Understanding the Use of Flow Cytometry for Monitoring of Drinking Water

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Finally, we would like to thank Ann Bunting, the project manager at the DWI, for her patience and guidance during the project.
Understanding the Use of Flow Cytometry for Monitoring of Drinking Water

Executive Summary

The aim of bacterial analysis of waters used for human consumption is to produce accurate and reproducible results. This in turn enables effective monitoring to promote reliable water treatment and distribution. Culture based methods for counting bacteria in drinking water give results which are inaccurate compared to ‘direct’ microscope counts or flow cytometry (FCM) methods. In addition, most final waters do not contain faecal indicators which can limit the scope for optimisation of these water treatment assets or improving the hygienic quality of product water due to limitations of culture based data.

Unlike culture based methods, FCM can provide rapid and accurate measurements of total and intact bacteria in water. FCM monitoring can provide early detection of changes in treatment works operation or in the drinking water storage and distribution systems. However, a current challenge with these alternative methods of bacterial monitoring, such as FCM, is data analysis and interpretation. This review and state of the art survey was carried out to determine how the FCM technique can help water companies determine microbial water quality for waters used for human consumption. This report presents information on the data obtained by FCM, the current limitations of the methodology and consideration of whether the method could be adopted for regulatory compliance monitoring.

A survey was undertaken to understand if (and how) each water company has approached FCM in England and Wales. Information about the nature of FCM use was presented with respect to the literature search and contextualised by a small international expert steering group. Determination of differences in investment level, opinion and local culture for ‘users’ and ‘non-users’ was compared. In total, 18 separate responses were received which represented all of the large water treatment and supply companies in England and Wales (a 95% response rate overall).

Most water companies are using FCM for monitoring of specific assets during investigations, notably samples from the water treatment works (WTW) inlet, post-coagulation / solids removal, post sand filter, or post chlorine contact tank. Final waters were the most commonly sampled location for routine sampling and using FCM for analysis. Where FCM has been used for monitoring distribution systems, most (~50 %) are using FCM on service reservoirs, highlighting the lack of information about these important assets. Fewer water companies (26 %) have been using FCM for monitoring distribution networks. Trend analysis and comparison with other datasets, alongside generation of prescribed values were the most common
ways by which FCM data was being used (each > 50 % of respondents). Both users and non-users were positive about the benefits of FCM over conventional heterotrophic plate count (HPC) methods. The perceived benefits included: ease of use, measurement of ‘active’ bacteria, and active management of water treatment and supply assets. 60 % of respondents considered that FCM was not useful for quantifying specific pathogens. Non-users were overall less positive about FCM but had similar views to users with respect to the benefits and limitations of the technology. Key challenges for implementing FCM for the industry included: method standardisation, data analysis and interpretation, difficulty of comparison with historic data, cultural change within water company institutions and instrument reliability. Speed, accuracy, data quantity and opportunity for online monitoring were thought to be the tangible benefits of using FCM.

Monitoring specific assets during water quality events and use of offline systems for routine analysis were identified as areas of opportunity. The use of online or discrete in-line automated FCM was seen as a practical approach for intensive root cause analysis of process deterioration and dynamic changes in microbial loading through a treatment works. Currently, regulatory monitoring requirements are clear that FCM is not required to for monitoring quality of drinking water. In addition, the lack of suitable prescribed concentration or value (PCVs), formal standard methods, or accreditation (e.g. through UK Accreditation Service) has so far limited the technology. As a result most water companies are using FCM as a sensitive measure of asset performance to reduce likelihood of compliance issues by detecting declines in asset performance. As FCM becomes increasingly used to make operational decisions, with potential financial and public health implications, it is important to have confidence in the results and therefore accreditation should be a desirable goal. It is considered that accreditation of FCM analysts competence is the most likely scenario with respect to standardisation.

However, water companies should ensure that data analysis is appropriate and standardised with consideration of the limits of FCM and the data generated. Data interpretation standardisation / guidelines are seen as the next big challenge for successful future use of FCM for the water industry. All water suppliers should continue to monitor assets for microbiological compliance using the approved indicator organism(s) of water quality to protect public health. As a broadly ‘industry-led’ initiative as opposed to ‘regulation-led’, FCM is an example of industry cooperation to attempt to solve the challenge of spot microbiological compliance events at generally well run and optimised water treatment and supply assets. Online FCM is identified as a key tool to reduce the risk to public health through optimised treatment and better asset understanding. The evidence suggests that FCM can produce information needed by operators to produce a more stable bacterial population which reduces the risk of pathogen penetration of water treatment barriers and subsequent regrowth within networks. While FCM has previously been proposed to be a panacea for microbial monitoring, the benefits of FCM come from the faster, cheaper and more reproducible bacterial analysis that enable it be used for diagnostic applications in water treatment.
# Glossary and abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>ACC</td>
<td>Aerobic colony count</td>
</tr>
<tr>
<td>ACV</td>
<td>Abnormal change values</td>
</tr>
<tr>
<td>ATP</td>
<td>Adenosine triphosphate</td>
</tr>
<tr>
<td>CAPEX</td>
<td>Capital expenditure</td>
</tr>
<tr>
<td>CFU</td>
<td>Colony forming units</td>
</tr>
<tr>
<td>CV</td>
<td>Coefficient of variation</td>
</tr>
<tr>
<td>Defra</td>
<td>Department for Environment, Food and Rural Affairs</td>
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<tr>
<td>DAF</td>
<td>Dissolved air flotation</td>
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<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
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<tr>
<td>DWI</td>
<td>Drinking Water Inspectorate</td>
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<tr>
<td>EQA</td>
<td>External quality assessment</td>
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<td>FCM</td>
<td>Flow Cytometry</td>
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<tr>
<td>HNA</td>
<td>High nucleic acid content (events)</td>
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<tr>
<td>HPC</td>
<td>Heterotrophic plate count</td>
</tr>
<tr>
<td>ICC</td>
<td>Intact cell counts</td>
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<tr>
<td>IMS</td>
<td>Immunomagnetic separation</td>
</tr>
<tr>
<td>LAMP</td>
<td>Loop mediated isothermal amplification</td>
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<td>LOB</td>
<td>Limit of blank</td>
</tr>
<tr>
<td>LOD</td>
<td>Limit of detection</td>
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<tr>
<td>LOQ</td>
<td>Limit of quantification</td>
</tr>
<tr>
<td>LNA</td>
<td>Low nucleic acid content (events)</td>
</tr>
<tr>
<td>MACS</td>
<td>Magnetic assisted cell sorting</td>
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<td>Abbreviation</td>
<td>Description</td>
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<tr>
<td>MALDI TOF MS</td>
<td>Matrix-assisted laser desorption/ionization, time of flight, mass spectrometry</td>
</tr>
<tr>
<td>MESF</td>
<td>Molecules of equivalent soluble fluorochrome</td>
</tr>
<tr>
<td>N/A</td>
<td>Not available</td>
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<tr>
<td>NEQAS</td>
<td>National External Quality Assessment Service (UK)</td>
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<tr>
<td>PCR</td>
<td>Polymerase chain reaction</td>
</tr>
<tr>
<td>PCV</td>
<td>Prescribed concentration value</td>
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<tr>
<td>PI</td>
<td>Propidium iodide</td>
</tr>
<tr>
<td>qPCR</td>
<td>Quantitative polymerase chain reaction</td>
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<tr>
<td>RGF</td>
<td>Rapid gravity filter</td>
</tr>
<tr>
<td>RNA</td>
<td>Ribonucleic acid</td>
</tr>
<tr>
<td>rRNA</td>
<td>Ribosomal ribonucleic acid</td>
</tr>
<tr>
<td>RT-qPCR</td>
<td>Reverse transcription quantitative polymerase chain reaction</td>
</tr>
<tr>
<td>SSC</td>
<td>Sideward scatter</td>
</tr>
<tr>
<td>SYBR Green I</td>
<td>N',N'-dimethyl-N-[4-[(E)-(3-methyl-1,3-benzothiazol-2-ylidene)methyl]-1-phenylquinolin-1-ium-2-yl]-N-propylpropane-1,3-diamine</td>
</tr>
<tr>
<td>TCC</td>
<td>Total cell counts</td>
</tr>
<tr>
<td>UKAS</td>
<td>United Kingdom Accreditation Service</td>
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<tr>
<td>UV</td>
<td>Ultra Violet Radiation</td>
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<tr>
<td>WQRS</td>
<td>Water quality risk score</td>
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<tr>
<td>WTW</td>
<td>Water treatment works</td>
</tr>
<tr>
<td>UV</td>
<td>Ultra Violet Radiation</td>
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Introduction

This literature review and water industry survey were carried out to determine FCM implementation across different supply regions (water treatment and supply companies) and for different FCM groups comprising ‘users’ and ‘non-users’. The literature review was carried out between March to September 2018. The questionnaire survey was undertaken between July and November 2018.

The ‘users’ and ‘non-users’ enabled an opportunity to assess the implementation of FCM across England and Wales. The Drinking Water Inspectorate (DWI) are part of the UK Government’s Department for Environment Food and Rural Affairs (Defra) and act as the drinking water quality regulator for England and Wales, enforcing the adherence to the water quality standards. The DWI commissioned this research to understand the potential of the FCM technique to be used for microbial water quality monitoring in both operational and regulatory contexts. The final report was completed between September 2018 and January 2019. The aim of the work was to determine how scientists, practitioners and water companies were using FCM as well as understanding the realised and perceived benefits of using FCM. The following aspects of FCM were considered: knowledge and interpretation of the technique and data, how it can be applied and implemented, what investment decisions have been made, and how is the approach being standardised? In addition, the review reports on previous sampling efforts using FCM, alongside the research developments that are taking the FCM technique forward.

The scientific objectives of the study were as follows:

1. Undertake a literature review to: 1) assess how FCM is being used in water quality areas including novel applications such as pathogen detection; 2) determine the accuracy of FCM compared to other ‘new’ technologies and 3) summarise previous attempts to generate PCVs for source water, treatment and supply when using FCM.

2. Determine limitations of: 1) available FCM technologies, 2) the methodology when applying FCM for drinking water analysis, including the accuracy compared to established and novel techniques.

3. Determine whether FCM can be applied to identify specific reference pathogens or compliance indictors including Campylobacter spp., Cryptosporidium spp., Legionella pneumophila, E. coli, and bulk or specific viruses (such as Enteroviruses).

4. Determine whether FCM can be used to identify higher risk populations of general bacteria. For example, can high nucleic acid (HNA) or low nucleic acid (LNA) differentiation provide suitable measures of this risk?

5. Describe future areas of research needed for FCM use and application in the water industry.
Methodology

Details of the literature review methodology are provided in Appendix A. The survey was implemented through the survey web application Qualtrics platform (Qualtrics, UK). The survey questions are presented within Appendix B. The survey questions were developed in collaboration with the international expert steering group for scientific and industry relevance. The steering group was a balance between industry and academic representatives who were active in the field of flow cytometry. The role of the steering group was to i) advise on the relevance of search terms used in the literature review ii) ensure that the survey questions were fit for purpose with respect to the project aims and objectives.

Literature Review

State of the art for FCM

Aim: To undertake a literature review to assess how FCM is being used in 1) water quality areas including novel applications such as pathogen detection, 2) accuracy compared to other ‘new’ technologies and 3) summary of previous attempts to generate PCVs for source water, treatment and supply.

It is not the aim of this review to repeat previous literature analysis as reviews exist on many different aspects of FCM (Müller and Nebe-Von-Caron 2010; Wang et al. 2010; Liu et al. 2013; Adan et al. 2017; Van Nevel et al. 2017a; Pecson et al. 2019; Safford and Bischel 2019). The aim was to provide context and direction on how to implement and extract practical value from FCM data from a regulatory and a public health perspective, something which to date has not been undertaken.

