Quantitative PCR (qPCR) techniques have been widely used to measure <I>Dehalococcoides</I> (Dhc) DNA in the groundwater at field sites for several years. Interpretation of these data may be complicated when different laboratories using alternate methods conduct the analysis. An improved data quality setting for qPCR quantification of Dhc has the potential to enhance the utility of these measurements in bioremediation practice and increased stakeholder confidence in the analysis. SERDP project ER-1561, Standardized Procedures for Use of Nucleic Acid-Based Tools for Microbial Monitoring, is focused on development of standardized methods for qPCR sampling, shipping and analysis with the goal of understanding and minimizing method variability.

As part of this project, a series of experiments has been conducted to establish the baseline variability and accuracy of qPCR quantification of Dhc by five laboratories through a "round robin" approach. Two of the participating laboratories perform this analysis commercially, two are academic and one is a government (DOE) laboratory. The Dhc 16S rRNA gene was used as the molecular target with a variety of qPCR instruments, qPCR chemistries, primers and probes and DNA extraction protocols utilized by the five laboratories. Three distinct test materials were circulated among the laboratories 1) plasmid DNA, to assess variability of the qPCR methods and instruments, 2) artificial groundwater spiked with Dhc, to determine variation associated with cell concentration, cell lysis and DNA extraction methods and 3) multi-method quantified Dhc (standardized Dhc reference culture) spiked into artificial groundwater, to assess the Dhc quantification accuracy of qPCR methods compared with other molecular and microscopic enumeration techniques.

The first Round Robin (Plasmid DNA) data collection has been completed and data interpretation is underway. The second and third experiments will be completed in the next 6 months. The results from these experiments will be used to determine critical points in the analysis where variation occurs and to identify methodological improvements that may improve agreement between laboratories.