Advances for the Rapid and Sensitive Biomonitoring of the Reductive Dechlorination's Biomarkers: Digital Droplet PCR

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Background/Objectives. Molecular biological tools (MBTs) are routinely employed to quantify biomarker genes during the characterization and biomonitoring of contaminated sites, the development of new biotechnologies and processes, or studies related to the culturing of dechlorinating consortia. In the last 20 years, quantitative real-time PCR (qPCR) has been the elective MBT allowing the quantification of *Dehalococcoides mccartyi* (Dhc) and reductive dehalogenase genes, essential biomarkers for the reductive dechlorination process. Indeed, in the frame of bioremediation projects of chlorinated solvents contaminated sites, quantitative data in certain range values provide a preliminary evaluation of the bioremediation potentialities of the site. Based on our experience with a large number of contaminated samples (≥ 3000 in the last 10 years) - including groundwater, soil, sediments, and microbial consortia – we observed that despite qPCR is a valid MBT for the quantification of dechlorinating biomarkers, the sensitivity, accuracy, and precision of the approach decrease at low abundances (i.e. ≤ 1E+03 gene copies/L). Thus, 1E+03 gene copies/L can be considered as a "detection limit" and values below this threshold need to be cautiously considered. This is relevant when contaminated environmental samples with low Dhc are analyzed, with the implication of an erroneous evaluation of the bioremediation potential of the site. In addition, another issue to face is the stability of the standards necessary for absolute quantification. Indeed, if long amplicons are used as standards, they must be freshly prepared at least every one or two months. Similarly, when plasmids are used as standards, they need to be routinely checked to ensure the integrity and to ensure that supercoiling does not happen. Digital droplet PCR (ddPCR) is a novel quantification method that does not require standards, or replicates, for the quantification and has been widely used in medical/clinical applications. Its applications in the environmental field and biodegradation monitoring are rarely reported. Here we report for the first time ddPCR targeting dechlorinating biomarkers.

Approach/Activities. The DNA in the ddPCR reaction mixture is partitioned into thousands of nanoliter-sized droplets, a PCR is run on a thermal cycler, and then droplets are read well by well. PCR-positive and negative droplets are counted to provide absolute quantification of the target. We developed the ddPCR protocol for the 16S rRNA Dhc, reductive dehalogenase genes (i.e. tceA, bvcA, vcrA), and some other functional genes used as biomarkers for the oxidative dechlorination (i.e., etnE, etnC). ddPCR protocol has been tested on several samples including dechlorinating consortia, environmental samples, and also long amplicons routinely used as standards for qPCR. Quantitative data obtained with ddPCR have been compared with qPCR data yielded in the same concentration range (from 1E+10 to 1 gene copies/µL).

Results/Lessons Learned. Quantitative data obtained with ddPCR showed high precision, sensitivity, efficiency, and low susceptibility to PCR inhibitors with all the samples tested. Below 1E+06 gene copies/µL ddPCR yielded higher reliability and reproducibility of the quantitative data compared to qPCR, up to 1 gene copy/µL. In the range 1E+10 ≤ gene copies/µL ≤ 1E+06, ddPCR quantification plateaued due to excessive targets and require sample dilutions. Diversely, qPCR results performed on the same samples yielded unreliable data below the threshold of 1E+03 or 1E+02 gene copies/µL. Overall, our comparative experiments suggested some advantages in using ddPCR as a quantitative tool for biomonitoring including enhanced sensibility, increased precision for low target concentrations, and improved reliability and

reproducibility in a large concentration range, in particular at low target abundances, implying a great potential for biomonitoring purposes. In the presentation, qPCR and ddPCR comparisons will be deeply presented and discussed.