Drinking waters are treated to ensure the absence of pathogenic bacteria in final waters and to limit uncontrolled regrowth of pathogens in distribution. To this end, treatment systems often make use of multiple treatment barriers such as coagulation, sedimentation, dissolved air flotation, ozonation, sand filtration, membrane filtration, and chlorine or Ultra Violet (UV) based disinfection (Hammes et al. 2008). In most countries there is a requirement for chlorine-based disinfection residuals to be maintained (Farrell et al. 2018) to ensure the microbial quality of drinking water as it reaches the customer’s taps (Van Nevel et al. 2017a). Monitoring of water that is used for human consumption is a legislated requirement in most countries based on standards enforced through regulators (for example in the UK the Drinking Water Inspectorate, Drinking Water Quality Regulator for Scotland, Drinking Water Inspectorate – Northern Ireland). This includes regular monitoring of microbes in drinking water.

However for many years, it has been known that bacterial survival and frequency are several orders of magnitude higher in final treated water when measured with direct ‘absolute’ counting methods compared to culture-based microbiological quantification methods (Hoefel et al. 2003; Nocker et al. 2017). It is known that bacteria and viruses occur naturally in the drinking water system, most are ubiquitous and do not constitute a risk to public health. However, there is a need to ascertain performance of assets through the
treatment train with respect to these populations. Bacteria and viruses, only become an issue for public health, when there are breakdowns in treatment, weaknesses in distribution, or optimum conditions for proliferation to occur.

Assessing the public health risk from microorganisms is important without relying on general culture based measures or indicator organisms (e.g. *E. coli* and coliforms) which do not always correlate well with disease causing organisms (Hassard et al. 2017). In the absence of suitable indicators of treatment performance which are easy to measure (Proposal for a Directive COM/2017/0753 final - 2017/0332 (COD) 2018), measures of bulk microbiological abundance change are a useful option (Prest et al. 2014), enabling operators and decision makers to understand performance of individual treatment barriers / steps. This is of particular importance given legislative requirements for conservatism of water quality despite climate change which, could, increase the risk of water-borne disease risk (Reasoner 1990; Semenza and Menne 2009; Liu et al. 2018).

Overall, data support the well-established paradigm; that the risk to public health from drinking waters is not always reflected in the presence of compliance organisms and less so by general HPC (Sinclair and Rizak 2004), possibly due to regrowth in distribution (Gillespie et al. 2014) and intrinsic resistance within microbial populations to treatments such as chlorine (Chiao et al. 2014). Rapid and specific measures of pathogen abundance in waters used for human consumption are needed but are usually too costly to implement at the sampling scale and frequency which is needed to monitor WTW (Hassard et al. 2016a).

Historically, laboratories have relied on HPCs to monitor water quality, but this method is unreliable and slow with analysis times of 1-3 days. It is also known that few bacteria (0.001 – 8.3%) are ever recovered on heterotrophic microbiological growth media (Hammes et al. 2008; Burtscher et al. 2009; Sekar et al. 2012). It is known, that most bacteria are not present as suspended bacteria but as biofilm based communities attached to pipe walls or contained within or on suspended particles. It is thought that these biofilm communities are not well quantified in water quality surveillance but could lead to water quality concerns during sloughing events (WHO 2003 a,b). There are no formal WHO recommendations on the methods, thresholds and interpretations required for monitoring bulk microbial abundance. As a result widespread adoption of HPC at 37 °C or 22 °C were applied through convenience and the lack of better alternatives (WHO, 2003 a, b).

However, this has led to the idea that more advanced analysis tools can and should be used to monitor microbial loading in drinking water (Hoefel et al. 2003). The FCM technology was originally developed in the 1960s for the analysis of mammalian cells in the medical industry (Wang et al. 2010). FCM is an automated technique that is able to rapidly (10,000-50,000+ cells per second) measure the optical properties (fluorescence, sideward scatter, forward scatter) of single cells suspended in liquid transport. This is done through particle interaction with light and conversion of an analogue to digital signal through a photomultiplier tube (Yang et al. 2019). Coupling this with bacterial stains provides a useful way of distinguishing bacteria from background particles. Further, FCM provides an assessment of the quantity
and also a measure of the viability, activity or type of bacteria within a sample (Wang et al. 2010; Van Nevel et al. 2017b; Berney et al. 2008 Yang et al. 2009; Nocker et al. 2011; Gillespie et al. 2014; Helmi et al. 2015; Hassard et al. 2016a, b).

One of the first applications of FCM in drinking water analysis, was for the detection of Cryptosporidium spp. oocysts (Vesey et al. 1991). FCM-based detection of bacteria in drinking water was used for studying the adsorption characteristics of E. coli to iron oxyhydroxide from a corrosion perspective (Appenzeller et al. 2002). Together these studies showed the potential FCM as a tool to study complex phenomena which occur in drinking water treatment and supply. An important finding occurred in 2003, showing the poor relationship between FCM data and HPC (Hoefel et al. 2003) backing up earlier studies which showed a similar phenomenon with other microbiological methods due to viable but non culturable bacteria and poor recovery efficiency of culture based methods (McFeters et al 1986; Oliver, 1993). Unfortunately, the difference between HPC and FCM is inherently non-linear between samples, this prevents corrections factors from being applied. The lack of correlation applies for both HPC vs intact cell counts (ICC) and HPC vs total cell count (TCC) (see Van Nevel et al. 2017b for a review).

Other studies have shown that FCM can help quantify important constituent concentrations such as assimilable organic carbon (AOC) and other nutrients important for regrowth (Hammes and Egli 2005). Biological stability and instability caused by decay, regrowth and sloughing of biofilm bacteria has also been studied in drinking water distribution networks (Hoefel et al. 2005a, b; Liu et al. 2018) and within buildings, which is thought to be an issue during extended stagnation (Lipphaus et al. 2014). Pathogens can be detected and enumerated and therefore the pathogen growth can be assessed in different waters using a combination of FCM and other methods (Vesey et al. 1994; Keserue et al. 2012). It was noted in Keserue et al. (2012) that there was a poor correlation between TCC and pathogens. Flow cytometry has been applied beyond potable waters to consider optimising water reuse/recycling systems (Whitton et al. 2018) as well as to study wastewater treatment systems (Foladori et al. 2007; Hassard et al. 2014, Brown et al. 2015).

The development of advancing molecular techniques such as polymerase chain reaction (PCR), has encouraged the development of cultivation free approaches for the quantitative and qualitative analysis of microbial communities. These techniques offered increased speed and reproducibility and can give more information about microbial communities than HPC, thereby offering alternative approaches for microbial characterisation to traditional cultivation-based techniques (Berney et al. 2008; Van Nevel et al. 2017b).

The continued development of these molecular biology techniques and recognition of limitations such as PCR bias reduced efficiency due to reagents, sequence being measured or inhibitors), cost and relative abundance (not quantitative from deoxyribonucleic acid - DNA sequencing data). As neither PCR or sequencing technologies provide an absolute cell count, this supported the further development of FCM as a tool for cultivation free microbial characterisation with present-day FCM technology. The technique can be easily utilised for routine analysis in a testing laboratory.
Counting TCC

The technological advances observed in FCM in recent years, has led to the development of protocols for the enumeration and characterisation of microbial populations for water applications (Berney et al. 2008; Hammes et al. 2008) through the use of staining of microbial nucleic acids or cell characteristics (Veal et al. 2000). These protocols enable the rapid enumeration of TCC and ICC (Gatza et al. 2013) (commonly referred to as live/dead staining and/or BacLight). TCC can be used to provide insight into water treatment and supply systems which do not contain chlorine. In addition, TCC can be used to monitor assets for breakthrough of low numbers of cells. The reproducibility of staining of bacteria for TCC was standardised and efforts to assess intra-lab variability in analysis was assessed (SLMB, 2012). A standardised protocol was developed and a guideline method was proposed in Switzerland. There is a requirement for measuring viability of cells (e.g. ICC as a proportion of TCC) which is particularly important in regions which chlorinate water supplies.

Counting ICC

SYBR Green I is commonly selected for TCC and a dual stain of SYBR Green I and Propidium Iodide (PI) is utilised for enumeration of ICC (Gatza et al. 2013) through the identification of ‘compromised’ cells. These cells are characterised as those which have a compromised cell wall permitting penetration of the PI dye. SYBR Green I is a DNA intercalating dye that can penetrate into cells with compromised and intact cell membranes (total). To determine the ICC of a sample, bacteria are stained with a combination of SYBR Green I (TCC based on green fluorescence) and PI. PI is another DNA intercalating dye, however, it can only penetrate into cells with compromised cell membranes. PI fluoresces red and so provides the intact cells (ICC based on increased red fluorescence in compromised cells). It is not recommended to count ‘dead’ cells by subtraction of ICC from TCC, this is because it is difficult to account for the background and fluorescence resonance energy transfer in the samples. Therefore a commonly calculated statistic is the % ICC of the TCC cells. One option for counting non-viable ‘dead’ cell counts is through single staining of cells separately with PI. Subsequently, the PI positives are counted (membrane compromised cells) on a 630 nm laser (red fluorescence) by sideward scatter (SSC) (Cheswick 2019, personal comm.). The alternative for determining dead cells using a dual stain is to undertake fluorescence compensation (e.g. fluorescence minus one analysis) to enable distinction between ICC, TCC and therefore dead cells.

Counting Virus

Numerous efforts have been made to count virus particles with FCM, mainly as virus are difficult to culture (days to weeks) (Safford and Bischel, 2019), are very important from a disease perspective (Gall et al. 2015) and often require significant pre-treatments prior to enumeration with PCR approaches, particularly from environmental water samples (Farkas et al. 2017). There is additional complexity due to
differences in staining efficiency with commercial stains due to virus species specific differences in genome sizes, genome types (e.g. Ribonucleic - RNA or DNA) and capsid protein coat structure (Pecson et al. 2019). Viruses sit within the ‘background’ in terms of fluorescence and sit below the detection limit in terms of size for most FCM analysers, although there has been some success recovering virus from wastewater (Brown et al. 2015).

Huang et al. (2016) used FCM and a different dye (SYBR Gold) to assess performance of a water reclamation centre performance in terms of TCC and total virus log reduction. Efforts have been made to separate viruses from the noise (e.g. Huang et al. 2015) although at present the methodology for virus enumeration from drinking water needs further research as only the largest types of virus have been effectively separated from the background in real environmental water samples (Lippé 2018). Nonetheless, it appears that flow virometry is likely to feature in future research to characterise and count viruses. For example El Bilali et al. (2017) showed that flow virometry can distinguish different levels of infectivity of Herpes Simplex Virus 1, suggesting that in principle infectivity studies could one day be feasible in drinking waters. Gaudin and Barteneva (2015) went further stating ‘flow virometry is a powerful and versatile tool to define virus particle profiles’.

