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Development and Application of a Rapid, User-Friendly and Inexpensive Method to Detect *Dehalococcoides* spp. Reductive Dehalogenase Genes from Groundwater

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Background/Objectives. A number of quantitative PCR (qPCR) protocols to enumerate *Dehalococcoides* spp. reductive dehalogenase (RDase) genes, such as *vcrA, tceA* and *bvcA,* are available. Many remediation professionals either detect RDase genes in-house or use the service of a commercial laboratory. However, there can be significant costs associated with both approaches (e.g., a thermal cycler for qPCR can be $20K). In this study, we developed an alternative method to qPCR for the detection of RDase genes. The approach involves loop mediated isothermal amplification (LAMP) and requires only low-cost laboratory equipment. In addition, the analysis time and cost per sample are lower than currently used methods. The method was tested with multiple groundwater samples from five different chlorinated solvent sites.

Approach/Activities. Previous research in our laboratory involved the validation of LAMP assays for *vcrA* and *tceA* using DNA extracted from two commercial bioaugmentation cultures (KB-1 and SDC-9). The current project used groundwater from 35 monitoring wells at five different sites (Kelly AFB -10, Tulsa OK -15, Raritan Arsenal – 2, NESDI/Quantico VA- 2, Indian Head - 6) for method development and testing. To establish that LAMP was comparable to qPCR for DNA extracted from groundwater, the first stage of the research involved a comparison of LAMP to qPCR for *tceA* and *vcrA* using a real time thermal cycler. Following this, experiments were conducted to optimize the sample concentration approach, so that the DNA extraction step could be removed. A final method was developed which only requires low cost equipment (bench top centrifuge and a water-bath) for RDase detection. In addition, guidelines were generated as to when this approach would be suitable for use.

Results/Lessons Learned. When qPCR and LAMP were compared using DNA extracted from groundwater, the results of both methods were almost identical (R2=0.991) over a range of approximately 10^4 to 10^{10} gene copies/L for tceA and 10^5 to 10^{10} gene copies/L for *vcrA.* We found that a method involving centrifugation provided the best approach for amplification directly from cell templates. Although amplification from cell templates resulted in lower values compared to DNA extracts, a strong correlation was obtained between the two sets of data. This equation could be used on cell template data and would thus save the time and cost associated with DNA extraction. The final approach (using LAMP/SYBR green and a waterbath) to detect RDase genes, without DNA extraction or a thermal cycler, was successful to 1.8×10^5 gene copies per L for vcrA and 1.3×10^5 gene copies per L for *tceA.* Both values are below the threshold recommended for effective in situ dechlorination.