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Opportunities and limitations of molecular methods for quantifying microbial compliance parameters in EU bathing waters

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ABSTRACT

The debate over the suitability of molecular biological methods for the enumeration of regulatory microbial parameters (e.g. Faecal Indicator Organisms [FIOs]) in bathing waters versus the use of traditional culture-based methods is of current interest to regulators and the science community. Culture-based methods require a 24–48 hour turn-around time from receipt at the laboratory to reporting, whilst quantitative molecular tools provide a more rapid assay (approximately 2–3 h). Traditional culturing methods are therefore often viewed as slow and 'out-dated', although they still deliver an internationally 'accepted' evidence-base. In contrast, molecular tools have the potential for rapid analysis and their operational utility and associated limitations and uncertainties should be assessed in light of their use for regulatory monitoring. Here we report on the recommendations from a series of international workshops, chaired by a UK Working Group (WG) comprised of scientists, regulators, policy makers and other stakeholders, which explored and interrogated both molecular (principally quantitative polymerase chain reaction [qPCR]) and culture-based tools for FIO monitoring under the European Bathing Water Directive. Through detailed analysis of policy implications, regulatory barriers, stakeholder engagement, and the needs of the end-user, the WG identified a series of key concerns that require critical appraisal before a potential shift from culture-based approaches to the employment of molecular biological methods for bathing water regulation could be justified.

1. The debate

The EU Bathing Water Directive (BWD) 76/160/EEC (CEC, 1976) engages stakeholder interest because of its impact on tourism, local

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economies and public health, and is well publicised through beach award schemes (Guimaraes et al., 2012). However, it also generates controversy across the scientific, regulatory and policy communities with regular debates being driven by scepticism of whether: (i) *Escherichia coli* is a suitable faecal indicator organism (FIO) to assess recent faecal pollution (Wu et al., 2011), (ii) the Directive is suitably protective of human health (Kay et al., 2004; Langford et al., 2000), and, more recently, (iii) the methods currently used to determine microbial water quality at bathing beaches are fit for purpose (Oliver et al., 2010).

These debates are healthy and, as is often the case, more questions are raised than definitive answers provided. However, what we do know is that from 2015 the number of EU designated bathing waters falling below the legally enforceable 'sufficient' standard (equivalent to a 90 percentile of >185 CFU/100 mL and >500 CFU/100 mL of intestinal enterococci and *E. coli*, respectively) could limit the use of EU bathing waters if the non-compliance continues beyond 2020 when the 2006 revised Bathing Waters Directive (rBWD) 2006/7/EC (CEU, 2006) in Europe takes full effect.

The enforcement of the revised BWD in Europe is likely to encourage member states to further improve wastewater infrastructure, and promote better integrated catchment management, as well as provide a significant impetus for the environmental regulators responsible for protecting our bathing waters as 'protected areas' as defined in Annex 4 of the Water Framework Directive (CEC, 2000) in Europe. This immediate focus, however, detracts attention from a more subtle, yet equally complex debate centred on the use of molecular biological testing and the transition of molecular methods from predominantly research tools to standardised protocols for evaluating water quality at bathing waters (Gooch-Moore et al., 2011; Griffith and Weisberg, 2011; Nevers et al., 2013). Current culture-based methods used to enumerate FIOs require a 24–48 hour turn-around time from receipt at the laboratory to reporting, whilst quantitative molecular tools provide a more rapid assay (approximately 2–3 h). Traditional culturing methods are therefore often viewed as slow and 'out-dated', although they still deliver an internationally 'accepted' evidence-base. In contrast, molecular tools have the potential for rapid analysis although they are not yet established enough in the EU for regulatory monitoring.

