Delivering Healthy Water:

building the science – policy interface to protect bathing water quality

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DELIVERING HEALTHY WATER

Delivering Healthy Water is a project funded by the Natural Environment Research Council (NERC) that promotes the exchange of knowledge and information between science providers, science users and the public with regard to advantages and limitations of different tools for compliance monitoring of bathing waters and other regulated waters.

The aim of the project is to develop a shared understanding of the science evidence base underpinning current and emerging microbial quantification techniques.

Briefing paper 1 of 2

Science, regulation and policy

May 2013

The quality of UK bathing waters is assessed by enumerating bacteria known as faecal indicator organisms (FIOs) throughout the bathing season. From 2015, the number of designated bathing waters of poor microbiological status is set to rise because of the introduction of more stringent standards associated with the revised Bathing Waters Directive (rBWD (2006)) in Europe.

Parallel debates over the suitability of traditional versus novel quantification methods add an extra layer of complexity for regulators. New molecular analytical techniques are beginning to offer a means of characterising microbial pollution that challenge traditional 'culture-based' reference methods.

Existing policy frameworks for microbial water quality standards around the world are centred on the culture-based approaches that are underpinned by rigorous science, reproducibility of results and an epidemiological evidence-base. However, the United States Environmental Protection Agency (USEPA) has already started to use molecular-based enumeration tools as an alternative to culture-based methods. This may increase pressure in the UK to consider a method shift as well. The difficulty lies in how to translate technological innovations into up-to-date regulation based on a firm and accepted policy evidence-base.

This is the first Briefing Paper from the Delivering Healthy Water (DHW) project. It focuses on the scientific, regulatory and policy questions surrounding a potential move to using molecular methods for compliance monitoring of bathing waters and other regulated waters. Briefing Paper 2 turns to the economic implications of such a move and the complexities of providing appropriate and timely public information on bathing water quality.

 Denotes a point at which information is linked to the decision making framework document.

1. Perceived advantages of molecular techniques

The applications which molecular techniques would benefit due to their rapidity, ability to distinguish dominant (human/ruminant) sources of faecal pollution and their potential for direct measurement of pathogens (particularly the viruses) are described in the table below. They are divided between the advantages associated with compliance monitoring and those relevant to identifying **FIO**s. It should also be noted that molecular tools are good for identifying hazards, but they are more limited in their ability to determine the risk of the hazard occurring.

	FIO identification	Compliance monitoring/ investigations
Rapidity	Bathing water sampled at 9am on a Monday morning can be reported by Tuesday using culture methods. If a molecular method such as **qPCR** is used in the UK, the same water sample could be reported within 2 – 3 hours excluding transport time. In particular, the speed associated with molecular techniques may afford the potential to define the end of a pollution incident.	The extra speed may not make much of a difference to the regulator for compliance monitoring of bathing waters in the absence of on-site testing (see section 2 below). However, there may be a role for **qPCR** in providing near-real-time information in the future if coupled with predictive monitoring although current EA validation of daily predictive methods with both single sample **qPCR** values and indeed culture-based enumerations has proven problematic.
Access to information otherwise unavailable	Molecular methods can provide a more meaningful and timely statement of risk than current methods. They can be used to identify the presence or absence of specific sources of faecal pollution, and to rank the most likely origin of faecal sources in catchments which can also inform assessment of human health risks linked to exposure from animal vs human sources. However the quantitative (i.e. percentage) *apportionment* of **FIO sources/MST targets** is not currently possible. Deciding when and how to deploy molecular methods is vital; stakeholders are often looking for a "magic bullet" to identify a source of faecal contamination when, in practice, multiple lines of evidence generally yield the best results.	The utility lies in rapidity – preventing exposure to pollution risks in near real time while maximizing utility. This is important from an economic perspective. Earlier advice to warn against bathing and earlier removal of that advice may be possible. This has the potential to refine the regulatory process and to determine more quickly when to end pollution incidents and tell people that it is safe to go back into the water.
Information can be saved for later use	When nucleic acid is extracted from a volume of water, the information in it does not need to be 'read' immediately. It could be frozen and used later. By identifying dominant pollution sources, investments (capital, operational and maintenance, management) can be more accurately targeted. The speed associated with the new techniques could assist in determining when a pollution incident has ended.	Filters and/or archived DNA extract may allow for regulatory decision making in the short term while providing samples for future source attribution/ apportionment studies at a later date. This also opens the possibility of developing a library of background data derived from molecular analysis.

