Delivering Healthy Water: building the science-policy interface to protect bathing water quality

Report from workshop 2

London, 27th and 28th March 2012.

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Participants:

Name	Organisation
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Andy Cummins (AC)	Surfers Against Sewage
Ana Maria de Roda Husman (AMdH) & Ciska Schets (CS)	National Institute for Public Health and the Environment (RIVM)
Lora Fleming (LF)	The European Centre for the Environment and Human Health (ECEHH)
Lidija Globevnik (LG)	European Topic Centre on Water
Valerie J Harwood (JH)	University of South Florida
Louise Heathwaite (LH)	Lancaster University
Chris Hodgson (CH)	Rothamsted Research
David Kay (DK)	Aberystwyth University
David Lees (DL)	CEFAS
Gordon Nichols (GN)	Health Protection Agency
Andreas Nocker	Cranfield University
David M Oliver (DO) & Melanie van Niekerk (MvN)	University of Stirling
Calum McPhail (CMP)	SEPA
Jonathan Porter (JP), Ian Dunhill (ID) & Amanna Rahman (AR)	Environment Agency
Richard Quilliam (RQ)	Bangor university
Ted Thairs (TT)	UKWIR
Tim Wade (TW)	USEPA

Overview

The first two workshops of the Delivering Healthy Water (DHW) project were hosted by UKWIR at their London headquarters and facilitated by David Oliver and Melanie van Niekerk of the University of Stirling. Workshop 1 was directed at the science user communities, predominantly regulators and policy makers while workshop 2 focussed attention on the science providers. This report provides a review of each seminar from **workshop 2** and a concise summary of the themes that developed over the day. It finishes with recommendations based on the key points that emerged across the workshop. In addition to members of the DHW Working Group, experts representing the science provider and science user communities were invited to participate. Four participants gave ten minute presentations and were asked to end their talk with the questions they considered needed addressing most urgently. For part of the workshop the participants divided into four groups to discuss a prepared set of questions that had been drawn up by the Working Group. These aimed to encapsulate the key problems facing science providers in assessing the pros and cons of moving towards molecular methods.

Aims

- To evaluate how science providers can align with the needs identified in day 1 by the regulatory, policy and other stakeholder communities.

- To identify pressing limitations or areas of uncertainty that may hinder meeting those needs now or in the near future.

Valerie J Harwood (JH) started proceedings with the presentation, **"MST, where are we now and where are we heading?"** She discussed the question of how hard it is to develop a marker and specificity was considered to be the key, for example beef vs dairy flora for MST are quite different. Determining performance of markers is important, but there are funding issues. JH gave an example of complexity from Tampa Bay involving different water types. Methods did not perform the same through the catchment transect and there was even variability among sampling dates - water changes through a storm event for example. There are many pathogens /indicators and we need multiple measurement platforms.. JH stressed that it is difficult to produce pie graphs of faecal source contributions from different hosts because you are not using and detecting a common marker across all livestock types. Therefore their abundances differ in the source matrices and that complicates the picture significantly.

Lidija Globevnik (LG) spoke next with a talk entitled "**Culture to molecular: perceived advantages and limitation for EU waters".** She described the current reporting process across the EU and outlined the way in which this is changing with the introduction of the revised BWD. LG introduced the "Eye on the Earth" portal that is used to present near real time data on bathing water quality in Europe. The purpose is to make data on bathing water quality available as soon as possible for the public via the internet. Concerning a move towards using molecular methods JG pointed out that the revised BWD, which sets the standards indicates no change to current culture based methods. However, she added that molecular methods could be promising and a better tool for point source pollution detection and therefore targeted management – and this surely is the purpose of the Directive. Using molecular methods bathing waters currently categorised as EXCELLENT may become GOOD (lower class). The reasons for this change should be efficiently and positively communicated to public.