An overview of where and how FCM is currently being used for water quality analysis, as found in the literature is summarised (Table 1). Further details of present-day use of FCM in the UK water industry (collated through survey responses) are detailed within ‘Current status of FCM within the water industry (objective 2)’.
<table>
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<th>Process/unit</th>
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<th>Parameters</th>
<th>Reason for use/decision</th>
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<td>(Elhadidy et al. 2016)</td>
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<td>Pre-coagulation</td>
<td>TCC, ICC</td>
<td>Inter-stage sampling using FCM to inform process control</td>
<td>(Hassard et al. 2018)</td>
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<td>Post-coagulation / settlement</td>
<td>TCC, ICC</td>
<td>Process optimisation</td>
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<td>Dissolved air flotation (DAF)</td>
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<td>Understanding bacteria growth and abundance</td>
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<td>Quantify bacteria in biofilms</td>
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<td></td>
<td>Slow sand filtration</td>
<td>TCC</td>
<td>Monitor biofilm performance</td>
<td>(Chan et al. 2018)</td>
</tr>
<tr>
<td></td>
<td>Membrane</td>
<td>TCC</td>
<td>Assess efficiency and integrity of membrane filtration.</td>
<td>(Park et al. 2018)</td>
</tr>
<tr>
<td>Disinfection</td>
<td>TCC, ICC</td>
<td></td>
<td>Evaluation of disinfection efficacy.</td>
<td>(Hammes et al. 2008)</td>
</tr>
<tr>
<td>Final Waters</td>
<td>TCC, ICC</td>
<td></td>
<td>Evaluation of treatment process.</td>
<td>(El-Chakhtoura et al. 2015)</td>
</tr>
<tr>
<td>Distribution/network</td>
<td>TCC, ICC</td>
<td></td>
<td>Evaluate regrowth, reinstatement of network following maintenance.</td>
<td>(Gillespie et al. 2014; El-Chakhtoura et al. 2015; Van Nevel et al. 2017a)</td>
</tr>
</tbody>
</table>

Predominately, the literature confirms that FCM is an appropriate technique for water quality analysis from surface waters through treatment to the final processes of treatment (including disinfection) and distribution. Fewer studies report the use of FCM throughout the treatment process for the purpose of process control and/or performance monitoring of assets. Distinguishing viable from compromised and active from inactive cells is a key aim of bacterial water quality monitoring, this is discussed in the next section.

**Actively growing bacterial populations**

In drinking water microbiology understanding the growth state of bacterial populations is of importance. For example, determining whether bacteria are actively growing, viable, culturable, pathogenic and / or decaying. This is because active and dividing bacteria may have different characteristics with respect to disinfection, represent more of a risk for regrowth and have different potential for human pathogenicity (Ramseier et al. 2011). Actively growing bacteria can also represent an early warning as ‘sentinels’ of contamination of nutrient rich environments such as wastewater contamination in cleaner nutrient poor water such as drinking waters (Winterbourn et al. 2016; Whitton et al. 2018). Similarly the presence of active bacteria can indicate a breakthrough of microorganisms into drinking water through compromised treatment barriers (Park et al. 2018). Suitable quantification of actively growing bacterial populations
have proven to be time consuming, can be specific to different strains or are prohibitively expensive to measure (Wang et al. 2009).

It has been suggested that the nucleic acid content of bacteria could be used as a proxy for this growth status (Li et al. 1995; Gasol et al. 1999; Longnecker et al. 2005). When using FCM it is possible to quickly determine the nucleic acid content of bacterial populations (Lautenschlager et al. 2014). Two arbitrary populations of bacteria tend to emerge, the so-called HNA and LNA bacteria (Gasol et al. 1999; Longnecker et al. 2005; Berney et al. 2008). These bacteria can be detected using SYBR Green I staining and FCM (sorted using SSC against green fluorescence). HNA therefore have high fluorescence intensity and high SSC, whereas LNA are considered the opposite (Wang et al. 2009; Prest et al. 2013). SSC signals are applied to estimate cellular size and fluorescence intensity provides a measure of the cellular nucleic-acid content. It is important to note that fluorophore fluorescent intensity is also governed by pH.

Due to the critical role in the quantity of genetic material in the growth of bacteria it has been proposed that the % HNA cells could be used as a reference for actively growing bacterial populations. HNA bacteria are thought to be larger and more active organisms (Gasol et al. 1999), whereas the LNA bacteria primarily consist of relatively small, less active bacteria in environmental water samples (Gasol et al., 1999; Wang et al., 2009). However, there is evidence suggesting that LNA bacteria are active and simply represent a different physiological state of HNA cells (Cheung et al. 2015; Hewitt and Nebe-Von-Caron 2004). For example, it has been shown that HNA bacteria have less than 10 times the adenosine triphosphate (ATP) content when compared to HNA cells (Zlatanović et al. 2017). Usually these two clusters are visible in most drinking water samples (Berney et al. 2008; Prest et al. 2013, 2014).

Microbiologists have argued that these groups contain similar assemblages and that bacteria change their phenotype from HNA to LNA depending on environmental conditions (Servais et al. 2003; Bouvier et al. 2007). Others propose that HNA bacteria are a diverse and different assemblage from LNA (Lebaron et al. 2002). Lebaron et al. (2001) labelled and sorted populations of aquatic bacteria into HNA and LNA, subsequently, they used leucine incorporation rates to demonstrate that LNA are less active.

The distinction into HNA and LNA therefore has potential to represent indicators of bacterial activity. The HNA/LNA ratio is widespread in FCM analysis. Water quality managers using FCM must therefore have a good understanding of the bacteria which constitute these fractions. Among those researchers and practitioners from water companies which measure nucleic acid content, there appears to be little agreement with respect to the location of the gates used to discriminate HNA from LNA. A commonly cited value of ~2 x10^4 arbitrary fluorescence units is cited (Prest et al. 2013; Chan et al. 2018), however after discussion with multiple water company FCM users, it is apparent that it is common practice to adjust gates for HNA and LNA determination. As a result, they do not rely on predetermined values, particularly as a result of the impact of pH and other interferences on fluorescent intensity (Shapiro 2005) which in turn changes the threshold for distinction between HNA and LNA. Minimising the impact of water chemistry, notably pH, through buffering represents a useful first step to standardising...
fluorescence based discrimination.

Therefore, there is a risk of adopting additional indicators which are not grounded to water hygiene or public health risk. From a water hygiene perspective, it has been posited that LNA bacteria could represent less of a risk than HNA bacteria (Zlatanović et al. 2017; Wilkinson 2015). This presumption seems without basis, particularly as many human pathogens are known to have very small genomes e.g. *Mycoplasma genitalium* or *Mycobacteria tuberculosis* (Cole et al. 1998; Baden-Tillson et al. 2008). This is because intracellular pathogens experience extensive genome reduction as some genes are transferred to the host nucleus (e.g. pathogenic virus), while others have simply been lost and their function replaced by host processes. This is something rarely considered when bulk HNA, LNA or ratios are reported. Finally, another consideration with respect to reliance on HNA and LNA to measure actively growing populations, is that bacteria appear able to regulate and control their activity at a community level through quorum sensing (Ayrapetyan et al. 2014), again compounding the difficulty in use of nucleic acid content for bacterial water quality monitoring purposes.

However, alongside absolute counts and fluorescent fingerprints, the HNA and LNA content could shed light on large changes to the composition of the bacterial community across the drinking water treatment train. For example, in through a water treatment works, a similar ratio of HNA/LNA bacteria was found in the rapid sand filters (RSFs) effluent and the granular activated carbon (GAC) effluent (Wang et al. 2009; Lautenschlager et al. 2014). Processes that use smaller media such as slow sand filtration, as well as membrane systems have been associated with an increase in the proportion of LNA bacteria compared to HNAs cells, despite declining cell counts overall (Lautenschlager et al. 2014; Park et al. 2018). This could be explained by the differences in size of the two types of bacteria as HNA cells are considerably larger than LNA cells (Wang et al., 2009). However, selective grazing of HNA bacteria (Boenigk et al. 2004), nutrient starvation or favourable treatment efficacy against HNAs has also been reported to change this ratio (Ramseier et al. 2011).

A study showed that ICC and %HNA were two significant parameters governing changes to the microbial abundance in source waters (Boi et al. 2016). However, when considering removal and inactivation the TCC is the appropriate parameter for determining treatment efficacy, while ICC should be used for disinfection processes that disrupt the cell wall of the bacterial cells (Hassard et al. 2018). This is with the exception of UV processes, where the mechanism of inactivation prevents use of the current stains to readily distinguish viability (Berney et al. 2007; Farrell et al. 2018).

A measurement error of approximately 5 % on the percentage of HNA cells and the nucleic acid content has been reported (Prest et al. 2014) although the error term in HNA/LNA ratios can be significantly higher 15-30% (Park et al. 2018) and should be propagated to account for error in both terms when reported. HNA/LNA ratios are being used by some water companies to assess changes in the character of water. Caution is recommended in using simple ratios to represent complex phenomena as: i) nucleic acid content does not determine whether a bacteria represents a health risk or vice-versa and ii) bacteria
can alter their nucleic acid content depending on growth phase. Insights provided by novel image data analysis (e.g. CHIC analysis – within Chan et al. 2018) could help to provide practical value from nucleic acid content data.

**Limitations of FCM**

In this section, FCM methods used for drinking water were assessed. This included their accuracy compared to established techniques for measuring drinking water bacteria as well as the limitations of FCM technologies. Conventional measures such as HPC results have coefficient of variation (CV – ratio of standard deviation to the mean) values of 7-50% for analysis of drinking water compared with FCM results with a CV value typically < 5% (Hassard et al. 2018; Whitton et al. 2018). This highlights the improved reproducibility of the FCM method over HPC (Van Nevel 2017b). From a statistical perspective, the HPC method detects between 25 and 250 CFU per plate, while FCM analysis detects between 100 and 40,000 events per sample and can be obtained without dilution of the sample (Table 2). For water which contains background material or has a cell number >40,000 events, dilution is required. HPC takes 3–7 days to obtain results, while FCM requires approximately 20 minutes per sample, averaged over a large number of samples (Helmi et al. 2014). The time required is significantly less per sample compared to most routinely applied methods for general bacterial analysis (Table 2).

The sensitivity of FCM is variable and should not be based on absolute values being analysed, but proportional to the initial cell concentration of the analysed water (Prest et al. 2013). Therefore absolute numbers are less important than relative change through a WTW for example. A change in cell concentration of 3% for most commercial flow cytometers has been reported, which results in a sensitivity of 97% (Table 2). Theoretical limits of blank (LOB) values have been calculated based on equation (1) (Forootan et al. 2017) working at the 95% confidence interval:

$$\text{LOB} = \text{mean of blank} + 1.645 \times \text{standard deviation of blank}$$

An LOB of around 116 cells ml\(^{-1}\) for ICC and TCC was calculated when 1000 µl was analysed (typically 50 or 100 µl used by water companies). In practise it has been found that the observed LOB values are greater for TCC as opposed to the value for ICC, possibly due to the increased specificity of this assay. The LOB of FCM will likely improve as instruments and fluorophores improve. In practise background particles within the cytometer and reagents contribute to this result for blank samples. Few water companies were reporting LOD at the time of this study (Table 3).

It is considered that the LOB for FCM is better than HPC with a theoretical LOB of 1-10 CFU ml\(^{-1}\) when 100 ml is filtered. If lower volumes are filtered (e.g. 1 ml) then this LOB will increase to 100-1000 CFU ml\(^{-1}\). Also, the low culturable recovery of most drinking water bacteria (Gensberger et al. 2015) means the actual LOB for HPC is several orders of magnitude higher than FCM when environmental bacteria are tested.
Samples from different sources or sample times can demonstrate different fluorescence fingerprints. This has allowed characterization and differentiation of samples based on these fingerprints (Chan et al. 2018). More information on this is provided in section ‘Actively growing bacterial populations’.

Another approach for quantifying both general and specific bacterial strains is quantitative polymerase chain reaction (qPCR). It is considered that qPCR can be more sensitive than FCM (theoretical LOB = 1.64 gene copies per reaction; limit of detection (LOD) = 3 gene copies per reaction). However the impact of inhibitors notably humic acids result in differential inhibition between samples, a phenomena not seen in FCM data (Farkas et al. 2017; Brandt and Albertsen 2018) and ultimately poor quantification despite greater relative sensitivity under idealised conditions. Other approaches such as enzyme linked growth quantification has shown promise (Bramburger et al. 2015), although concerns exist with respect to sensitivity and long incubation times which often restrict utility in final drinking waters. Automated visual quantification methods such as BACMON have shown promise for online, automated analysis. However most methods which rely on visual analysis only (no staining) do not have the ability to distinguish viable bacteria from non-viable bacteria, something of importance for most bacterial water quality monitoring.

