson et al., 2002). A positive Gene-Trac® Dhc test indicates that Dhc DNA was detected in the sample and is encouraging for dechlorination of chlorinated ethenes to ethene. Note not all Dhc are capable of conversion of vinyl chloride to ethene; this capability can be determined by the Gene-Trac® VC test (see Section 2) which is commonly performed as a follow-on analysis after positive Gene-Trac® Dhc tests. In most cases Dhc must be present at sufficient concentrations in order for significant dechlorination to be observed, guidelines for expected impacts at various Dhc concentrations are indicated below.

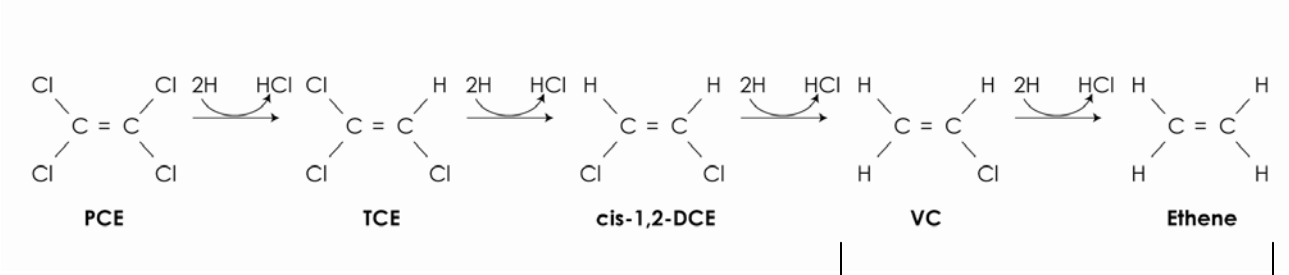
> **Values of 10^4 Dhc gene copies per liter (or lower):** indicates that the sample contains low concentrations of Dhc which may indicate that site conditions are suboptimal for high rates of dechlorination. Increases in Dhc concentrations at the site may be possible if conditions are optimized (e.g., electron donor addition).
>
> **Values of 10^5-10^6 Dhc gene copies per liter:** indicates the sample contains moderate concentrations of Dhc which may, or may not, be associated with observable dechlorination activity (i.e., detectable ethene).
>
> **Values at or above 10^7 Dhc gene copies per liter:** indicates that the sample contains high concentrations of Dhc that are often associated with high rates of dechlorination (Lu et al., 2006) and the production of ethene.
>
> **Values of 10^9 Dhc gene copies per liter** are generally the highest observed for groundwater samples with rare exceptions.

Gene-Trac® VC- Vinyl Chloride Reductase (*vcrA*) Test

Background

Gene-Trac® VC is a qPCR test for the vinyl chloride reductase (*vcrA*) gene that codes for a Dhc enzyme that converts (VC) to ethene, a critical step in reductive dechlorination of chlorinated ethenes. Gene-Trac® VC is commonly used where Gene-Trac® Dhc test results are positive to confirm that the Dhc detected are capable of complete dechlorination to ethene.#

The vinyl chloride reductase gene (*vcrA)* (Müller et al., 2004) produces an enzyme that is found in many (but not all) Dhc and is reported to be the most common identified VC reductase in the environment (van der Zaan et al., 2010).



Key activity of vinyl chloride reductase vcrA gene/enzyme

Interpretation of Gene-Trac® VC Results

Detect in Gene-Trac® VC Test

A detect in the Gene-Trac® VC test indicates that a Dhc population has the *vcrA* gene and the prospects for complete dechlorination to ethene are good. As a minimal requirement, *vcrA* copies exceeding 10^5/L combined with observed increases over time (i.e., cell growth) are required for robust VC dechlorination (van der Zaan et al., 2010). Also the guidelines for detection of ethene provided under Gene-Trac® Dhc are conservative for interpretation of Gene-Trac® VC (i.e., > 1 x10^7 gene copies/L indicate a high likelihood of detection of ethene). In one study, more than 90% of samples where *vcrA* enumeration exceeded 1 x10^7 gene copies/L had detectable ethene (Dennis, 2009). In cases where *vcrA* gene copies are lower the likelihood of detectable ethene decreases.