Ana Maria de Roda Husman (AMRH) gave a presentation called **"Delivering Healthy Water - waves ahead"** in which she suggested that the question should be 'how to not use qPCR'. In terms of deciding whether there is a use for qPCR for indicators AMRH suggested that using qPCR for FIOs makes things very complicated – it is useful but not used enough to look for hazard identification. She did not think that in 5-10 years we will be moving to qPCR because we will have moved to the next thing as new methods will keep coming along. This creates a problem. qPCR as an investigative tool is good, but not as a legally supportive tool for recreational water. The membrane filtration and most probable number example shows that two approved ISO methods can vary considerably in their quantitative capability. As we move down the molecular route it becomes more about chemistry.

Dave Kay (DK) gave the final **presentation "A roadmap of science challenges ahead"**. He gave an overview of the problems associated with measuring FIOs. Natural variability is a major issue and while an order of magnitude difference in concentration would be acceptable for some academic studies (example cited of virology), regulators may not be happy presenting such data in court. DK considered the key requirements of qPCR to be a good indicator of risk, accurate and cheap. The fact that qPCR does not identify the effectiveness of UV was thought to be a problem. DK suggested that the best policy at the moment is to stick with culture (more precision) and use qPCR/MST for investigative (IF we have enough samples).

Key Questions evaluated by the Workshop Participants

How realistic in terms of a fit to regulators needs is the deployment of rapid testing methods?

- They can provide more a meaningful statement of risk than the current technologies that we have.
- There's little appetite for it. We currently measure everything based on the directives that tell us what to do and that's based on the growth medium.
- It's only useful if it's used very regularly ie opening and closing a beach. There's no point in knowing within hours - if you measure something once a month
- A rapid test has got to be something that can be done in the field; in the back of a van, for logistics. If the EA takes 20 samples in a day that then go to a centralised lab there are economies of scale in what is done in the lab. If you want to have something that's rapid and it's going to a lab overnight and then it's getting analysed, a rapid technique is not going to give you anything of an advantage.

How are indicator markers (e.g. faecal bacteroidetes) measured by qPCR affected by physical & environmental factors?

- The more data we get the better we can get but at the moment we can't come up with a definitive answer.
- We know that the markers are likely to have a very different persistence throughout the day because the viability of an organism is going to vary
- The bacterial markers that we've mentioned looking for probably aren't as persistent in the environment as the viruses that are the pathogens. So, something that gives you that persistence is probably going to give you some considerable benefit.
- JP expanded on a point from his presentation saying that when plotting concentrations of bacteroidetes and bacteroidales against coliform concentrations they get a stronger relationship than when they plot them against intestinal enterococci concentrations..

We know that the general characteristics of water quality from source to sea can impact on the quality of microbial enumeration using the same qPCR technique. How problematic is this?

- It is very problematic.
- You need a number of controls (e.g. process/inhibition/recovery etc)
- Continuous sampling may bring about more consistent results: but might be difficult to do – hard to fund method development. There is of course added value in moving away from spot samples e.g. learn about ecology, would be able to sample larger volumes so bringing in pathogens as well as indicators.

Q2 What are the most important performance characteristics to evaluate (e.g. sensitivity of the technique, host specificity) across different methods for MST deployment?

- You can't do performance characteristics until you have defined your method – there is no consensus.

- If a method is defined then standard and well defined approaches can be taken from other fields.
- For accreditation it would be key that they follow standards used in other fields and these are the things that we would associate in terms of sensitivity, linearity, limited detection, specificity, robustness and inter-lab comparisons.
- Would need to work through in similar lab setting followed by inter-lab comparison (at least 12)

What are the debates/contradictions/issues in the existing evidence base of our understanding of MST marker sources and behaviour (e.g. bacteroidales)?

- We need to know more about the fate and transport of the organisms that we are using as the basis for MST markers. That would include the survival of the organism and/or the persistence of the target, meaning the persistence of the DNA, the potential growth of the organism in the environment (attachment to particles)
- For the FIO research agenda we don't really know enough about the fate and transport of FIOs and this is now echoed in the MST agenda.
- We need markers for a lot more hosts.
- From a risk-management perspective, do we really need more markers from a lot more different hosts or do we just needs ones for the hosts that will help us differentiate risk the most? Like human and cattle? If we have a good knowledge of human, avian and cattle, in some watersheds then that would give us a lot of information. But, it still won't take us back to the relationship with the faecal indicator bacteria.