With respect to FCM, the sensitivity and LOD are likely to improve; for example faster flow rates will allow more sample volume to be filtered in less time. Flow cytometers which allow faster data capture (event acquisition) and better detection of molecules of fluorophore over background are also coming to market. A limitation to this incremental improvement will likely be problems with discerning signal from noise due to background sample matrix effects creating more noise when trying to resolve the cell count signal. Higher power lasers should help to overcome some of this but a limit is the quenching/decomposition of current dyes. Better dyes which have improved fluorescent quantum yield could help ameliorate this. Therefore, dyes which suffer reduced decomposition under higher excitation dose could be useful to move towards single cell analysis (Yang et al. 2009). Ultimately, once lower limit of quantification (LOQ) extends to single or double digits per assay, (e.g. 1’s, 10’s) random fluctuations in the number of events in the observation volume will contribute a major source of the error in measurement (Shapiro 2005).
Table 2 – Limitations of different methods used for bacterial analysis of drinking waters.

<table>
<thead>
<tr>
<th>Method</th>
<th>Acceptance criteria</th>
<th>Precision (R²) §</th>
<th>Speed †</th>
<th>Theoretical Limit of blank (volume measured) ¶</th>
<th>LOD (lower-upper) ‡</th>
<th>LOQ (lower-upper) ‡</th>
<th>Specificity‡</th>
<th>kₐ</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPC</td>
<td>7-50 %</td>
<td>50-96 %</td>
<td>Days</td>
<td>N/A</td>
<td>1 CFU / 100 ml</td>
<td>N/A</td>
<td>Yes</td>
<td>78-99.5 % ↓</td>
<td>(Edberg and Edberg 1988; Rompré et al. 2002; Allen et al. 2004; Bramburger et al. 2015; Hassard et al. 2017)</td>
</tr>
<tr>
<td>Enzyme linked measurement</td>
<td>5-20 %</td>
<td>72-99 %</td>
<td>Hours</td>
<td>1 CFU / 100 ml (100 ml)</td>
<td>20 CFU / 100 ml</td>
<td>N/A</td>
<td>No</td>
<td>-</td>
<td>(Bramburger et al. 2015)</td>
</tr>
<tr>
<td>Imaging (fluorescent) ***</td>
<td>6-18 %</td>
<td>91-99 %</td>
<td>Hours</td>
<td>N/A</td>
<td>N/A</td>
<td>520</td>
<td>No</td>
<td>60-72 %</td>
<td>(Anguish and Ghiors 1997; Bouvier and Del Giorgio 2003; Coskuner et al. 2005; Lopez et al. 2005; Vital et al. 2007; Waters 2009)</td>
</tr>
<tr>
<td>qPCR ◊ ***</td>
<td>0.1-35 %</td>
<td>&gt; 99 %</td>
<td>Day</td>
<td>&lt; 1.64 gene copies per reaction (20 µl)</td>
<td>3 gene copies per reaction</td>
<td>No</td>
<td>67-99.9 %</td>
<td>(Fontaine and Guillot 2003; Mushar et al. 2004; Farkas et al. 2017; Forootan et al. 2017; Brandt and Albertsen 2018)</td>
<td></td>
</tr>
<tr>
<td>FCM ***</td>
<td>&lt; 5 %</td>
<td>99 %</td>
<td>Minutes</td>
<td>116.4 cells ml⁻¹ (1000 µl)</td>
<td>200-cells ml⁻¹</td>
<td>100 – 40,000** cells ml⁻¹</td>
<td>Yes</td>
<td>97 %</td>
<td>285 MESF</td>
</tr>
</tbody>
</table>

* Coefficient of variation is the ratio of the standard deviation to the mean typically between 3 or more values. † = speed is the estimated time for analysis of one sample, ‡ = specificity is the ability of the method to relate a measure uniquely to a particular subject. § = definition of method precision adapted from Hammes et al. (2008). ¶ = if known, ◊ = dependent on DNA extraction method used see (Brandt and Albertsen 2018), ‡ = if different from LOD, ↓ = based on chromogenic agars, ** = without dilution assumes 50 µl volume, *** = detects non-culturable bacteria, LOD = Limits of detection, LOQ = Limits of quantification, kₐ = method sensitivity, which is a measure of the ability of a method to establish significant differences in concentration. MESF = Molecules of equivalent soluble fluorochrome, N/A = Not Available.
Table 3 – limits of detection from water companies carrying out FCM analysis in England and Wales

<table>
<thead>
<tr>
<th>Company</th>
<th># WTW</th>
<th>Instrument(s) Manufacturer; (Model)</th>
<th>Reported lower LOD</th>
<th>Method for gate selection</th>
<th>Blank used - Yes (details) / No</th>
<th>Calibration of instrument Yes (details) / No</th>
<th>Validation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Company A</td>
<td>146</td>
<td>BD Accuri (C6)</td>
<td>-</td>
<td>User selected</td>
<td>No</td>
<td>Yes - Fluorescent beads</td>
<td>No</td>
</tr>
<tr>
<td>Company B</td>
<td>17</td>
<td>N/A&lt;sup&gt;a&lt;/sup&gt;</td>
<td>N/A&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Software assigned</td>
<td>Yes&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Yes - Fluorescent beads</td>
<td>-</td>
</tr>
<tr>
<td>Company C</td>
<td>68</td>
<td>Thermofisher (Attune Nxt)</td>
<td>-</td>
<td>-</td>
<td>Yes filtered (0.2 μm) Evian water</td>
<td>Yes - Fluorescent beads</td>
<td>No</td>
</tr>
<tr>
<td>Company D</td>
<td>57</td>
<td>Thermofisher (Attune Nxt)</td>
<td>-</td>
<td>User selected</td>
<td>Yes filtered (0.1 μm) Highland Spring Water&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Yes - Fluorescent beads</td>
<td>Yes - pure culture</td>
</tr>
<tr>
<td>Company E</td>
<td>16</td>
<td>BD Accuri (C6), Thermofisher (Attune Nxt), Sigrist (BactoSense)</td>
<td>200 cells per ml</td>
<td>User selected</td>
<td>Yes&lt;sup&gt;2&lt;/sup&gt;</td>
<td>Yes - Fluorescent beads</td>
<td>No</td>
</tr>
<tr>
<td>Company F</td>
<td>36</td>
<td>Thermofisher (Attune Nxt)</td>
<td>-</td>
<td>User selected</td>
<td>Yes&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Yes - Fluorescent beads</td>
<td>Yes - NEQAS</td>
</tr>
<tr>
<td>Company G</td>
<td>-</td>
<td>BD Accuri (C6)</td>
<td>200 cells per ml</td>
<td>User selected</td>
<td>Yes filtered (0.2 μm) Evian water</td>
<td>Yes - Fluorescent beads</td>
<td>Yes bottled water</td>
</tr>
<tr>
<td>Company H</td>
<td>84</td>
<td>BD Accuri (C6), BD (FACSVia)</td>
<td>-</td>
<td>User selected</td>
<td>Yes MilliQ water&lt;sup&gt;c&lt;/sup&gt;</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Company I</td>
<td>53</td>
<td>N/A&lt;sup&gt;a&lt;/sup&gt;</td>
<td>N/A&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Software assigned</td>
<td>No</td>
<td>Yes - Fluorescent beads</td>
<td>-</td>
</tr>
</tbody>
</table>

<sup>a</sup> – analysis undertaken by contract laboratory details not provided, <sup>b</sup> – details not provided, <sup>c</sup> – blank lower limit of detection of 200 cells per ml. One company reported 2 cells per ml.

* - 40,000 – 1,000,000 cells per ml upper limit reported by respondents, † - maximum sample storage of 24 hours practised by most (Water Company D allow up to 48 hours for final waters).

‡ - any standardised, filtered (<0.22 μm) isotonic solution (including bottled water) is appropriate for dilutions. Deionised water is not appropriate as it damages cells.

§ - incubation time averaged 14.1 minutes with most respondents incubating for 15 minutes. ‖ - incubation temperatures ranged from 30-37°C most respondents incubating at 35°C

LOD = limit of detection (-) not reported.
Use of FCM for regulatory compliance monitoring

Describe approaches for correlating FCM data to regulatory compliance information, including pass/fail criterion, insight into different methods of disinfection, PCVs (threshold or regulatory standards), speed and cost comparison.

In this section, the question is addressed as to whether absolute numbers FCM are of importance for assessing compliance? This is due to the established paradigm that TCC and ICC bacteria do not necessarily lead to higher numbers of compliance indicator organisms (Van Nevel et al. 2017b). Due to a number of limitations, no legislative standard exists for FCM data in waters used for human consumption. It is also not possible to establish single standards for all sites due to the unique composition of different waters which may have identical bacteriological compliance rates. However other industries have made progress standardising molecular biology testing such as qPCR detection of virus in shellfish (Anon., 2013, 2017).

Threshold/guideline values were rarely applied for HPC data (Sartory 2004; Gensberger et al. 2015), therefore it is logical to suggest that FCM data will be the same in drinking water. The question of ‘how many cells are needed for safe and unsafe water?’ remains elusive. It is considered that individualised, utility-specific and site specific limits to support decision-making could be established after detailed monitoring of the particular systems. Implementation of FCM by water companies would therefore require several years of parallel FCM and HPC measurements (HPC 22 °C and E. coli / coliform analysis), thereby building-up a solid database and gaining confidence in the FCM data from their own system. This could enable water companies which utilise FCM to detect abnormal change (considering seasonality in drinking water sources) to appropriately monitor the quality of final water through FCM testing. Some water companies have made progress on implementing a bacterial control standard based on FCM for both WTW and service reservoirs (Table 4). It is beyond the scope of this review to ascertain what scientific basis there are for these standards. However, ensuring that standards or thresholds are appropriate for different waters and assets is of upmost importance. In the next section it is hypothesised as to what abnormal change in FCM measurements might look like.

What might abnormal change look like in FCM data?

Abnormal change values (ACV) (likely log or percentage not absolute number) serve as triggers to review previous data and make an assessment of any significance of the increase (Sartory 2004). Factors such as the difference between the types of source would need to be considered for example groundwater supplies would have lower ACV than lowland river source supplies due to the naturally lower populations of bacteria. To readily assess a suitable ACV, natural variability and data driven insight should take precedence, although local operator knowledge of water quality change should be reviewed and
considered. In practice, FCM counts in treated water in UK public water supplies vary across 6 orders of magnitude. Any assigned PCV or ACV would need to be site specific and represent a marked rise (or decline) in bacterial populations which indicates significant change. Several suppliers, however, have established arbitrary levels of increase ranging from 0.5 to >2 log increases over previous results (Table 4).

This has the advantage that it automatically takes into account the variability in bacterial populations that occur during the seasons. Statistical approaches which utilise confidence intervals are recommended as opposed to mean values, which have little practical value for data of this scale. As for HPC data, it is recommended that FCM data derived for groundwater sourced supplies can be calculated from annual datasets (providing not a shallow aquifer) as the counts tend to be more stable in water which has lower productivity. In contrast FCM data applied to more nutrient-rich surface water derived supplies multiple ACV’s might be required (e.g. summer and winter) to reflect the greater natural change expected at these sites. This is particularly relevant for sites which blend different water sources in different proportions throughout the year.

From an operational perspective, FCM counts are most useful for assessing trends in changes of water quality and some distribution management issues (e.g. biofilm development, stagnation and reduced chlorine levels). General heterotrophic bacteria populations are not considered to be of significant health concern (Drinking Water Inspectorate, 1998, Standing Committee of Analysts, 2002). Currently the hygiene risk of drinking water samples based on FCM data is not known. When considering decision making from FCM, a precautionary principle should be utilised. Risk assessment (e.g. quantitative microbial risk assessment) is challenging on general bacterial FCM data (ICC and TCC). Although risk management through water safety plans appears on the horizon using these datasets. Quantitative microbial risk assessment on specific pathogens quantified using flow cytometry can be practised, attention should be given to sensitivity and specificity of the method (Taguri et al. 2011).