2. Arguments against changing to the use of molecular techniques as routine regulatory monitoring tools for bathing waters

Absence of a convincing driver for change and doubts over robustness of evidence

Through the course of this project the message that has consistently emerged from regulators is that they would embrace a change in technology if presented with compelling evidence to support the need for it. To date, culture-based methods have satisfied regulatory requirements, they are available worldwide, the infrastructure is in place and data can be interpreted and compared. It is widely held that such evidence has not been presented to support a change to other methods at this time (see below for more information on the current knowledge gaps).

For bathing waters that consistently meet regulatory standards, there is no imperative to move to more rapid methods of monitoring. There can be no legal standard for a new method until it is recognised by a body such as ISO and this will add to the reluctance of regulators to adopt change.

The advantages of speed afforded by molecular methods may only be realised if sampling is carried out very regularly. There is little advantage in reporting results within hours if a bathing water is only sampled once a week (for England and Wales). A two hour turnaround time for molecular methods to provide a quantitative result is possible. However, if a laboratory is processing 250 samples a day then there is a need to factor in sample processing time thus increasing the duration involved from sample preparation through to reporting of a result. Transit time from the point of sample collection to the laboratory can increase the turn-around time considerably and so the logistics of sample collection and transport may also present challenges.

The advantage is that a high volume of samples should mean reducing overall costs. Rapid methods are only useful for people who want to use the beach on that day as information relating to any single sample gives an indication of water quality at that moment in time and may not be representative of water quality conditions a short while later that same day. The use of a rapid method only makes sense if the sample can be analysed within a reasonable time from its collection and probably by on-site analytical equipment.

Costs and availability of resources are other factors that serve to limit enthusiasm for a move to using rapid methods. These are also addressed in Briefing Paper 2.

Concerns over accuracy and precision of new methods

The key requirements of **qPCR** as a bathing water assessment tool are for it to be: (i) a good indicator of risk; (ii) accurate and reproducible; and (iii) cost effective. The accuracy of **qPCR** has been questioned by some studies with inter-lab variability of **qPCR** analysis in water where target levels are very low, raising some matters that need to be addressed by training and standardisation of methods.

The natural variability of **FIO** concentrations in water samples is in itself a major challenge for water quality reporting. A high frequency monitoring programme undertaken as part of a research programme has highlighted two $\log_{10}$ orders of magnitude in the daily range observed in multiple enumerations (i.e. triplicate enumeration CFU values, daily *n*=20-24) taken within the bathing day (60 days in the 2011 bathing season at the Swansea Bay **DSP** (Figure 1)). This, coupled with a considerable imprecision in **qPCR** enumeration data, raises serious questions over, for example, weekly spot enumerations of **FIO**s, whether by CFU or qPCR, for bathing water quality assessment. Furthermore there is contradictory evidence over the effectiveness of UV disinfection impacting on **qPCR** targets (WERF vs Stapleton et al.). This would pose additional problems for risk assessment if it is assumed that UV disinfection causes inactivation of microbial indicators and pathogens: i.e. DNA from inactivated sewage microbes could impact on compliance despite effective infrastructure investment to reduce microbial risk.

The increasing body of research developing around this area has highlighted the complexity surrounding the use of molecular methods**.**

The best strategy at the moment would appear to be the use of existing culture-based approaches for regulatory work and use qPCR / MST for qualitative source tracking investigations.

As the **rBWD** is implemented and four years of water quality data are used for compliance monitoring, some of the variability discussed above will be accommodated.