Non-Detect in Gene-Trac® VC Test (U qualified)

A non-detect in the Gene-Trac® VC test indicates that *vcrA* gene sequences in the sample are below the detection limit of the assay (typically 4 x 10^3 *vcrA* gene copies/L). This indicates VC accumulation (VC stall) is possible. Note negative Gene-Trac® VC test results do not indicate with 100% certainty that a VC-stall will occur as there are other vinyl chloride reductase genes, such as *bvcA* (van der Zaan et al., 2010) that also convert VC to ethene.

Comparing Gene-Trac® VC and Gene-Trac® Dhc Test Results

Sites may contain different types of Dhc populations. At some sites the Dhc population is homogenous while other sites have Dhc populations that are mixtures of different types of Dhc. This can lead to differing results for Gene-Trac® Dhc and Gene-Trac® VC.

In many cases, the numerical results of Gene-Trac® VC test are identical to those obtained in the Gene-Trac® Dhc test, indicating that the entire Dhc population contains the *vcrA* gene. In other cases, Gene-Trac® VC results may differ significantly (i.e., more than an order or magnitude) from the total Dhc for a number of reasons.

Table 3 provides some common scenarios for Gene-Trac® VC and Gene-Trac® Dhc test results. In general, where Gene-Trac® VC results are non-detect, or significantly lower than Gene-Trac® Dhc, accumulation of VC is more likely.

Table 2: Interpretation of Gene-Trac® VC in Relation to Gene-Trac® Dhc

Gene-Trac® Dhc (16S rRNA gene copies/ L)	Gene-Trac® VC (*vcr*A gene copies/L)	Results Summary	Interpretation	Potential Site Implications
2×10^8 /L	3×10^8/L	Total Dhc and *vcrA* are ~the same (within 3-fold)	Entire Dhc population has *vcrA* gene	Potential for complete dechlorination high. VC stall unlikely-sites with *vcr*A above 1×10^7/L typically have detectable ethene
1×10^8/L	Non-detect	Total Dhc high; *vcrA* non-detect	High concentration of Dhc and entire population lacks the *vcrA* gene	Likelihood for VC accumulation high as *vcrA* non-detect
1×10^8/L	1×10^6 /L	Total Dhc is significantly higher (100 fold) than *vcrA*	*Dhc* population consists of different types, some with the *vcrA* gene (~1%) and some without (~99%)	VC-accumulation possible; Dhc/*vcrA* proportions may change over course of remediation
1×10^6/L	1×10^8/L	*vcrA* orders of magnitude higher than Dhc	Significantly higher *vcrA* may indicate the presence of populations of non-Dhc microorganisms with *vcrA* like genes	Potential for VC-stall likely low

Gene-Trac® Dhb-Total *Dehalobacter* Test

Gene-Trac® Dhb is a qPCR test targeting the 16S rRNA gene sequences unique to *Dehalobacter* (Dhb). Dhb are implicated in the biodegradation of 1,1,1-trichloroethane (to chloroethane), 1,1,2-trichloroethane and 1,2-dichloroethane to ethene (Grostern and Edwards, 2006) and chloroform (to dichloromethane) (Grostern et al., 2010) as well as incomplete dechlorination of PCE and TCE to cis-DCE (Holliger et al.,1998). Gene-Trac® Dhb may also be used as a tool to assess the impact of bioaugmentation with the KB-1® Plus cultures which contain high concentrations of Dhb.

Positive Gene-Trac® Dhb Test Results (Detects)

A positive Gene-Trac® Dhb indicates that a member of the *Dehalobacter* (Dhb) genus was detected in the sample. The detection of Dhb indicates that some or all of the dechlorination activities attributed to Dhb may be present at the subject site. Increasing concentrations of Dhb are indicative of increased potential to degrade some or all of these compounds.

Note: the Gene-Trac® Dhb test will not differentiate the type of Dhb*;* therefore, observations of the specific biodegradation pathways and end products based on chemical analytical methods in conjunction with Gene-Trac® Dhb will increase the interpretability of Gene-Trac® Dhb results.