Table 4 - Definitions of ‘abnormal change’ and operational limits PCV values for plate counts and equivalent values for FCM used by UK water companies

<table>
<thead>
<tr>
<th>Water Company</th>
<th>PCV method</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Company H</td>
<td>if &gt; 5% of the total cells are ‘intact’ cells in final water post WTW, &gt; 50,000 ICC per ml in service reservoirs</td>
<td>PCVs are flagged for inspection, with relevant Operational scientists, Process Controllers and Network analysts receiving auto email notification</td>
</tr>
<tr>
<td>Company E</td>
<td>have defined operational limits on a site specific basis*</td>
<td></td>
</tr>
<tr>
<td>Company D</td>
<td>have developed a risk score involving calculation containing ICC, HNA and LNA*</td>
<td></td>
</tr>
</tbody>
</table>

#(adapted from Sartory 2004); *No further information provided
Specific pathogen analysis

Can FCM identify specific reference pathogens or compliance indicators including Campylobacter spp., Enterovirus, Cryptosporidium spp., Legionella pneumophila and E. coli or virus?

There are a number of methods that can be used to detect pathogens and indicator organisms in drinking water including DNA microarray, qPCR, enzyme-linked immunosorbent assay, magnetic nanoparticle based immunoassay and lab-on-chip style biosensors amongst others (Yang et al. 2009). Most do not have the sensitivity, are not absolute or can be inhibited by sample matrix effects. Detection of reference pathogens and indicator organisms is possible using FCM and has recently gained increased attention. This is principally through cell sorting from the background material. This involves sorting based on a defining characteristic (intracellular, size, shape, fluorescence, specific binding to dye etc). Sorting by FCM is known as fluorescence activated cell sorting. This method is most appropriate when targeting non-specific intracellular characteristics or bulk properties such as shape, size or fluorescence (Reynolds et al. 1999; Wang et al. 2009). However, the method which has received most interest for specific bacteria analysis involves sorting with magnetic beads known as immune-magnetic separation (IMS) or magnetic assisted cell sorting (MACS). This method has been used by the water industry for Cryptosporidium analysis for decades, usually coupled with laborious, costly and time consuming microscopy (Vesey et al. 1994; Reynolds et al. 1999). Therefore, this leads to the assumption that if FCM is more accurate than conventional microscopy (Safford and Bischel 2019) and less biased than culture based methods (Van Nevel et al. 2017b), why not use it for specific pathogen analysis (e.g. Cryptosporidium spp. etc)?

There have been several reports which suggest that FCM is more appropriate than current international standards techniques for quantification of Legionella spp., Cryptosporidium spp., Campylobacter spp., Vibrio spp. (Vesey et al. 1994; Anguish and Ghiorse 1997; Reynolds et al. 1999; Vital et al. 2007; Keserue et al. 2012) as the readily accepted limits of culture and microscopy do not apply to FCM (Table 2, Table 3). It is well established that the IMS-FCM method is limited by the specificity of antibodies. Usually specific staining is achieved using an antibody conjugate with magnetic nanoparticle and a fluorophore. Detection limits of 2000 cells mL⁻¹ have been reported recently (Van Nevel et al. 2013), which suggests IMS-FCM has improved from historic LODs of 10⁴ cells L⁻¹ (Tyndall et al. 1985).

Most antibodies tend to be too specific for targeting bacteria at serovar level (sub-species differences in bacteria) for indicator organisms. Recent advances in polyclonal antibody techniques enable targeting of antigenic serotypes of E. coli O/K (Thermo Fisher Scientific, catalog # PA1-7213, RRID AB_558822), however this is considered more selective than conventional culture based selective quantification of E. coli. Further non-specific reaction with other related enterobacteriaceae (Family of gram negative bacteria) limit suitability of these systems for hygiene determination.
Further, a recent study showed specific quantification of *Legionella pneumophila* serogroup 1–12 using IMS-FCM. However, conventional plate counts often exceed those seen through IMS-FCM (Keserue et al. 2012), as plate counts are more inclusive of clinically relevant serogroups. This is the opposite when comparing plate counts to other molecular methods such as qPCR which tend to have greater flexibility for probe design (Hassard et al. 2017). Ultimately, rapid and reproducible staining procedures coupled with advanced fluidics permit quantification of target pathogens in minutes as opposed to hours or days (Table 3). Therefore it is the view of the authors that it is a matter of when, not if, FCM-like technologies are used to count hygiene relevant pathogens and indicator organisms.

**Future development and research: combining FCM with other approaches to answer novel questions.**

The recent development of imaging FCM will facilitate continued optimization of FCM applications. The ImageStream system (Amnis, https://www.accela.eu/discover/identification-and-measurement-of-bacterial-size-using-the-imagestream) provides a valuable means of measuring bacteria concentration, species and growth stage. Using high-resolution multispectral cell image analysis, the ImageStream can identify and count cell subpopulations based on complex morphological features (Phanse et al. 2012). It has been suggested that future FCMs may incorporate a number of technologies (Table 3) such as imaging, sorting, Matrix Assisted Laser Desorption/Ionization (protein fingerprints) or sequencing technologies (Wang et al. 2010; El-Chakhtaura et al. 2015). These approaches create opportunity to extract more information about the microbiological character of waters used for human consumption, but compound the issues with respect to data analysis and are expensive (Table 5).

Microfluidics-FCM could change the way we count bacteria. Microfluidic sample handling systems operating in micro-fabricated structures provides higher speeds, smaller sizes and lower costs. An example has used co-staining with SYTO 9 (membrane-permeable, similar to SYBR Green I) and PI. Differentiation of live and dead bacterial cells was demonstrated on a microfluidic chamber when a colour CCD camera was used for detection (Inatomi et al. 2006; Jiang et al. 2015). These devices could one day be disposable or truly handheld devices further improving the appeal of FCM based technologies. Compared to other technologies the relatively low per sample cost (despite relatively high capital expenditure - CAPEX) and low labour costs are the main advantages of FCM compared to other technologies for bacterial detection (Table 5).
Table 5 - Methods used for general bacterial quantification / bacterial identification in drinking water (Adapted from Van Nevel et al. 2017b).

<table>
<thead>
<tr>
<th>Method</th>
<th>CAPEX(£)</th>
<th>Measures</th>
<th>Principle of test</th>
<th>Viability</th>
<th>Labour</th>
<th>Time</th>
<th>Use with FCM?</th>
<th>Commercial examples</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>General bacterial quantification</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HPC*</td>
<td>£</td>
<td>Cultivable bacteria</td>
<td>Growth</td>
<td>Yes</td>
<td>Medium</td>
<td>Days-weeks</td>
<td>No</td>
<td>-</td>
<td>Reasoner 1990; Van Nevel 2017a,b</td>
</tr>
<tr>
<td>FCM‡§</td>
<td>££££</td>
<td>Cell concentration</td>
<td>Staining</td>
<td>Yes</td>
<td>Low</td>
<td>Minutes</td>
<td>N/A</td>
<td>BD Accuri (C6) Oncyte (OC-300) Sigrist (Bactosense)</td>
<td>Prest et al. 2013</td>
</tr>
<tr>
<td>Imaging§</td>
<td>£</td>
<td>Cell concentration</td>
<td>Staining</td>
<td>Yes</td>
<td>High</td>
<td>Minutes</td>
<td>No</td>
<td>-</td>
<td>Burtscher et al. 2009</td>
</tr>
<tr>
<td>ATP‡</td>
<td>£</td>
<td>ATP concentration</td>
<td>Enzymatic</td>
<td>Yes</td>
<td>Low</td>
<td>Minutes</td>
<td>Yes</td>
<td>Luminultra (Quench-Gone Aqueous (QGA™)</td>
<td>Nescerecka et al. 2016</td>
</tr>
<tr>
<td>qPCR†§</td>
<td>££££</td>
<td>16S rRNA gene copies</td>
<td>Gene amplification</td>
<td>Yes</td>
<td>High</td>
<td>Hours-days</td>
<td>Yes</td>
<td>-</td>
<td>Lopez-Roldan et al. 2013</td>
</tr>
<tr>
<td>Nucleic acid quantification</td>
<td>£</td>
<td>Total DNA/RNA, electron transfer</td>
<td>Fluorescence/Absorbance</td>
<td>Yes</td>
<td>Medium</td>
<td>Hours-days</td>
<td>No</td>
<td>ThermoFisher (MagMAX) or FCM fingerprint</td>
<td>(McCoy and Olson 1985; Farabegoli et al. 2003)</td>
</tr>
<tr>
<td><strong>Specific bacterial quantification</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Selective Plate count*</td>
<td>£</td>
<td>Cultivable bacteria</td>
<td>Growth, enzymatic</td>
<td>Yes</td>
<td>Medium</td>
<td>Days-weeks</td>
<td>No</td>
<td>Lab M (Harlequin E. coli / coliform)</td>
<td>(Hassard et al. 2017)</td>
</tr>
<tr>
<td>Enzyme linked measurement‡</td>
<td>£££</td>
<td>Cultivable bacteria enzyme kinetic</td>
<td>Growth, enzymatic</td>
<td>Yes</td>
<td>Low</td>
<td>Minutes</td>
<td>No</td>
<td>Idexx (Coliert), PDS (Tecta B16), microLAN (BACTcontrol),</td>
<td>(Pickett 2018)</td>
</tr>
<tr>
<td>Antibody staining</td>
<td>£££</td>
<td>Specific bacteria based on surface antigen properties</td>
<td>Binding / Fluorescence</td>
<td>No</td>
<td>High</td>
<td>Hours-days</td>
<td>Yes</td>
<td>Rqmicro (Cellstream)</td>
<td>(Keserue et al. 2012)</td>
</tr>
<tr>
<td>Technique</td>
<td>Cost</td>
<td>Description</td>
<td>Duration</td>
<td>Cost</td>
<td>Device/Method</td>
<td>Reference</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-----------------------------------------------</td>
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<td>-----------------------------------------------------------------------------</td>
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<td>------</td>
<td>------------------------------------------------------------------------------</td>
<td>-------------------------------------</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Immunomagnetic separation/quantification §</td>
<td>££££</td>
<td>Identify and compare bacteria present based on a single gene relative abundance</td>
<td>No</td>
<td>High</td>
<td>Hours-days Yes</td>
<td>Pacbio (SMRT)</td>
<td>(Wagner et al. 2016)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>16S rRNA Sequencing†</td>
<td>£££££</td>
<td>Next generation Amplicon sequencing</td>
<td>No</td>
<td>High</td>
<td>Days-weeks Yes</td>
<td>Illumina (MiSeq Series sequencing)</td>
<td>(Perrin et al. 2019)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whole Genome sequencing†</td>
<td>£££££</td>
<td>Next generation based microbial genome sequencing</td>
<td>No</td>
<td>High</td>
<td>Days-weeks Yes</td>
<td>Biorad (S3 Cell sorter)</td>
<td>(Wang et al. 2010)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fluorescence activated cell sorting§</td>
<td>££££ £</td>
<td>Cell concentration</td>
<td>Yes</td>
<td>Medium</td>
<td>Minutes N/A</td>
<td>Biosearch (LavaLAMP)</td>
<td>(Hassard et al. 2016b)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LAMP†, ‡</td>
<td>£££££</td>
<td>Very specific amplification of DNA</td>
<td>Yes</td>
<td>Low</td>
<td>Hours Yes</td>
<td>Biosearch (LavaLAMP)</td>
<td>(Hassard et al. 2016b)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>qPCR / RT-qPCR†</td>
<td>£££££</td>
<td>Gene copies of DNA or RNA target</td>
<td>Yes</td>
<td>High</td>
<td>Hours-days Yes</td>
<td>Primerdesign (Genesig)</td>
<td>(Farkas et al. 2017)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>pre-RNA analysis†§</td>
<td>£££££</td>
<td>Gene copies of rRNA precursors</td>
<td>Yes</td>
<td>High</td>
<td>Hours-days Yes</td>
<td>-</td>
<td>(Cangelosi et al. 2010)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MALDI - TOFMS §</td>
<td>££££££</td>
<td>Protein fingerprint</td>
<td>Yes</td>
<td>High</td>
<td>Hours-days Yes</td>
<td>Sciex (TOF/TOF™ 5800 System)</td>
<td>(Singhal et al. 2015)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mass cytometry §</td>
<td>££££££</td>
<td>Antibody Isotope labelling of cells</td>
<td>No</td>
<td>High</td>
<td>Hours-days N/A</td>
<td>Fluidigm (CyTOF)</td>
<td>(Singhal et al. 2015; Guo et al. 2017)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Imaging flow cytometry §</td>
<td>£££££</td>
<td>Combine FCM with imaging</td>
<td>Yes</td>
<td>Low</td>
<td>Hours N/A</td>
<td>Amnis (ImageStream XMark II)</td>
<td>(Wang et al. 2010)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Digital PCR†§</td>
<td>££££</td>
<td>Gene copies of DNA or RNA target</td>
<td>Yes</td>
<td>High</td>
<td>Hours-days Yes</td>
<td>Biorad (QX200 Digital Droplet PCR)</td>
<td>(Monteiro and Santos 2017)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* - culture dependent, a = the labour is the estimated effort for analysis of one sample, † - polymerase chain reaction (PCR) dependent, ‡ - Online / Inline compatible, § - absolute quantification, ¶ = not exhaustive list, MALDI = Matrix Assisted Laser Desorption/Ionization, TOFMS = Time of flight mass spectrometry, qPCR = quantitative polymerase chain reaction, RT-qPCR = reverse transcription qPCR, LAMP = Loop mediated isothermal amplification. CAPEX cost estimate in GBP (£) = £ (<100), ££ (101-1,000), £££ (1,001-10,000), ££££ (10,001-100,000), £££££ (100,001-1,000,000), ££££££ (>1,000,000)
Data analysis and interpretation

