APPENDIX H

MICROBIOLOGICAL ANALYSIS RESULTS

SiREM
Site Recovery & Management
130 Research Lane, Suite 2
Guelph, Ontario N1G 5G3
Phone: (519) 822-2265
Fax: (519) 822-3151
www.siremlab.com

Gene-Trac-VC, Vinyl Chloride Reductase Assay
Certificate of Analysis

Customer: Ralph Morse, O'Brien & Gere

SiREM Reference #: S-0552

Project: Old Erie Canal Site, Clyde, NY

Report Issued: 16 September 2005

Customer Reference #: 35313

Gel Image(s): DHC-UP-0211, VC-0013 B

Date Sampled: 24 August 2005

Test Results:

Customer Sample ID	SiREM ID	Non-*Dehalococcoides* Bacterial DNA	Vinyl Chloride Reductase Test Intensity (% of Positive Control)	Intensity Score	Test Result: Vinyl Chloride Reductase DNA:
MW4B-F1	VCR-0056	Detected	31%	+	Detected
MW6S-F1	VCR-0057	Detected	48%	++	Detected
MW1S-F1	VCR-0058	Detected	23%	+	Detected
MW9S-F1	VCR-0059	Detected	0%	-	Not Detected
Not applicable	-ve control	Not applicable	0%	-	Not Detected
Not applicable	+ve control	Not applicable	100%	+++	Detected

A positive (+ to ++++) result indicates that DNA corresponding to the vinyl chloride reductase gene of *Dehalococcoides* group was detected.

"Vinyl Chloride Reductase Test Intensity" = quantitative assessment of electrophoresis band intensity of PCR product as a percentage of the corresponding positive control reaction. This value provides a semi-quantitative assessment of the amount of VCR genetic material present in the sample.

"Intensity Score", categorizes PCR product quantity based on the "intensity (% of positive control)":++++ = Very high band intensity (greater than 100% of positive control), +++ = high band intensity (67-100%), ++ moderate band intensity (34-66%) + = low band intensity (4-33%), -/+ = inconclusive (1-3%), - = no detectable band (0%)

Analyst: _____
Ximena Druar, B.Sc.
Molecular Biologist

Reviewed by: _____
Philip Dennis, M.A.Sc.
Technology Manager

Leading Science. Lasting Solutions

Case Narrative

SiREM received four groundwater samples from the Old Erie Canal Site on 25 August 2005. The samples arrived in good condition in a cooler with a measured temperature of 9.4°C. Samples were stored at 4°C until DNA extraction. Genomic DNA was extracted from the samples on 30 August 2005. The suitability of extracted DNA for testing using universal bacterial primers was determined on 30 August 2005; all of the samples were determined to contain PCR–Testable DNA suitable for vinyl chloride reductase testing. Vinyl chloride reductase testing was performed on 7 September 2005; positive, negative and DNA extraction controls were within laboratory control limits.

SiREM
Site Recovery & Management
130 Research Lane, Suite 2
Guelph, Ontario N1G 5G3
Phone: (519) 822 2265
Fax: (519) 822-3151
www.siremlab.com

Certificate of Analysis:
Quantitative Gene-Trac *Dehalococcoides* Assay

Customer: Ralph Morse, O'Brien & Gere

SiREM Reference #: S-0552

Project: Old Erie Canal Site, Clyde, NY

Report Issued: 20 September 2005

Customer Reference #: 35313

Date Sampled: 24 August 2005

Test Results

Customer Sample ID	SiREM ID	Sample Matrix	% *Dehalococcoides* in Microbial Population (% *Dhc*) [A]	*Dehalococcoides* 16S rRNA Gene Copies (*Dhc* Count) [B]
MW4B-F1	DHC-1697	watera filter sample	17-42%	2.7×10^7/liter
MW6S-F1	DHC-1698	watera filter sample	12-30%	3.7×10^7/liter
MW1S-F1	DHC-1699	watera filter sample	0.3-0.9%	1.6×10^5/liter
MW9S-F1	DHC-1700	watera filter sample	Not applicable[(1)]	Inconclusive

Notes

[A] This value is calculated by dividing the number of *Dehalococcoides* 16S rRNA Gene Copies by the total number of bacteria as estimated by the mass of DNA extracted from the sample. Range represents normal variation in *Dehalococcides* enumeration.