Note: Dhb have been reported to contain multiple copies (up to 4 per cell) of the 16S rRNA gene (Grostern and Edwards, 2008). This means that, unlike Dhc, there is not a 1:1 ratio between the 16S rRNA gene copy and the number of Dhb cells in a sample. Calculating the number of Dhb cells requires dividing the Gene-Trac® Dhb test result by the 16S rRNA gene copy number (often 3-4 copies/cell).

Non-detect Gene-Trac® Dhb Results (U qualified)

In cases where Gene-Trac® Dhb is not detected (e.g., 4,000U) this indicates that *Dehalobacter* species were not identified in the sample and that anaerobic reductive dechlorination of 1,1,1-TCA, 1,1,2-TCA, 1,2-DCA or chloroform, which are dechlorinated by *Dehalobacter*, may not be observed. This activity can be introduced at sites through the addition of bioaugmentation cultures containing *Dehalobacter* such as KB-1® Plus.

Key Elements of Gene-Trac® Data

Gene-Trac® test results include two key values (a) Target Gene Enumeration, an enumeration of target gene sequence by quantitative PCR (e.g. "Dhc Enumeration" "Dhb 16S Gene Copies" or "*vcrA* gene copies") and (b) Target gene percent (e.g. "Percent Dhc"), an estimated percentage of the microbial population comprised by microbes harboring the target gene and other microbes present in sample. Further explanation of these values is provided below.

a) Target Gene Enumeration

This value is the concentration of Dhc or Dhb 16S rRNA or *vcrA* gene copies detected in the sample. Results may be reported as either gene copies per liter (for groundwater) or per gram (for soil). In general, the greater the number of gene copies in a sample the greater the likelihood of related dechlorination activity. Dhc 16S gene copies are typically equivalent to the number of Dhc as they have 1 gene copy per cell this is not necessarily true for Dhb or *vcrA* which have the potential be present in multiple gene copies per cell. Guidelines for relating target gene presence and concentration to observable dechlorination activity for groundwater samples are provided below in previous sections.

b) Target Gene Percent (%Dhc, %Dhb, %*vcrA*)

This value estimates the percentage of the target gene (e.g., %Dhc) relative to other microorganisms in the sample based on the formulas/assumptions presented below. For example, %Dhc is a measure of the predominance of Dhc and, in general, the higher this percentage the better.

$$\%Dhc = \frac{Number\ Dhc}{Number\ Dhc + Number\ other\ Bacteria}$$

Where:

$$Number\ other\ Bacteria = \frac{Total\ DNA\ in\ sample\ (ng) - DNA\ attributed\ to\ Dhc\ (ng)}{*4.0 \times 10^{-6}\ ng\ DNA\ per\ bacterial\ cell}$$

*Paul and Clark, (1996).

Percent Dhc (and % *vcrA*) values can range from very low fractions of percentages, in samples with low numbers of Dhc and a high number of other bacteria (incompletely colonized by Dhc), to greater than 50% in Dhc enriched locations (highly colonized by Dhc).

In addition to determining the predominance of the target gene target gene percent is also useful for interpretation of Dhc counts from different sampling locations, or the same location over time. For example, the %Dhc value can be used to correct Dhc counts where samples are biased due to non-representative sampling. Example 1 illustrates a hypothetical scenario where the %Dhc value improved data interpretation.

Example 1, use of %Dhc to interpret enumeration data

Table 2 presents results from MW-1 sampled in April, May and June. Based on the Dhc enumeration alone one would conclude that the concentration of Dhc held steady between April and May; however, the %Dhc indicates the proportion of Dhc actually increased from April to May and the unchanged count in May could be a case of low biomass recovery during sampling or other losses such as sample degradation in transit. The higher raw count and the higher percentage of Dhc in June confirm the trend of increasing Dhc concentrations over time.