Recent advances in FCM offer new and exciting ways of understanding water treatment and supply assets. However, these technological advances introduce considerable challenges with respect to data interpretation. It is largely considered that the methods for acquisition are optimised for current equipment and methodology (Table 3). Serious misinterpretation or incorrect processing of FCM datasets is possible, particularly considering the ‘user-defined’ nature of assigning gates. The next challenge of FCM implementation is using and interpreting this data which is of a different order to traditional microbial datasets. Therefore, the goal of this section was to provide some universal guidance with respect to data acquisition and interpretation.

FCM data is commonly presented as one dimensional or two dimensional displays with logarithmic axis which extend from 1-6 fold decade range. These axis and data within represent values which can differ 10,000 – 100,000 fold between the upper and lower ends of the scale. Basic guidelines for FCM datasets is provided within Box I. It is recommended that provision of an international standard method is a long term aspiration for the continued use of FCM for waters used for human consumption.

<table>
<thead>
<tr>
<th>Box I - First look at guidelines for minimum FCM data and metadata for presentation and analysis in Drinking Waters used for human consumption</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Instrumentation</strong>: identify instrument, make, model, serial number, software (including version). Identify the template file used and if standard methods for FCM methods have been followed (e.g. incubation, staining etc.).</td>
</tr>
<tr>
<td><strong>Graphics</strong>: identify the parameters used to generate graphic, where possible consistency across displays is recommended. A scale axis is an essential requirement for contour or coloured plots.</td>
</tr>
<tr>
<td><strong>Gating</strong>: display the gate used on graphic. Display of different gates should be shown sequentially when subjective manual gating is used. If automated gating through an algorithm is used, this should be clearly defined and what sensitivity analysis has been undertaken to show the algorithm is fit for purpose. It is suggested that predetermined gating is not appropriate for FCM analysis and that each samples requires analysis to demonstrate ‘suitability’ (Afshari et al. 2016).</td>
</tr>
<tr>
<td><strong>Frequency of measurements</strong>: percentages of cells in gates should be shown. Compute the TCC, ICC, HNA, LNA and % HNA. Compute these values relative to the total number of cells presented on the graphic on which the values appear (Herzenberg et al. 2006).</td>
</tr>
<tr>
<td><strong>Measurements</strong>: define the statistic measurement applied (mean, 50th percentile etc) and quality control parameters (e.g. CV values). The number replicates, calibrations, or control experiments should be provided within the metadata (Lee et al. 2008).</td>
</tr>
<tr>
<td><strong>Data processing</strong>: it is the responsibility of the provider of the data to ensure that a minimum amount of data is provided to ensure relevance. It is suggested, that methods for standardisation of data acquisition (including laboratory analysis) and the</td>
</tr>
</tbody>
</table>
data interpretation is undertaken (Hassard et al. 2018).

**Competency of FCM laboratories to count bacteria in drinking water.**

External quality control schemes will be vital for comparison between different instruments, labs and water companies e.g. (The Laboratory Environmental Analysis Proficiency Scheme – LEAP and Public Health England’s proficiency testing for food and water microbiology). It is recommended that guidance within ISO 17994:2014 and ISO 13843:2017 be considered and adapted for FCM. National External Quality Assessment Service (UK) (NEQAS) are an example of an organisation which can provide proficiency training of staff using FCM. This is critical as errors in instrument operation can influence the quality of data from FCM. It is recommended that the requirements of ISO 15189:2012 are adapted with respect to training FCM analysts. A two tiered approach could be adopted to those trained to collect FCM data and those competent analysing FCM data. Assessors (to measure and record competency) would need to be verified themselves of fitness to assess, this could be every two years. Web based programmes to assess whether instrument and individual are fit for analysis show particular promise e.g. VERIQAS™ show particular promise. A similar scheme is recommended for FCM analysis of drinking waters (Barnett et al. 2012).

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**Current status of FCM within the water industry (objective 2)**

Two countries which have heavily invested in the use of FCM for monitoring of drinking waters are the United Kingdom and Switzerland. The United Kingdom is of particular interest, as its governing and regulatory environment has given rise to companies implementing FCM at different rates or not at all. This is due to independent decision making between the different water company regions with respect to investment, and different levels of investment linked to priorities within each water company. This strata of variation in approach and level incorporation FCM (alongside other microbiological monitoring tools) presents a unique study opportunity, issues that are explored further in subsequent sections.

An online questionnaire using Qualtrics software (Appendix A) was distributed to the water companies through the DWI day-to-day contacts with the aim of ascertaining the current status of FCM within the water industry. The questionnaire received a 95% response rate with participants representing both users and non-users of FCM. Participants represented water companies across England and Wales, operating between 16 to 146 WTW.

Eight responses were received from companies currently using FCM. Nine responses were received from companies which were not using FCM. These companies represent a range of experiences including: earlier adopters through to recent adopters of FCM; and two water companies who utilise an external analytical service partner. One water company which identified as a ‘non-user’ also reported that they were in the process of implementation of FCM. The majority of respondents (37.5%) reported using FCM on < 10 WTW (Table 7), with those participants identified as early adopters found to use FCM on a larger number of WTWs (i.e. > 20), presumably due to their experience in using the method/technology and subsequent inclusion of more sites.
Table 6 How many water treatment works is FCM used on?

<table>
<thead>
<tr>
<th>No. of sites</th>
<th>&lt; 10</th>
<th>10 – 19</th>
<th>20 – 50</th>
<th>51 - 100</th>
</tr>
</thead>
<tbody>
<tr>
<td>% of respondents</td>
<td>37.5</td>
<td>25</td>
<td>25</td>
<td>12.5</td>
</tr>
</tbody>
</table>

Respondents reported owning and/or renting between 1 – 3 FCM units per company, with the dominant brands and manufacturers identified as the BD Accuri C6\(^1\) and Thermo Scientific Attune NxT\(^2\) followed by the Sigrist BactoSense \(^3\) (automatic) and BD Facsvia\(^4\). The majority of respondents (75 %) reported that they had not adapted or modified their primary FCM instrument for example making the instrument online or inline. The other 25% reported that they had made minor modifications to their FCM instruments. It was not evident from the responses what modifications were made.

Respondents were further asked to rate the service provided by the identified manufacturers, through their agreement to a series of statements regarding maintenance, servicing etc. The majority of participants (> 50 %) strongly agreed the level of service provided in supporting maintenance and repairs to be satisfactory (Figure 1), with participants reporting either < 48h (38 %) or – 1 week (38 %) turnaround time to repair a malfunctioning unit. A reduction in satisfaction was found for general servicing and customer service with the lowest satisfaction found for method development with 37.5 % of respondents satisfied with the support available. However, it should be noted that the manufacturers identified within the survey are classified as a technology provider, and not do not specialise in method development.

![Figure 1 Level of satisfaction with the service provided by FCM manufacturers](image)

Responses received from the non-FCM users confirmed 56% of these participants intended to undertake FCM for monitoring drinking water in the future. A number of these respondents detailed their plans for

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1. [https://www.bdbiosciences.com/instruments/accuri/](https://www.bdbiosciences.com/instruments/accuri/)
4. [https://www.bdbiosciences.com/eu/instruments/clinical/cell-analyzers/bd-facsvia/m/3694812/overview](https://www.bdbiosciences.com/eu/instruments/clinical/cell-analyzers/bd-facsvia/m/3694812/overview)
adopting FCM, with some companies currently in the process of procuring a unit and/or exploring the option of a research project to assist in establishing and validating the method. The remaining 44% of non-users who did not anticipate the adoption of FCM were received from smaller companies (i.e. total WTW < 19), who questioned ‘the cost of implementation compared to the size of the business’ and some uncertainty of the benefits the technology had to offer.

**Data collection**

Participants were asked to identify where FCM was used within a WTW and for what purpose using the following categories:

- **a)** Routine monitoring: *defined as sampling and analysis undertaken for routine purposes using FCM.*
- **b)** Operational/enhanced monitoring during normal conditions: *defined as elevated frequency sampling campaign and analysis using FCM for ‘normal’ operational or diagnostic purposes.*
- **c)** Root cause analysis during operational issues: *defined as a sampling campaign and analysis using FCM for root cause analysis or operational optimisation.*
- **d)** Investigations during a water quality event: *defined as sampling campaign and analysis using FCM to investigate during a water quality or potential compliance event.*

Responses received found FCM to be predominantly used for root cause analysis and investigations during a water quality event throughout the WTWs (Figure 2 c, d) with > 25% of participants identifying its use through every stage of the treatment process. A greater number of participants targeted specific assets within the WTW during root cause analysis and investigations. These assets which were focused on included the WTW inlet, post-rapid gravity filter (RGF), post-slow sand filter and pre and post-chlorine contact tank.

Routine monitoring and enhanced monitoring were found to be predominantly completed for final waters by 63% of the respondents (Figure 2 a, b) and this is in agreement with the areas of FCM analysis and application identified in the literature. The data therefore suggests that upon the identification of a water quality event through routine analysis of the final waters, FCM is subsequently utilised as an investigatory tool to identify which assets within the treatment process may contribute to the event. Analysis of the RGF and slow sand filter are targeted to determine potential breakthrough. This is interesting as it suggests water companies are showing interest in breakthrough of organisms that may lead to non-compliance. Further analysis of the chlorine contact tank informs on the efficacy of the disinfection process and the possibility of ingress. Interestingly, FCM use following UV treatment was also identified by 25% of respondents (Figure 2). The current method of cell preparation (cell staining) with SYBR Green I and PI prior and cell membrane integrity using FCM is not believed to be effective at evaluating the performance of UV treatment (Hassard et al. 2018; Safford and Bischel 2019), however 25% of respondents suggested they have successfully adapted the methodology to be able to successfully evaluate efficacy of the process. This requires further investigation for confirmation. The development of novel dyes could aid in
evaluating UV disinfection.
a) **Routine monitoring**

b) **Operational/enhanced monitoring during normal conditions**

c) **Root cause analysis during operational issues**

d) **Investigations during a water quality event**

Figure 2 Where FCM is used across the WTW and for what purpose
In addition to the WTWs, respondents confirmed FCM analysis extended to the distribution system. 50% of the FCM users are using the technology to monitor their service reservoirs, distribution networks and customer taps (Table 7). This demonstrates the perceived ability of the technology/methodology to monitor a wide range of assets. In most cases when FCM was used in the extended storage and distribution systems, service reservoirs were the asset which received the most focus. This is probably due to a combination of ease of sampling, importance for monitoring chlorine residuals, regrowth and ingress. FCM is also useful for monitoring and optimising assets where bacterial indicator organisms are expected to be absent (e.g. chlorine contact tanks and service reservoirs).