Do we have sufficient evidence that MST signals from a specific faecal source can be detected throughout different seasons?

- The answer was 'no, but there is no strong evidence to the contrary'
- The vital element here was the organisms that have the markers but are not entities in and of themselves. Little is known about the stability of the organisms that carry the markers at the individual level, at the herd level or population level.
- We do know that cattle herds for example, can differ a lot in cattle-specific, but there was no seasonal study in that case.
- Factors that influence faecal flora include diet, antibiotic treatment, pregnancy and lactation and there could very well be some distinct temporal trends in this
- Human markers like the bacteroidetes and HF183 are always in large sewage systems and in municipal sewage, it doesn't matter what time of year. But what if we went to a small package plant, maybe we would see some seasonal differences there. Again, that's something that has not been explored.
- Would changes in temperature in water bodies affect the persistence of the organisms that carry the markers? This is the case for specific RNA coliphages, for example - they don't survive well in warmer waters. It also might affect the activity of the polymerases.

- Changes in water turbidity (as well as pH and UV) may influence the efficiency of the PCR reaction and may also affect the fate and transport of the organism. But this all hinges around PCR or qPCR inhibition and how that might differ seasonally even in a given water body.

What are the key academic concerns associated with the use of qPCR for bathing water quality assessment?

- How **quantitative** it actually is able to be-controls and interpretation
- Whether it is possible to establish **dose thresholds** that are useful for regulators
- The issue of **inhibition** and whether it could be too specific or was it specific enough in terms of strain pathogenicity.
- There could be **variance** depending on how sampling was carried out in the environment, and the method of sample preparation.
- Drivers that could concern academics for example if legislation or law suits are pushing for a certain type of test, why should academics concentrate on something else that's not being driven economically?
- There is no consensus in terms of the methods or even whether qPCR should be used in a regulatory context.

What are the costs and benefits of molecular technologies?

Costs

- Different methods – lack of standardisation
- Training - Equipment, space
- Communication-managing expectations
- Dynamic field – changing techniques and equipment

Benefits

- Use in MST & investigations
- Speed & specificity
- Flexibility – multiple targets
- Limitations of culture methods

How prohibitive is the current lack of understanding of the ecology, the fate and transfer, abundance distribution and prevalence of target material in waters throughout the catchment continuum for reliable deployment of molecular-based tools?

- Fundamentally can do it but there needs to be the will behind it
- Faecal source variability, and complexity of samples
- Need epidemiological studies that demonstrate relationship to human health
- How does the length of time an organism has been in the environment affect the ratio of culturable to total or qPCR cells?

- Important to understand the ecology of the microbe and what is happening in the ecosystem, intra and inter variability in environments is important. Need background information to inform the decision as to when and where to get samples. The knowledge gaps on ecology apply to culturable FIOs as well

What are the biggest challenges facing regulators in moving towards using molecular methods in the regulation of recreational waters?

SCIENCE USERS	SCIENCE PROVIDERS
To support "informed" decisions of bathers, surfers and other visitors	Deciding what to measure. What is more important, connection with health risk or indication of contamination source?
To support risk management with timely answers	Can we use qPCR for source tracking?
Regulators are led by directives so may not be able to use new technologies for regulation	How quantitative is qPCR?
Identifying the trigger for addressing a contamination problem • Human health risk? • Ecological risk • Anticipated difficulty of remediation action?	Information • Speed • Confidence in • Understandable format • Should science resources be spent on better & more intensive monitoring or should it be spent on clean ups?
	Cost
	Too little funding for technology development
	Education of regulators/ management over advantages and disadvantages of molecular data
	Viable versus non-viable issues
	Inter and intra lab variability in recovery of organisms?
	qPCR – too many variations?
	How do we communicate/ interpret complex results and deliver to public in an understandable form
	Can new technologies deliver high confidence in outcomes of health significance?