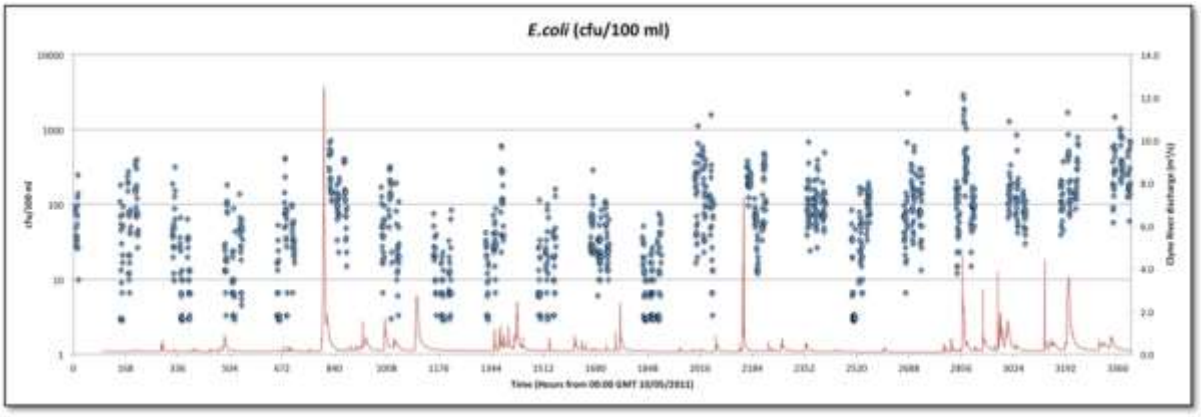


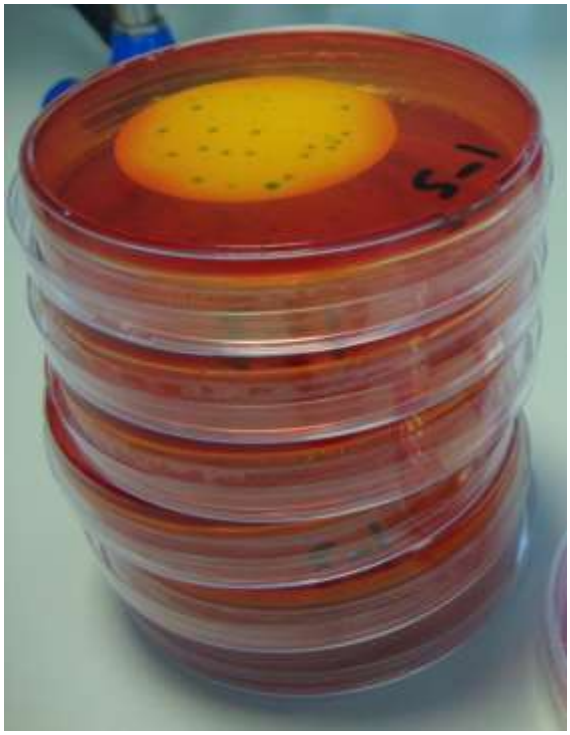
Figure 1 Raw data on 60 bathing days at the Swansea DSP in 2011 (n=20-24/day). The continuous line represents flow in the river Clyne. Each column represents 19-25 water samples each sample having been analysed 3 times with 2-3 dilutions per samples. [1]

[1]**This is unpublished data, reproduced with the permission of the EU funded Smart Coasts project team comprising Aberystwyth University, the Environment Agency, City and Council of Swansea and Dwr Cymru Welsh Water (contact: David Kay dvk@aber.ac.uk)**

A lack of consensus over what might replace current methods

As yet, there is no single molecular approach to advocate or even consensus over whether **qPCR** should be used in a regulatory context at all.

Regulators are concerned over how evidence gathered using molecular techniques would stand up in court. This reinforces a move towards the need to use as a suite of methods as opposed to any one option. This would mean building on the large body of knowledge and public understanding relating to current methods of bathing water quality assessment. Ultimately the most useful scenario may be where we use both culture and molecular methods along with local knowledge to indicate whether faecal pollution sources for an impacted area are likely to be ruminant or human.



It must also be remembered that sample location and environmental conditions may affect the relationship between colony forming units (**CFUs**) and **qPCR cell equivalents (CE)** and that this would complicate cross-comparison studies between culture and molecular approaches (e.g. proximity to constant sources of DNA, sample inhibition, precipitation, stormwater contributions, tertiary wastewater treatment etc.).

Site specific feasibility studies would need to be conducted to determine whether it would be appropriate to use a molecular method at a particular site.