[B] *Dehalococcoides* are generally assumed to contain one 16S ribosomal ribonucleic acid (rRNA) gene copy per organism; therefore, this number can be interpreted to represent the number of *Dehalococcoides* present in the sample.

[1] Not applicable as *Dehalococcoides* not detected and/or quantifiable DNA not extracted from the sample.

Additional explanation provided in: *Interpretation of Quantitative Gene-Trac Dehalococcoides Test Results.*

Analyst: _____
Ximena Druar, B.Sc.
Molecular Biologist

Reviewed by: _____
Philip Dennis, M.A.Sc.
Technology Manager

SiREM
Site Recovery & Management
130 Research Lane, Suite 2
Guelph, Ontario N1G 5G3
Phone: (519) 822-2265
Fax: (519) 822-3151
www.siremlab.com

Gene-Trac-VC, Vinyl Chloride Reductase Assay
Certificate of Analysis

Customer: Ralph Morse, O'Brien & Gere

Project: Old Erie Canal Site, Clyde, NY

Customer Reference #: 35313

Date Sampled: 24 August 2005

SiREM Reference #: S-0552

Report Issued: 16 September 2005

Gel Image(s): DHC-UP-0211, VC-0013 B

Test Results:

Customer Sample ID	SiREM ID	Non-*Dehalococcoides* Bacterial DNA	Vinyl Chloride Reductase Test Intensity (% of Positive Control)	Intensity Score	Test Result: Vinyl Chloride Reductase DNA:
MW4B-F1	VCR-0056	Detected	31%	+	Detected
MW6S-F1	VCR-0057	Detected	48%	++	Detected
MW1S-F1	VCR-0058	Detected	23%	+	Detected
MW9S-F1	VCR-0059	Detected	0%	-	Not Detected
Not applicable	-ve control	Not applicable	0%	-	Not Detected
Not applicable	+ve control	Not applicable	100%	+++	Detected

A positive (+ to ++++) result indicates that DNA corresponding to the vinyl chloride reductase gene of *Dehalococcoides* group was detected.

"Vinyl Chloride Reductase Test Intensity" = quantitative assessment of electrophoresis band intensity of PCR product as a percentage of the corresponding positive control reaction. This value provides a semi-quantitative assessment of the amount of VCR genetic material present in the sample.

"Intensity Score", categorizes PCR product quantity based on the "intensity (% of positive control)":++++ = Very high band intensity (greater than 100% of positive control), +++ = high band intensity (67-100%), ++ moderate band intensity (34-66%) + = low band intensity (4-33%), -/+ = inconclusive (1-3%), - = no detectable band (0%)

Analyst: _____
Ximena Druar, B.Sc.
Molecular Biologist

Reviewed by: _____
Philip Dennis, M.A.Sc.
Technology Manager

Case Narrative

SiREM received four groundwater samples from the Old Erie Canal Site on 25 August 2005. The samples arrived in good condition in a cooler with a measured temperature of 9.4°C. Samples were stored at 4°C until DNA extraction. Genomic DNA was extracted from the samples on 30 August 2005. The suitability of extracted DNA for testing using universal bacterial primers was determined on 30 August 2005; all of the samples were determined to contain DNA suitable for *Dehalococcoides* testing. Vinyl chloride reductase testing was performed on 7 September 2005; positive, negative and DNA extraction controls were within laboratory control limits. Quantitative *Dehalococcoides* testing was performed on 16 September 2005; positive, negative and DNA extraction controls were within laboratory control limits.