Discussion Themes

Throughout the workshop a number of key themes emerged during the discussion sessions. Those themes are highlighted below and excerpts from the discussion included demonstrating the importance of these themes to the different science user and provider

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communities. Paragraphs in italics represent comments reproduced directly from transcripts of recordings made at the workshop:

THE NEXT BIG THING

LF queried if the US or EU goes to qPCR how much are the UK regulators being forced because it's the 'next big thing, it's going to be better' even though it may be at a great cost to do it?.

JP commented that if the US EPA pushes for qPCR we don't know whether Europe will follow that. And if it does it will need consensus from a lot of different European countries all agreeing on it.

Next generation sequencing: JH has some concerns here because a pathogen or two would be picked up by this approach but it wouldn't pick up the minority bugs

FIO vs PATHOGENS

If we can prove a disconnect between the behaviour of an FIO and the behaviour of a pathogen and the molecular assay can detect the pathogen directly, then you have a driver towards looking for that pathogen. But if the FIO and the pathogen behave in a very similar way and we can do the FIO then there isn't a big regulatory driver, at the moment, to move towards looking for the pathogens with a molecular assay.

Two reasons for not looking for pathogens:

1. A small fraction of the population is actually infected with pathogens, so they're rare targets, which means that if you start looking for pathogens then you have this incredible patchiness in distribution that makes it even more difficult than looking for indicator bacteria which are themselves patchy.
2. The question of which one do we look for and their ecology is so different. If you look at a cryptosporidium versus a campylobacter or in a salmonella, versus even a norovirus, which is an RNA virus and adenovirus, which is a DNA virus, and they're incredibly different in their responses to environmental stresses. And so the conundrum that's been holding us back for many years is which ones do we target and how do we concentrate them effectively without mucking up our assays to the point where we can't detect them. And so, that brings us back to the advantages of the indicators.

LF undertook work that found no relationship between qPCR and FIOs at a non-point source beach. The EPA studies were always at beaches impacted by point sources, where the relationship works.

COST

'*If you do qPCR there are a lot of controls you have to use to make sure that you're doing your job right. So, even if you automate the methods you've still got to have somebody to look over everything, you're still going to have expensive expendable re-agents that are*

going to be involved, the number of controls that you have to run for every water sample, and when you factor in the labour costs I'm just not sure if it's ever going to be as cheap as culture methods. But it could be made a lot cheaper AND more reliable for multiple users'.

'*There are pressures to try and reduce the number of bathing water samples taken. Because there are some sites that we never fail, the question gets asked 'what's the point of taking a sample every week, oh it's another pass' and reducing sampling frequency could be seen as a cost saving. However there was a strong opinion that in fact we don't do enough monitoring, modelling and sampling. This was supported as an important view to balance the alternative school of thought that 'we're doing enough and doing too much in some cases'*.

NEW APPROACHES

DL suggested that the direction of travel was towards the prediction of risk and management around prediction rather than retrospectively following analysis? If you got weekly samples, with a long data-set, you'll have covered, over time, most of the risky scenarios. So hind casting, what were the predictive risks and then managing around that risk will be useful. He also said that rapid methods are only useful for people who want to use the beach on that day.

LF thought that the situation was more complex because it would be better to have more samples being taken in places that are having problems and fewer samples, with maybe some modelling, in places that aren't.

FUTURE RESEARCH

The following are items extracted from the presentations, feedback sessions and discussions that represent opportunities for future research in the area of emerging molecular tools:

1. Survival of pathogens and indicator data is scarce. There is a clear need for systematic studies of fate and transfer of microorganisms of concern (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 pathogens.
2. An underexplored area of research is the question of whether pathogens from humans are more dangerous to human health than pathogens from animals.
3. There is much to learn about the fate and transport of, and predation and competition among MST markers and qPCR targets. A more detailed understanding of behaviour dynamics is urgently needed. This includes investigation of seasonal variability in MST signals from specific faecal sources.
4. There is a need for research to distinguish viable/non-viable organisms to be able to measure the efficacy of wastewater treatment processes if molecular methods are to be adopted.