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Acceptability of the EPA qPCR Test at Your Beach

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Section 5.2 of the 2012 Recreational Water Quality Criteria (RWQC) states that "EPA encourages a site-specific analysis of the method's performance prior to use in a beach notification program or adoption of WQS [water quality standards] based on the method." [1] This document provides guidance on how to evaluate the acceptability of the quantitative polymerase chain reaction (qPCR) test at your beach.

The methods referred to in this document are two qPCR *Enterococcus* spp. as measured by qPCR methods, EPA Methods 1611 and 1609 [2-3]. The former method was released simultaneously with the 2012 RWQC. The latter method is an improved version of the method that uses a newer formulation of PCR reagent (environmental master mix) that has shown reduced susceptibility to inhibition compared to the reagent used in Method 1611 (universal master mix). In addition, Method 1609 includes a competitive internal amplification control (IAC) assay to help specifically identify false negative reactions or reduced amplification efficiency due to Taq DNA polymerase inhibition[4]. Although either method is acceptable for use, EPA recommends the use of Method 1609 due to these enhancements.

This document does not address performance acceptability criteria of the method itself, as these are stated within the method (see Section 14 in Methods 1609 and 1611) as based on a nationwide method validation. Additionally, this document assumes that the testing laboratory has been able to perform the method within the acceptance criteria, and now wishes to ascertain whether or not the method would be acceptable for use at a particular site.

The demonstration should have the following characteristics:

1. At least 10 samples should be taken on different days for site evaluation in advance of using the method for beach action decisions. Among these samples, a maximum of 10% can fail the Salmon DNA SPC control assay criterion (see Section 9.12 in Method 1611) or the SPC and IAC control assays criteria (see Sections 9.12 and 9.13 in Method 1609). For any samples that fail the initial analysis, one or both of the interference mitigation approaches: extract dilution (see Section 9.12 in Methods 1609 and 1611); higher Salmon DNA (see reference 17.5 in method 1609), can be used to assess for mitigation of the interference. If mitigation by one of these approaches is successful (i.e., samples now pass the control assay criteria specified above), these samples can be considered as not having failed in the site evaluation. As an option, beach managers localities can choose to sample for an entire beach season or year for initial site evaluation so that their data is more representative, but this is not required.

2. Particularly if beach closures are not mandated by local standards after a heavy rain event, site evaluation sampling should include a representative number of samples collected after such events. In general sampling should represent the conditions when recreation is expected or known to occur.

3. Sites should be re-evaluated every year, preferably prior to using the method for beach action decisions, since water characteristics, including the appearance and disappearance of inhibitors to the method, have been shown to change over time.

Some localities may be inclined to compare the frequency of beach advisories based on the qPCR test versus culture test. EPA neither encourages nor discourages such comparisons; however, it is noted that the results should be interpreted carefully. Comparisons between exceedances are not reflective of the respective method performance assuming all the controls in the methods are performing properly. Caution should also be exercised because comparing exceedances does not take into account the uncertainty of the respective health relationships between the two methods. Also, it is noted that the same day notification potential for qPCR more accurately reflects water quality for beach goers compared with methods where results are not available until the following day because water quality is known to fluctuate day-to-day.

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References

(1) US EPA. Recreational Water Quality Criteria. 2012. EPA-820-F-12-058.
(2) Method 1611: Enterococci in Water by TaqMan® Quantitative Polymerase Chain Reaction (qPCR) Assay. October 2012. EPA-821-R-12-008.
(3) Method 1609: Enterococci in Water by TaqMan® Quantitative Polymerase Chain Reaction (qPCR) with Internal Amplification Control (IAC) Assay. March 2013. EPA-820-R-13-005.
(4) Haugland, R.A., Siefring, S., Lavender, J., Varma, M. Influences of sample interference and interference controls on quantification of enterococci fecal indicator bacteria in surface water by the qPCR method. *Water Research* **2012**, 46(18): 5989–6001.