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Interlaboratory calibration study of *in situ* automated qPCR for *Enterococcus* versus commercial benchtop instruments

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Abstract

Background: The next major advance in microbial water quality monitoring may arise from innovations in automation and portability of technology. An *in situ* automated qPCR instrument that delivers data in real time has previously been developed and deployed in both open ocean and pier mooring studies (the Environmental Sample Processor, or ESP). This study evaluated the inter-laboratory repeatability of data collected from these instruments and commercial bench top thermalcyclers.

Materials: Four laboratories tested 2 ESP qPCR modules and 5 benchtop instruments (4 models from 3 manufacturers) using 4 versions of mastermix chemistry for the Entero1A assay. The protocols varied by enzyme and probe quencher, and all variations were run on each instrument using the same standard reference material. Analysis of covariance (ANCOVA) was used to compare standard curves generated among laboratories, instruments and chemistries.

Results: There was a significant effect of laboratory ($F = 20.1$, $p<0.001$) and instrument ($F=60.1$, $p<0.001$). The two ESP modules were statistically indistinguishable, but they were significantly different from benchtop instruments used in the same laboratory ($F = 231.9$ and 32.7, both $p<0.001$). The difference between ESP and benchtop instruments varied with the chemistry used, with a maximum difference of ~6 Cq. The ESP modules detected 72% of samples containing 12.6 *E. faecium* genome copies per reaction.

Conclusion: The difference between ESP qPCR modules and benchtop instruments is on par with the difference among laboratories using thermalcyclers from different manufacturers. The ESP qPCR module is sensitive enough for routine water quality monitoring applications.