<table>
<thead>
<tr>
<th>Service reservoirs</th>
<th>Distribution networks</th>
<th>Customer taps</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>75</td>
<td>37.5</td>
<td>12.5</td>
<td>25</td>
</tr>
</tbody>
</table>

When questioned on whether FCM methodology was more suited to a certain water type, a general agreement was observed by the majority of the participants that the methodology was suitable for all water types with water quality events identifiable once a baseline trend has been established. It is thought that the baseline trend however, is required for each site/asset and cannot necessarily be generalised across sites. One response went further:

*FCM is not necessarily impacted by water type. However, it is important to have a background profile for different water systems. Beyond very general observations, it is not possible to assess the importance of single result with reference to the normal variation of that system. The reference does need to be done on site-by-site basis*

**Data interpretation**

Participants were asked to provide information on how the data collected through FCM was used within their companies. Responses confirmed a wide range of uses (Figure 3), with the data predominantly used for trend analysis (63 %), generation of internal targets (i.e. TCC) (50 %) and combining with other data sets to make decisions (50 %). Data sets identified included turbidity, pH, ammonia, chlorine, HPC, coliforms and *E. coli*. The responses received suggests each participant is using the data differently, with individuals displaying creativity/originality in how the data is used and interpreted in the absence of a standardised protocol. For example:

*We use FCM data to produce a water quality risk score (WQRS), this is an automatic calculation involving the number of intact cells and the median florescence. This acts as a very basic scatter analysis, as the diversity of the population increases the population profile broadens increasing the median florescence, increasing the WQRS. This is a more sensitive indicator than cell count alone as the WQRS change in response to cell type/condition as well as cell numbers. Using a raw water dilution series we establish values at which we would expect to see colonies begin to*
form on our HPCs. We also established a value at which we would expect to see enough colonies on the HPC to constitute a fail and a value at which we would expect to start seeing coliforms. This allows us to set universal limits at WTW as we'd expect 0 colonies in final waters. We also look for trends and atypical patterns of behaviour and as such the WQRS helps us to prioritise work.

Notably, 37.5% of the participants are not currently using the data generated by FCM (Figure 3), this is believed to be due to individuals still undergoing method validation and/or deciding how the generated data is used/incorporated with other data sets to assist with performance monitoring and decision-making. The importance of trend analysis (60% of respondents) highlights the perceived value for long term raw water quality assessments and asset management (Figure 3).

The implementation of FCM the water industry

Individuals were further questioned on their knowledge of the FCM methodology and their confidence in adapting the method and troubleshooting. The 'level of confidence' was rated on a scale of 0 – 5 with 0 = no confidence and 5 = complete confidence (Figure 4). Noticeably, no category rated highly, with the greatest level of confidence observed for ‘using a method developed by others’ (3.43 ± 0.93). This is presumably due to all participants adopting a version of the fixed single gate template and methodology as described by Gatza et al. (2013) with only minor modifications in relation to incubation temperature. Instrument failures and anomalies in results were mentioned by two water companies as concerns should
they be a future requirement for FCM.

A possible consequence of relying on the method devised by Gatza et al. (2013) has resulted in the lowest average level of confidence in ‘troubleshooting the method’ (1.71 ± 0.55) and the highest average level of confidence in ‘using a method developed by others’ (3.52 ± 0.61) (Figure 4). This suggests that the method is potentially being followed without detailed appreciation of the underlying FCM principles. It is suggested that recommendations of ISO 15189:2012 are implemented with respect to FCM instrument users, with multiple levels of competency and assessors to ensure quality of data generated (see Box 1). What was of interest was the relatively low level of confidence in ‘gating different populations of cells’ at 3.00 (± 0.60) and ‘threshold to remove background’ at 2.14 (± 0.37). Both of these aspects of the methodology are completed through use of standard templates (from Gatza et al. 2013), confirming the idea that most users are comfortable applying but not modifying standard methods.

When comparing the individual participants’ responses of early adopters in comparison to recent users, a higher level of confidence for method development, adapting templates and method troubleshooting is observed for early adopters. This presumably was from their experience of using the methodology. Interestingly, the more recent adopters reported an increased level of confidence for ‘thresholding to remove background’ and ‘troubleshooting the method’. However, one of the identified recent users has undertaken specific research into thresholds for background removal in addition to experiencing numerous difficulties with their FCM which has enhanced the overall confidence level of this particular group of users for these categories. However, most users who considered themselves more experienced reported a greater degree of confidence in using FCM.

‘[We have] created a new gate, changed PMTs and thresholds - optimising the operation’

**Perspectives of FCM: challenges and opportunities**

All respondents of the online questionnaire (i.e. non-users and users) were asked to rate their perspective/agreement with a series of statements regarding the use of FCM for monitoring drinking water quality (Figure 5 a, b). These responses provide a snap-shot of the current perspective of both ‘users’ and ‘non-users’ whilst also enabling a comparison between the two groups.
As expected, the non-user group demonstrated a more neutral perception (neither agree nor disagree) of the methodology across all of the posed statements (Figure 5 a). Overall, the non-user group appeared well informed of the FCM methodology and its limitations for example the inability to target specific pathogens.

a) FCM Non-Users

b) FCM Users

The FCM user group were found to have a more favourable view (Figure 5 b) with varying levels of agreement in terms of reliability (75%), ease of use/operation (87.5%), ability to measure active bacterial populations (100%), application in control charts/operational ranges (87.5%) and management of water treatment supply assets (87.5%).
Interestingly, both groups demonstrated a varied perspective on the use of FCM as a monitoring tool for compliance purposes with 44.4% and 37.5% in agreement for the ‘non-users’ and ‘users’ groups respectively. The challenges and opportunities associated with FCM for regulatory compliance monitoring was further explored with both user groups through an open question. The challenges and opportunities identified by the user groups were summarised within themes and ranked according to the number of responses received from both user groups (Table 8). The ‘non-users’ considered that method standardisation and data interpretation are key challenges for their uptake of FCM in the future (Figure 6).

**Table 8 Challenges and opportunities for regulatory compliance monitoring**

<table>
<thead>
<tr>
<th>Challenges</th>
<th>Opportunities</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Data collection and interpretation</td>
<td>1. Water quality deterioration (pre-empt failure/asset management)</td>
</tr>
<tr>
<td>2. Long-term data collection to establish a baseline and setting prescriptive limits</td>
<td>2. Future potential of indictor organism tags</td>
</tr>
<tr>
<td>4. Method not limited to indicator species</td>
<td>4. Possibility of online monitoring</td>
</tr>
<tr>
<td>5. Methodology (reproducibility and repeatability)</td>
<td>5. Advantageous troubleshooting tool</td>
</tr>
<tr>
<td>6. Regulatory support/understanding</td>
<td></td>
</tr>
<tr>
<td>7. Combining with complementary technologies</td>
<td></td>
</tr>
<tr>
<td>8. Practical use</td>
<td></td>
</tr>
</tbody>
</table>
Figure 6 Web diagram showing companies’ perspective of FCM for monitoring drinking water quality split by users and non-users. Quantitation of responses undertaken by a 3-person panel, independently. Scores represent the mean score allocated from the panel.
Many participants cited data collection (including long-term data collection to establish a baseline and set limits) as the most significant challenge for FCM implementation, further enhanced through the need to establish data sets for individual WTW and assets for example:

‘There are significant challenges with different baselines from each individual works’

‘We need a decent data set before limits can be set- and limits when set may need to include interpretation of fingerprints’

As such, a common theme throughout the responses suggested FCM would be more appropriate as a troubleshooting tool for root cause analysis rather than for compliance monitoring with 50% of responses from users and 40% of responses from non-users believing there were opportunities for FCM in pre-empting failures and supporting asset management through a proactive monitoring programme.

‘Apart from in gross contamination cases, we have been unable to identify a direct link between raised FCM results and any evidence of impact on health. A single high result therefore does not necessarily imply a contamination and would not be a helpful compliance tool’

‘I see FCM doing what aerobic colony count (ACC) analysis should do, i.e. indicate a deterioration in water quality and pre-empt a statutory failure by prompting an early investigation. This is where it will really help with compliance’

It should be noted however, that as the size and scope of datasets develop, it is expected that general trends emerge with respect to the order of magnitude of FCM cell counts compared to compliance organisms. Following data collection; the lack of a standardised protocol and the perceived limitation that the method enumerates all bacteria and not specific indicator organisms were identified as the next significant challenges. This was further identified as a barrier for using FCM for drinking water quality monitoring through the requirement of ‘cultural change [as results] cannot be linked to standard methods’ and provides different data sets than typically observed during standard analysis.

Conversely, opportunities for regulatory compliance monitoring by FCM identified that developments of the technology/methodology may result in the possibility of being able to tag indicator target organisms (Table 4). Such developments would support compliance monitoring and enable the technology to be more than a monitoring tool.

Further challenges identified, though not observed consistently by all participants, included the reproducibility and repeatability of the method, regulatory support, combining with complementary technologies and the practical use within the field.

‘The lack of an external quality assessment (EQA) means we have no confidence in reproducing results across sites’

‘There are also issues related to reproducibility and therefore assurance across different instruments and therefore across industry’

‘The regulator needs to understand there are other methods and they are being worked on- so FCM may
Overall conclusions

FCM is a relatively new technology for the water industry and it is being fairly widely used for operational/investigational purposes. Of the FCM users in this study, most are monitoring specific assets for TCC and ICC during water quality events and use of offline FCM systems for routine analysis. These two areas are seen as key immediate benefits of FCM for water companies. The use of online or discreet in-line automated FCM is seen as a practical approach for intensive root cause analysis or measurement at sites where sub-daily variability in microbial loading or water treatment works performance is seen. However this may not be cost effective in the long term for most water treatment works (based on current pricing models). Given that FCM cannot be applied for regulatory compliance monitoring, (no regulatory requirement or PCVs, formal standard method, UKAS accreditation etc), most water utilities are using FCM as a more sensitive measure of asset performance and to spot declines in water quality.

There is no good correlation between FCM results and HPC which limits its applicability for compliance under the current regulations. There is no direct link between FCM result and risk to public health (similar to HPC) and at present there are no robust FCM methods for pathogens or E. coli, although it is thought that this could be possible in the future. Any possible future standard for FCM would have to be site specific or more descriptive. There is a need for standardisation of methods and data analysis / interpretation. Based on this report the following is recommended:

1. Water companies should ensure that data analysis is appropriate and standardised where possible (bearing in mind limitations and restrictions around suitable FCM data interpretation)

2. Data interpretation standardisation / guidelines is seen as the next big challenge for successful future use of FCM for the water industry.

3. All water suppliers should continue to monitor assets for microbiological compliance using the approved indicator organism of water quality to protect public health.

4. As a broadly ‘industry-led’ activity as opposed to regulator-led, FCM is an example of industry cooperation to attempt to solve the challenge of spot microbiological compliance events at generally well run and optimised water treatment and supply assets.