SiREM
Site Recovery & Management
130 Research Lane, Suite 2
Guelph, Ontario N1G 5G3
Phone: (519) 822-2265
Fax: (519) 822-3151
www.siremlab.com

Interpretation of Quantitative Gene-Trac *Dehalococcoides* Test Results

1) Background:

Dehalococcoides group organisms (*Dhc*) are the only known microorganisms capable of complete dechlorination of chloroethenes (i.e., tetrachloroethene, trichloroethene, *cis*-dichloroethene, vinyl chloride to non-toxic ethene. The detection of the *Dhc* 16S ribosomal ribonucleic acid (rRNA) gene has been correlated with the complete biological dechlorination of chlorinated ethenes to ethene at contaminated sites (Hendrickson et. al., 2002, *Applied and Environmental Microbiology*, 68: 485-495). The Quantitative Gene-Trac *Dehalococcoides* test is a quantitative polymerase chain reaction (PCR) test used to determine the concentration of the *Dhc* 16S rRNA gene in soil and groundwater samples.

2) Interpretation of Test Results:

The Quantitative Gene-Trac test reports two types of results, "*Dehalococcoides* 16S rRNA Gene Copies" is a raw value whereas "% *Dehalococcoides* in Microbial Population" is the raw value expressed as percentage of total microbial population. A detailed explanation of the two types of results is provided below.

a) *Dehalococcoides* 16S rRNA Gene Copies

This value is the direct number of *Dhc* 16S rRNA gene copies detected in the sample. Results may be reported either per liter (for groundwater) or per gram (for soil). This number is generally interpreted as equivalent to the number of viable *Dhc* present in the sample when certain reasonable assumptions are made, including that the DNA quantified belongs to viable *Dhc* (i.e., not from dead *Dhc*) and that each *Dhc* cell contains only one 16S rRNA gene. Guidelines for relating this value to observable dechlorination impacts for groundwater samples are provided below.

- **Values of 10^3 gene copies per liter or lower,** indicate the sample contains low concentrations of *Dhc* organisms which may indicate that site conditions are sub-optimal for high rates of dechlorination. Increases in *Dhc* concentrations at the site may be possible if conditions are modified (e.g., electron donor addition).

- **Values of 10^4-10^6 gene copies per liter,** indicates the sample contains moderate concentrations of *Dhc* which may, or may not, be associated with observable dechlorination impacts (i.e., ethene).

- **Values at or above 10^7 gene copies per liter,** indicate the samples contains high concentrations of *Dhc* which is often associated with high rates of dechlorination and the production of ethene. Test results exceeding 10^9 gene copies/liter are rarely observed.

b) % *Dehalococcoides* in Microbial Population (% *Dhc*)

This value presents the percentage of *Dhc* (% *Dhc*) relative to other microorganisms in the sample based on the formulas below. % *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:

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

The number of non-*Dhc* bacteria is estimated by assuming each non-*Dhc* bacterium contains 4.0 x 10^{-6} nanograms (ng) of DNA (Paul and Clark. 1996. *Soil Microbiology and Biochemistry*). Because the total mass of DNA in a sample is determined (by fluorometry) the total number of bacteria present can be estimated. For perspective, the % *Dhc* can range from very low fractions of percentages, in samples that have low numbers of *Dhc* and high numbers of other bacteria (incompletely colonized by *Dhc*), to greater than 50% in *Dhc* enriched cultures such as KB-1™ (fully colonized by *Dhc*).

In addition to determining the predominance of *Dhc*, this value is also used for interpretation of *Dhc* counts from different sampling locations or the same location over time, because it is normalized to total bacteria. In particular, the % *Dhc* value can be used to correct *Dhc* counts where samples are biased low due to non-representative sampling of biomass (bacteria). Example 1 below illustrates a scenario where the % *Dhc* value improves the interpretation of data where one sampling event was biased.

Example 1, use of % Dhc Value to interpret raw data

Example 1 presents results from monitoring well MW-1 sampled in April, May and June. Based on the raw *Dhc* counts alone (*Dehalococcoides* 16S rRNA Gene Copies) it might be assumed that the number of *Dhc* decreased 10-fold between April and May; however, based on the percentage of *Dhc* it is clear that the proportion of *Dhc* actually increased from April to May and that the low count is probably a case of sampling variability (biased low). The higher raw count and the higher percentage of *Dhc* in June confirms the trend of increasing *Dhc* concentrations over time.