3. Gaps relating to molecular methods and future research opportunities

Epidemiological evidence

Given the development of internationally accepted analytical standard operating procedures there is a need for a series of robust international epidemiological studies that demonstrate the relationship of molecular markers to human health (including, importantly, epidemiological studies of non-sewage impacted recreational waters) and to determine whether it is possible to establish threshold doses that are useful for regulators. For example, research is needed to determine whether qPCR measures of FIOs are better indicators of swimmer-health risk than culture based measures and If so, why?

An essential requirement is to undertake qPCR and culture-based epidemiological studies in parallel, across the EU, to provide a back-to-back comparison.

Existing data on this matter carries many uncertainties. Many more organisms can be looked for from nucleic acid preparations than by culture based methods therefore any proposed or future dose-response studies should look at as wide a spectrum of targets as possible within available resource constraints. We have to explore the relative merits of using FIOs versus infective pathogens (eg. adenovirus) and discover whether the pathogen behaves differently to the faecal indicator. If it does there is a driver towards moving to a molecular method but if it does not then we need to question any motivation for change. However, knowledge of a single pathogen, whilst interesting, may not provide any information on risk from the numerous pathogens potentially present, a pattern driven by the health status of the contributing population.

Rapidity and logistics

Molecular methods are attractive in part because of the speed of analysis they offer. However to maximise the benefits of this fast turnaround time, any molecular analysis for bathing waters would need to be conducted close to the beach rather than be transported to regional or national laboratories as the extra time taken for transport to regional or national laboratories negates the shorter analysis time. However this option increases the number of laboratories needed to do the work and increases costs. It should also be remembered that a single sample taken early in the day might not characterise the bathing day at a UK bathing water (Figure 1).

Markers and targets – choice, fate and transport

There is much to learn about the fate and transport of, and predation and competition among MST markers and qPCR targets (for example viruses/pathogens and bacteria). This includes the survival of the organism in the fresh and salt water environment, the persistence of the target DNA and the potential growth or reservoirs of the organism in the environment (including favourable persistence via attachment to particles). Data on the survival of pathogens and indicators are scarce. The three key challenges in selecting and using MST markers and qPCR targets are listed below.

- There is a need for systematic studies into the fate and transfer of microorganisms of interest and more detailed investigation of the ecology of pathogens and indicators across different environments – we only know the tip of the iceberg for indicators and even less for key pathogens such as norovirus and adenovirus. This is a point that is frequently acknowledged but has yet to be followed through with the necessary research.

- The inability to distinguish viable from non-viable organisms is a limitation of **qPCR** at present and presents problems in relating **qPCR** targets to human health risk. More research is needed to optimise qPCR protocols for discriminating viable and dead targets.

- Investigations into environmental factors such as the seasonal variability in **MST** signals from specific faecal sources are also required. For example would changes in the temperature of water bodies affect the persistence of the organisms that carry the markers? This is the case for specific RNA coliphages - they do not survive well in warmer waters and it also might affect the activity of the polymerases. Variability in responses to environmental stress also poses a conundrum for what should be targeted.

Determining the performance of markers is important and developing marker specificity is the key, for example beef and dairy flora markers for MST are different and vary in source magnitude. This means that it is difficult to apportion faecal source contributions from different hosts because you are not using and detecting a common marker across all livestock types that originated in equal abundance in the source matrix. In addition, there is a need to ensure a continued programme of cross-comparison of patterns and magnitudes of FIO vs molecular markers detected throughout a range of aquatic environments impacted by different catchment sources.

Precision and reproducibility

There is a need to prove the precision and reproducibility of the qPCR methods for surface waters using international ring-trials, first with analyses of seeded stock solutions by multiple laboratories and then in different environmental matrices. There have been some national trials conducted in the USA (Kinzelman, 2011[2]) but more work is needed across the EU. A science-policy evidence base is needed to design and support enforcement of standard methods for sample concentration and analyses before regulatory adoption could be accepted, particularly where the resultant data could be used for legally enforceable compliance, as is the case in bathing, drinking and shellfish harvesting waters. The process of transferring this through to regulatory utility would take a number of years.

Complex samples, or those likely to inhibit quantification, need particular attention. We need to know how to maximise quantification efficiencies for these types of samples to ensure accurate risk characterisation can be achieved for bathing waters.