Table 3: Use of % Dhc* Value to Diagnose Sampling Bias

Sample	Dhc Enumeration	%Dhc	Interpretation Based on %Dhc
MW-1, April	1.0 x 10^5/Liter	0.1%	Dhc is a low proportion of total microbial population
MW-1, May	1.0 x 10^5/Liter	1%	Dhc proportion increased 10-fold from April. Dhc enumeration was unchanged possibly due to low biomass recovery from monitoring well, non-biased sample would be [(1.0/0.1) x 1.0 x10^5] = 1.0 x 10^6/Liter
MW-1, June	1.0 x 10^7/Liter	10%	Dhc has increased 100-fold from April and confirms May sample was likely low biased

**Note: the above approach is also applicable to the "%vcrA" and "%Dhb" values provided on their respective test certificates*

References

Dennis, P., X.M. Druar, A. Waller and E. Edwards, 2006. Advantages of Vinyl Chloride Reductase Gene Testing in Bioremediation. Abstract and platform presentation, Presented at Fifth International Conference on Remediation of Chlorinated & Recalcitrant Compounds, Monterey, California May 22-25, 2006.

Dennis, P**.,** 2009. Lessons Learned from Interpreting the Quantification of *Dehalococcoides* - Platform Presentation-Clemson Hydrogeology Symposium, Clemson University, Clemson, South Carolina, April 2, 2009.

Grostern, A. and E.A. Edwards, 2006. Growth of *Dehalobacter* and *Dehalococcoides* spp. during Degradation of Chlorinated Ethanes. *Appl. Environ. Microbiol*. 72: 428–436.

Grostern, A. and E.A. Edwards, 2008. Characterization of a *Dehalobacter* Coculture that Dechlorinates 1,2-Dichloroethane to Ethene and Identification of the Putative Reductive Dehalogenase Gene. *Appl. Environ. Microbiol*. 75: 2684–2693.

Grostern, A., M. Duhamel, S. Dworatzek and E.A. Edwards, 2010. Chloroform respiration to dichloromethane by a *Dehalobacter* population. *Environmental Microbiology* 12(4) 1053-1060.

Holliger, C., D. Hahn, H. Harmsen, W. Ludwig, W. Schumacher, B. Tindall, F. Vazquez, N. Weiss, and A.J.B. Zehnder, 1998. Dehalobacter restrictus gen. nov. and sp. nov.,a strictly anaerobic bacterium that reductively dechlorinates tetraandtrichloroethene in an anaerobic respiration *Arch Microbiol* (1998) 169 : 313–321.

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. *Appl. Environ. Microbiol.* 70: 6347–6351.

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

Paul, E.A. and F.E. Clark, 1996. *Soil Microbiology and Biochemistry* Academic Press, Inc., San Diego, CA.

Müller, J.A., B.M. Rosner, G. von Abendroth, 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. A*pplied and Environmental Microbiology* 2004 August; 70(8): 4880–4888.

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 Chloroethene-Reductive Dehalogenase Genes with Geochemical Conditions in Chloroethene-Contaminated Groundwater. *Appl. Environ. Microbiol*. 76(3) 843–850.

Appendix A: Data Qualifiers

Data Qualification

Data qualifiers and notes are used to clarify Gene-Trac® test results. Additional explanation beyond that provided on the test certificate is provided below.

"**U" Not detected, associated value is the quantitation limit.** Indicates that the target gene (microbe) was not detected in the sample above the quantitation limit of the assay. Note the quantitation limit value can change between samples as the volume filtered can vary; thus, a sample in which 100 ml was tested would have a 5–fold higher quantification limit compared with a sample in which 500 ml was tested.

"J" The associated value is an estimated quantity between the method detection limit and quantitation limit. Indicates that the target gene was conclusively detected but the concentration is below the quantitation limit where it cannot be accurately quantified.

"I" Sample inhibited the test reaction. This means universal primers were incapable of amplifying DNA from this sample. The inability to amplify with universal primers suggests that the sample may be imparting matrix interference. Matrix interference is commonly attributed to humic compounds, polyphenols and metals. Non-detects with an "I" qualifier are more likely to be false negative.

"B" Analyte was also detected in the method blank. Indicates that DNA was detected in a method blank or negative control; detectable contamination of the blanks with microbes or DNA containing the gene of interest is not uncommon as the test reaction is extremely sensitive. In most cases, blank contamination is at a very low level relative to test results (often orders of magnitude lower). In these cases, blank contamination is not relevant to interpretation of test results. The potential of test samples being contaminated (i.e. false positives) should be considered in cases where blank results are within 1 order of magnitude of test results.