

Application of ddPCR for detection of *Enterococcus* spp. in coastal water quality monitoring

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Droplet digital polymerase chain reaction (ddPCR) was evaluated for the detection of fecal indicator bacteria (FIB), *Enterococcus* spp., in San Diego County beach water samples collected under diverse conditions, from multiple pollution sources, as part of regulatory monitoring activities over 20 months. Two US EPA-approved methods, qPCR (EPA 1609.1) and Enterolert (SM9230D), were used as reference comparator methods. A total of 361 samples were assayed by both ddPCR and qPCR and yielded an acceptable Index of Agreement (IA) of 0.89, based on EPA Site-Specific analysis guidelines. A Pearson's correlation coefficient of $r = 0.87$ ($p < 0.001$), further indicated a strong relationship between the methods results. From the 361 samples, 185 split samples with ddPCR and Enterolert values within the limits of quantification, were used as a 'training' data set to derive an intrinsic copy number equation (ICE) for scaling ddPCR gene copy number to Enterolert most probable number (MPN). Of the 1993 samples that comprised the complete 'test' data set assayed by ddPCR and Enterolert, 1086 generated results that fell within the limits of quantification for Enterolert and yielded an overall IA of 0.64. Re-analysis using median as a measure of central tendency to account for significant skewing of Enterolert data yielded an IA of 0.72. Beach grouping-specific IA values ranged from 0.63 to 0.93. Pearson's correlation coefficient, r , ranged from 0.13 to 0.94 within beach groupings and generated a combined value of 0.60 for all groupings. Using the ICE, a ddPCR advisory threshold of 1413 DNA copy number/100 mL was empirically determined to be the equivalent to the California Enterolert beach action threshold of 104 MPN/100 mL, based on comparison with all 1993 paired ddPCR and Enterolert results. Using the 1413 DNA copy number/ 100 mL as a beach action threshold for ddPCR resulted in a 90.4% agreement with Enterolert (6.0% false negative and 3.7% false positive). Together these findings support the conclusion that ddPCR readouts align closely with Enterolert MPN for identifying FIB exceedance levels of *Enterococcus* spp. in coastal waters of San Diego, CA.

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