However, it is important to note that microbial water quality testing at designated bathing waters in the EU can serve two separate purposes. The first is the provision of a monitoring framework for reporting and regulation of microbial water quality and the second is in helping control the public health risk from microbiological contamination of bathing waters. The first purpose is effectively 'state of the environment' monitoring to collect sufficient data to produce information on general status of bathing water quality and infer how well our management practices and policies are working, and whether environmental outcomes are being achieved. This data is collected over the longer term and can be summarised into a bathing water classification and may contribute to a beach award. The second purpose is about assessing the risk of an individual bathing event. Thus, the time delay of culture-based approaches leads some scientists to question whether rapid molecular methods could play a more effective role in assessing the risk of individual bathing events. This is a debate that is international in scope, but which was driven principally by the need for new recreational water quality criteria in the US. The US movement was prompted by a lawsuit against the US Environmental Protection Agency (USEPA) filed by the Natural Resources Defence Council (NRDC) which argued that the USEPA had not delivered on its intention to explore new or revised water quality criteria linked to 'rapid test methods' (Gooch-Moore et al., 2011). This led to the publication of revised standards based on the voluntary use of molecular biological methods, principally quantitative polymerase chain reaction (qPCR) analyses. Thus, the crux of the debate centres on the relevance and effectiveness of existing (culture-based) methods compared with promising (qPCR-based) quantification methods for enumerating microbial compliance parameters at designated bathing waters and whether either relates to human health risk.

If, in time, qPCR is adopted widely in the US as a method of choice for quantifying levels of faecal pollution then pressure may begin to build in the UK and the rest of Europe to follow suit for enumerating these regulatory microbial parameters within the EU Directives (Oliver et al., 2010). In response, a Working Group (WG) was established in the UK, under the auspices of the 'Delivering Healthy Water' project. The WG drew on international expertise via a series of workshops to debate the utility of qPCR methods versus culture-based approaches for microbial water quality analysis linked to regulatory monitoring. The overarching aims of the WG were to: (i) interrogate the existing evidence-base and (ii) provide a balanced evaluation of the associated uncertainties, benefits and limitations surrounding such a shift in methodological approach for bathing water monitoring and regulation.

2. From research tool to standardised protocol: five hurdles to overcome

The WG identified a series of key recommendations needed to underpin adoption of the new molecular biological methods by regulatory bodies. These reflect generic scientific considerations but focus the lens of debate on a European policy perspective. Each recommendation is dealt with in the sections below.

2.1. Recommendation 1: building the epidemiological evidence-base

Demonstrating a robust relationship between (a) molecular marker(s) and human health outcomes (i.e. infection or illness in bathers) via an epidemiological evidence base is of fundamental importance before any shift from a culture-based to a qPCR-based approach can be considered across the EU. This priority recommendation was also identified by a group of international experts convened to debate the transitioning of new methods from research and development to an operational phase as part of the US recreational water quality criteria (Boehm et al., 2009). Recent epidemiological studies in the US have explored the relationship between FIO concentrations and gastrointestinal infections using qPCR methods (Wade et al. 2006, 2010), however, these studies focus only on beaches impacted by human sewage and consequently their generic relevance to bathing waters in Europe (which are more likely to be impacted from diffuse sources) is unclear.

It is critical that we understand how transferable the dose–response relationships from epidemiological studies at locations dominated by point sources are, particularly when differences between the risks associated with human and ruminant wastes are so poorly characterised (Boehm et al., 2009; Dufour et al., 2012; Gooch-Moore et al., 2011; Till et al., 2008) and the relationship between levels of exposure and incidence of illness in the wider population fraught with unknowns (Bridge et al., 2010; Soller et al., 2010). Others have begun to investigate the role of qPCR versus culture in sub/tropical diffuse source recreational marine waters and proposed further epidemiological studies in order to explore possible dose–response relationships between human illness with indicator organisms (Sinigalliano et al., 2010). We advocate the need for a series of robust international epidemiological studies that span a number of European bathing water types that are impacted by point sources (e.g. sewage contributions), diffuse source inputs, and sites that experience a mix of both sewage-derived and diffuse source contributions to the overall microbial load. We also argue that it would be essential to undertake such epidemiological studies by measuring culture and qPCR-based targets in parallel and in the same sample to provide a definitive back-to-back comparison of the methods across a suite of international waters. The provision of a cross-comparison data set derived using both culture based and molecular methods to quantify microbial parameters would allow for some exploration of parity to historical data sets. In time, these studies