Regulatory results will then need to be examined for equivalence between methods where a switch is proposed from culture to qPCR. The two methods show variable correlation and measure fundamentally different states of FIOs.

qPCR and tertiary treatment of wastewater

A fundamental question that needs to be addressed is how can molecular methods such as qPCR be used for beach monitoring, permitting and compliance when they may be insensitive to tertiary waste water treatments such as UV disinfection (Stapleton *et al.* 2009[3]) The UK relies on UV disinfection as the main tertiary treatment method. There are major implications for the cost of improving bathing waters and interpretation of the data. We need a more detailed understanding of the viability of organisms after wastewater treatment when enumerated as qPCR CEs (and hence the efficacy of UV and other treatment methods in removing viable organisms).

[2] Comparative Evaluation of Molecular and Culture Methods for FIB for Use in Inland Recreational Waters. WERF Report PATH7R09

[3] Evaluating the operational utility of a Bacteroidales quantitative PCR-based MST approach in determining the source of faecal indicator organisms at a UK bathing water

4. Policy & Practice - questions and matters to address

Some bathing waters will see their status altered because of tightening bathing water standards in the rBWD. Further changes in the form of method transition for quantifying microbial compliance parameters could have the same effect and would be difficult to co-ordinate across agencies and explain to the public.

If the US EPA goes ahead with plans to use qPCR will the World Health Organisation and EU follow suit and change the measurement approaches required in the rBWD under future revisions? The rBWD is up for review in 2020 but any changes would need to be supported by a majority of the member states of the European Union.

There are challenges ahead in changing policy. The regulatory community has concerns about when and how to trust new technology and requires a solid evidence-base prior to implementation of any changes in this area.

All stakeholders are subject to potential bias in any new development. The bias may take the form of political pressure, academics wanting to pursue their own method of interest, pressure to reduce costs or public pressure to get information quickly.

Resource implications

If molecular methods are adopted on a routine basis, it may well be in addition to culture-based techniques. Is there enough expertise and facilities available and, if not, what extra staff, training and equipment would be needed? Where should these resources be sited to make proper use of the rapidity of the molecular techniques? Are mobile units feasible?

While there are budget pressures on all regulators (discussed further in **Briefing Paper 2**), there is also pressure from bathing water users groups to do more monitoring and improve quality, as well as the legal requirements in Europe to meet the new standards of the Bathing Waters Directive. In the USA public pressure from beach users helped to drive forward new molecular standards.

If MST was used as a tool to explain different pollution source contributions it would be necessary to sample at a sufficient spatial and temporal resolution to account for natural variability in the environment and this may have significant cost implications given the total cost per sample involved of £ 150-250.

Sampling

Regulatory sampling for bathing water compliance only provides a snapshot in time of that sample environment. Although this may be well understood by the Water Industry it may not be public knowledge. Meanwhile there are also pressures to reduce the number of bathing water samples taken, particularly at sites that always pass. However, as mentioned above consistent 'pass' only means that the sample spot at the beach reaches the standards consistently. Water quality elsewhere at the beach may be different. Before sampling is reduced, we should have a better understanding of the bathing water quality (not just the sampling spot) and how the public understands this.

One direction of travel may be towards the prediction of risk rather than retrospectively following analysis. Forecasting, asking what the predictive risks are, and then managing around that risk could be useful. We should be trying to predict risks to health rather than trying to predict a spot sample but we need intensive datasets to do this. Alternative views are that the situation is more complex and it would be better to take more samples in places that are having problems and fewer samples, with maybe some modelling, in places that are not. Current predictive models of FIO fate and transfer are driven by culture-based data and the determination of culturable numbers is essential for the modelling of culturable responses of bacteria to environmental conditions. New techniques would carry with them implications for these models.

Questions arise over whether composite sampling has a role to play to iron out the variations in FIOs/ pathogens or whether this would also iron out the peaks where, arguably, public health is most at risk.

Summary

We need to build consensus regarding the use of new and emerging tools through robust evaluation of key performance criteria for a range of environmental matrices under variable conditions. This requires a continued programme of laboratory and field based experimentation to build a policy an evidence base to underpin credible decision-making framework for considering future technology transition for enumeration of regulatory microbial parameters.