Sample	*Dehalococcoides* 16S rRNA Gene Copies	% *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 10^4/Liter	1%	*Dhc* predominance increased 10-fold from April, low count from low biomass sampled, non-biased sample would be [(1.0/0.1) x 1.0 x10^5] = 10^6 /Liter
MW-1 June	1.0 x 10^7/Liter	10%	*Dhc* predominance moderate and has increased 100-fold from April

3) Explanation of Notes

Quantitation limit: The quantitation limit of Gene-Trac test is 2,150 *Dhc* 16S rRNA gene copies per liter. Note, the specific quantitation limit for each test varies depending on the volume of sample used in the DNA extraction process. For example, if only a ½ liter of water was used the quantitation limit would increase two-fold to 4300 gene copies per liter. The specific quantitation limit is provided only where *Dhc* is not detected.

Value is an estimated quantity between the quantitation limit and detection limit: This is applicable in situations where *Dhc* DNA is detected above the detection limit, but below the quantitation limit, of the standard curve. In such cases an estimated value is provided which is based on extrapolation of the standard curve.

Sample inhibited testing: Each Quantitative Gene-Trac test includes a quantification of the amount of DNA extracted from the sample and a second test to determine if the extracted DNA is suitable for *Dhc* testing (PCR with a universal Bacteria primer). If a sample is determined to contain DNA and PCR with universal primers is negative, it suggests that the extracted DNA inhibited the PCR. Inhibition may be caused by compounds present in the original groundwater sample (e.g., humic acids). Where inhibition occurs there is an increased likelihood of false negatives since *Dhc* DNA, if present, may not be detected.

DNA not extracted from the sample: If DNA is not detected in the sample then "DNA not extracted from the sample" is reported. This is commonly due to samples that contain little or no biomass (bacteria). In some cases sampling may not capture bacteria (e.g., when attached bacteria are not dislodged from the aquifer matrix).

4) Converting Standard Gene-Trac to *Dhc* 16S rRNA Gene Copies/Liter

Quantitative Gene-Trac provides quantitative results in *Dhc* 16S rRNA Gene Copies/Liter, whereas standard Gene-Trac provides semi-quantitative results using a plus scale. Based on parallel analysis of standard versus Quantitative Gene-Trac estimates of the number of *Dhc* gene copies for each + score in the standard test were determined. Note, the conversion factors do not apply in all cases and are meant to be used as a rule of thumb for relating standard Gene-Trac results to Quantitative-Gene-Trac.

Estimated 16S rRNA Gene Copies/Liter for Standard Gene-Trac Intensity Scores

Standard Gene-Trac Intensity Score	Approximate Range of 16S rRNA Gene Copies/Liter
+	10^3-10^5
++	10^4-10^6
+++	10^5-10^7
++++	10^6-10^8

5-0552.

O'BRIEN & GERE

CHAIN OF CUSTODY

COOLER TEMP = 9.4°C

Sheet 1 of 1

Contact: Ralph Morse
Address: 435 New Karner Road
Albany, New York 12205
Phone: (518) 452-9392 x12

Job No.: 35313

Laboratory: SiREM

CLIENT: Parker Hannifin
PROJECT: ADD #1- SiREM Plugged Filter Sampling
LOCATION: Old Erie Canal Site, Clyde, NY

COLLECTED BY: (Signature) James A. Moore

SAMPLE IDENTIFICATION	2005 Date	Time	Total Vol (L)		No. of Containers	ANALYSIS REQUESTED
MW4B-F1	8/24/05	1032	4.6		1	GENE-TRAC &
MW6S-F1	8/24/05	1500	6.4		1	GENE-TRAC-VC
MW1S-F1	8/24/05	1106	6.65		1	
MW9S-F1	8/24/05	1400	4.25		1	

[1] Matrix = water, wastewater, air, sludge, sediment, etc.

[2] Type = grab, composite

Relinquished by: [signature] of: OBG	Date: 8/24/05	Time: 1900	Received by: J Webb of: SIREM.	Date: 8/25/05	Time: 12:30
Relinquished by: of:	Date:	Time:	Received by: of:	Date:	Time:
Use this space if shipped via courier (e.g., Fed Ex) Relinquished by: of:	Date:	Time:	Courier Name: FedEx 8497 6898 4040	Date:	Time:
			*Attach delivery/courier receipt to Chain of Custody		