Glossary

Cell equivalent (CE) Each CE being measured is the amount of DNA one would expect to find in a single cell.

Colony forming unit (CFU)

CFU is an estimate of bacterial numbers and is used to determine the number of viable bacterial cells in a sample per millilitre of water. Unlike direct microscopic counts where all cells, dead and living, are counted, CFU estimates viable cells. The appearance of a visible colony requires significant growth of the initial cells - and it is not possible to determine if the colony arose from one cell or 1,000 cells. Therefore, the results are given as CFU/mL (colony-forming units per millilitre) for liquids such as bathing water samples.

Compliance monitoring

The sampling and analysis of water at designated bathing waters to assess compliance with the standards set by the current Bathing Water Directive and to be used in the four year data set that will provide the first set of classifications under the rBWD in 2015.

Culture-based enumeration

Established methods for growing or culturing FIOs for the purpose of monitoring water quality. A common method is the membrane filter technique. This consists of filtering a water sample on a sterile filter with a 0.45-mm pore size which retains bacteria, incubating this filter on a selective medium and enumerating typical colonies on the filter.

Designated sample point (DSP)

A water quality sampling point which is representative of the overall bathing water set by the competent authority. (The "Competent authority" means whoever has the legal responsibility for the local implementation under the Directive).

Designated bathing water. Beaches that attract a large number of visitors may be designated as bathing waters. In 2012 there were 83 designated BW sites in Scotland, 416 in England, 100 in Wales and 23 in Northern Ireland

Faecal indicator organism (FIO)

Bacteria normally present in large numbers in faeces whose presence above standard levels is viewed as a sign that levels of all bacteria from faeces are too high.

International Organisation for Standards (ISO) ISO develops and publishes international standards for products and services. For example ISO 8199:2005 Water quality -- General guidance on the enumeration of micro-organisms by culture . **http://www.iso.org/iso/home.htm**

Microbial Source Tracking (MST). The tracing of a pollution indicator organism in order to identify the host group of organisms from which it originated. This allows for the identification of the source of pollution and the potential pathways for transfer of microorganism in the environment.

Molecular Marker

Markers that may be biochemical, morphological or related to DNA. In the latter case a marker refers to specific fragments of DNA that can be identified within the whole genome and used to identify an organism.

Molecular technique

Molecular methods, such as quantitative polymerase chain reaction (qPCR) that have the potential for rapid analysis of samples.

Quantitative Polymerase Chain Reaction (qPCR).

A biochemical reaction in which a selected piece of DNA is copied and amplified. This then gives a copy number per unit volume allowing for the accurate quantification of the number of copies of an organism in a sample.

Revised Bathing Water Directive (rBWD) Research into bathing water and human health since the original Directive's introduction in 1976 has led to the development of the revised Bathing Water Directive (2006/7/EC), which will be implemented in stages between now and 2015, when the original Directive will be repealed. The revised Directive uses

two parameters to assess water quality, Escherichia coli and intestinal enterococci, using a four year data set for each set of results, and sets much tighter standards than the original Directive.

http://www.defra.gov.uk/environment/quality/water/water-quality/bathing/

Threshold dose

The minimum dose a substance that will produce a detectable degree of any given effect

Tertiary treatment of waste water

The purpose of tertiary treatment is to provide a final treatment stage for waste water to mprove the effluent quality before it is discharged to the receiving environment (sea, river, lake, ground, etc.). Disinfection using ultraviolet light (UV) is one form of tertiary treatment used to reduce substantially the number of microorganisms in the water.

Regulated water

For the purposes of this document the term Regulated Water refers to any water body where the water quality is protected by legislation that sets out standards that must be achieved for the purposes of a compliance regime. This includes but is not limited to bathing waters and shellfish harvesting waters.

Further information and contacts

This project has been funded by the Natural Environment research Council (NERC) and led by the University of Stirling. It is supported by Lancaster University and Aberystwyth University.

The core Working Group includes a membership of representatives from UKWIR, SEPA, EA, Defra, and Surfers against Sewage but has also drawn on a breadth of knowledge and experience from across the UK and the international community as well.

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Briefing Paper 2 concerned with economics and public information can be viewed and downloaded from the DHW website www.deliveringhealthywater.net