5. Use of online FCM is identified as a key strategy to reduce the risk of public health concerns through optimised treatment and better asset understanding to produce a stable bacterial population in water to reduce the risk of pathogen penetration of water treatment barriers and risk of regrowth within networks.
References


Appendices

Appendix A: Literature review methodology

The review was conducted to identify all available evidence, while reducing the impact of bias on the review findings related to the principal questions aforementioned. The review will involve the following tasks: scoping search, structured search combining a first and a second screening, documentation, verification and report. The scoping search will centre on identifying relevant databases and grey literature sources as well as primary documents that provide a good understating of the review challenges. Expected outcomes of the scoping stage will be keywords to be used during the structured search, inclusion/exclusion criteria for the initial screening and quality criteria for the second screening (e.g. applicability to review questions, appropriateness of study design).

Keywords defined in the scoping search will be organized into search strategies to be used in the structured search. With this purpose, search strings combining keywords with Boolean operators (AND, OR, NOT) will be used on each selected database (e.g. flow cytometry*, water, aquatic, “treatment plant”, reservoir*, river*, lake*, effluent, quantification, analysis); proximity operators may be needed as well. The first screening will target words in fields such as the title and abstract. Then inclusion/exclusion criteria will be applied to retrieved documents to procure the evidence base for the second screening. At that stage, full text references will be read and their quality appraised in terms of validity, reliability and applicability to the three postulated search questions. Results from the individual references will be compiled to identify patterns pertaining to the use of flow cytometry for monitoring waters used for human consumption. This information will be summarized in a document that will support the survey questions and overall interpretation of these findings.

An accurate account of the review is required so that each task will be documented. Detailed records will include sources searched (database and database provider), search strategy, number of references found for each source/strategy, and search date. Electronic copies of each search strategy (a text file to be copied and pasted into an Office document) will be provided. References (e.g. articles, reports, studies) will be recorded and organized. Management of references will greatly help the process of the systematic review since all references retrieved in the search should be accounted for. A copy of the pdf files used in this study shall be catalogued and stored for a period of five years. With this purpose, Mendeley, a free electronic reference management system available at Cranfield, was used.
Appendix B: Survey, Understanding the Use of Flow Cytometry (FCM) for Monitoring Drinking Water (Defra-DWI)

A questionnaire was constructed to capture a ‘snapshot’ of the status of FCM implementation in water companies within England and Wales. Participants from each water company (excluding network only suppliers) were recruited to the intake study by the researchers. These participants were asked to complete a short survey representing a single water company ‘response’. Participants were provided with carefully written instructions on how to fill out the survey. A copy of the survey is provided below:

You are invited to participate in a research study conducted on behalf of the Drinking Water Inspectorate (Defra-DWI) by Cranfield Water Science Institute (CWSI) at Cranfield University. Your participation in this survey is entirely voluntary.

The purpose of the survey is to: (1) Examine the uptake of flow cytometry (FCM) in water utilities in England and Wales (2) Understand how water utilities are using the information generated from FCM (3) Investigate the potential future developments of FCM for monitoring drinking water quality.

The survey forms part of an information gathering exercise which will inform the DWI on the current position of FCM for monitoring microbial water quality and the potential of using FCM to supplement both operational and regulatory monitoring. The overall objectives of the research study will assist the DWI in making informed decisions with respect to FCM and the generated data.

The intention of the survey is to obtain a company response on FCM operation and data use. The survey is therefore intended for individuals who are able to respond on behalf of their company and not a personal reflection of the methodology. Responses are also welcomed from those who do not currently undertake FCM to gauge the potential of the technique for monitoring drinking water quality.

The survey can be paused and saved at any point to allow time to find any required information. However, if you feel you are not the most appropriate person to complete please feel free to forward to another representative within your company.

Once you have agreed to participate you will be directed to the online survey and guided through a series of questions which take approximately 10 – 30 minutes to complete (depending on requirements for data-gathering).

The survey conforms to the provisions and requirements of the General Data Protection Regulation (GDPR 2018) with ethics approval from Cranfield University’s ethics committee. All responses will be anonymised in any published reports. If you have any questions regarding your participation in this survey, please contact Dr Francis Hassard; francis.hassard@cranfield.ac.uk; Tel: 01234 750 111
STATEMENT BY PERSON AGREING TO PARTICIPATE IN THIS SURVEY
I have read and understood the informed consent document and I freely and voluntarily choose to participate in this study.

- Yes I consent  (1)
- No I do not consent  (2)

End of Block: Introduction Questions
Start of Block: Have/use FCM
Which company are you responding on behalf of?

________________________________________________________________

Does your company use flow cytometry (FCM) for monitoring drinking water?

- Yes  (1)
- No  (3)

Approximately how many drinking water treatment works does your company operate (including surface, ground water and boreholes)?

________________________________________________________________

Please rate the following with respect to your company's perspective of using FCM for monitoring drinking water.
<table>
<thead>
<tr>
<th>Strongly agree (1)</th>
<th>Agree (2)</th>
<th>Somewhat agree (3)</th>
<th>Neither agree nor disagree (4)</th>
<th>Somewhat disagree (5)</th>
<th>Disagree (6)</th>
<th>Strongly disagree (7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FCM is a very reliable approach (1)</td>
<td></td>
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<tr>
<td>FCM is more uncertain than traditional cultivation methods (2)</td>
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<tr>
<td>FCM is easy to use / operate (3)</td>
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<tr>
<td>FCM will not be useful as a monitoring tool for compliance purposes (4)</td>
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<tr>
<td>FCM does not provide more information than total viable counts (5)</td>
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<tr>
<td>FCM is useful for measuring specific pathogens or compliance indicator organisms (e.g Escherichia coli or Cryptosporidium spp.) (6)</td>
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<tr>
<td>FCM is useful for measuring active populations of bacteria (7)</td>
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<tr>
<td>FCM data can be applied to setting control charts/operational ranges (8)</td>
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<tr>
<td>FCM can be used to actively manage water treatment or supply assets (9)</td>
<td>Strongly agree (1)</td>
<td>Agree (2)</td>
<td>Somewhat agree (3)</td>
<td>Neither agree nor disagree (4)</td>
<td>Somewhat disagree (5)</td>
<td>Disagree (6)</td>
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</table>

Please state the manufacturer, model and procurement date of each of your flow cytometers used for the primary purpose of monitoring drinking water quality.

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________________________________________________________________
________________________________________________________________

Have you adapted/modified your primary FCM from the original manufacturer set up? (i.e. online, inline, custom lasers etc.) If so please describe changes.

- Yes (1)________________________________________________________________________________

- No (2)
Thinking about your primary operational FCM supplier, are you satisfied with the service that is provided?

<table>
<thead>
<tr>
<th></th>
<th>Strongly agree (1)</th>
<th>Somewhat agree (2)</th>
<th>Neither agree nor disagree (3)</th>
<th>Somewhat disagree (4)</th>
<th>Strongly disagree (5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maintenance (1)</td>
<td>○</td>
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<td>○</td>
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<td>Servicing (2)</td>
<td>○</td>
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<td>○</td>
<td>○</td>
<td>○</td>
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<tr>
<td>Repairs (3)</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
</tr>
<tr>
<td>Customer service (4)</td>
<td>○</td>
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<td>○</td>
<td>○</td>
<td>○</td>
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<tr>
<td>Method development support (5)</td>
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<td>○</td>
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</table>

Hypothetically if your primary operational FCM were to breakdown, how long do you estimate the down time to be before the unit is back in service?

○ < 48 hours (1)

○ 48 hours - 1 week (2)

○ 1 - 4 weeks (3)

○ 1 - 3 months (4)

○ > 3 months (5)

○ Never (7)
Please rate your knowledge of the FCM methodology and trouble shooting (where 0 = no knowledge, 5 = expert)

<table>
<thead>
<tr>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Using a method developed by others ()</td>
<td>()</td>
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</tr>
<tr>
<td>Comfortable adapting / developing method templates ()</td>
<td>()</td>
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<tr>
<td>Gating different populations of cells ()</td>
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<tr>
<td>Thresholding to remove background ()</td>
<td>()</td>
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<tr>
<td>Trouble shooting flow cytometry instrument ()</td>
<td>()</td>
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<tr>
<td>Trouble shooting of method ()</td>
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</table>

Has your company adopted the BD White Paper protocol "Assessing Water Quality with the BD Accuri C6 Flow Cytometer" for the pre-treatment of samples prior to analysis?
- SYBR Green I (10,000X dilution of DMSO stock) for total cell concentration (TCC) - SYBR Green I (10,000X dilution of DMSO stock) + PI (0.3 mM) for intact cell concentration (ICC) - Incubated at 35 (degrees Celsius) for 10-15 min in the dark.

- Yes (please describe any modifications you have made to this protocol) (3)

- No (please provide a reference for your protocol) (4)
Which method is used for selecting 'gates' to discriminate between populations of events? (Please select all that apply)

- Downloaded template e.g. BD water template (please provide a reference) (7)
- Automatic gate selected by software (1)
- User selected (2)
- Other (please provide information) (4)

Although not mandatory, as the method is currently not accredited by UKAS and not required for compliance monitoring, do you include blanks when analysing your samples?

- Yes (please provide details of the blank and frequency) (1)
- No (2)

Although the method is currently not accredited by UKAS, do you undertake any validation of your FCM against an external standard (e.g. bead validation)?

- Yes (please provide details of frequency and type of validation) (1)
- No (3)

Thinking about water treatment works where routine monitoring is in place, do you use FCM to monitor any of the following?
<table>
<thead>
<tr>
<th></th>
<th>Routine monitoring (e.g. alongside regulatory monitoring) (1)</th>
<th>Operational / enhanced monitoring during normal conditions (2)</th>
<th>Root cause analysis during operational issue (e.g. asset failure) (3)</th>
<th>Investigations during a water quality event (4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Source waters / raw waters (1)</td>
<td>[ ]</td>
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<td>[ ]</td>
<td>[ ]</td>
</tr>
<tr>
<td>Water treatment works inlet (2)</td>
<td>[ ]</td>
<td>[ ]</td>
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<td>[ ]</td>
</tr>
<tr>
<td>Pre-coagulation (3)</td>
<td>[ ]</td>
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<td>[ ]</td>
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<tr>
<td>Post coagulation / settlement (4)</td>
<td>[ ]</td>
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<tr>
<td>During dissolved air flotation (5)</td>
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<tr>
<td>Post rapid gravity filter (6)</td>
<td>[ ]</td>
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<tr>
<td>Post slow sand filter (7)</td>
<td>[ ]</td>
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<tr>
<td>Before a membrane separation (please state which membrane e.g. micro filtration, ultra filtration, Reverse osmosis) (8)</td>
<td>[ ]</td>
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<tr>
<td>Post-membrane separation (please state e.g. micro, ultra filtration, Reverse osmosis) (9)</td>
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<tr>
<td>Post UV system (10)</td>
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<tr>
<td>Pre-chlorine contact tank (11)</td>
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<tr>
<td>Within or post-chlorine contact tank (12)</td>
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</tbody>
</table>
In your experience, is FCM more suited to a particular raw water type, e.g. groundwater, upland impounding reservoir, upland river abstraction, low land abstraction, if other please specify.

__________________________________________________________________
__________________________________________________________________
__________________________________________________________________
__________________________________________________________________

Do you see more potential for FCM for particular water treatment processes? Please explain.

__________________________________________________________________
__________________________________________________________________
__________________________________________________________________
__________________________________________________________________
Approximately how many water treatment works does your water company have FCM data for?

<table>
<thead>
<tr>
<th>Number of treatment works (1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;10 (1)</td>
</tr>
<tr>
<td>10-19 (2)</td>
</tr>
<tr>
<td>20-50 (3)</td>
</tr>
<tr>
<td>51-100 (4)</td>
</tr>
<tr>
<td>101-500 (5)</td>
</tr>
<tr>
<td>&gt;500 (6)</td>
</tr>
</tbody>
</table>

How does your company use the data derived from FCM analysis? (please select any that apply)

- Used in isolation to make decisions (1)
- Used with other data sets to make decisions (please state which datasets) (2)
- Used to generate universal internal targets/PCVs which apply across assets. If so please describe the targets or standards. (3)
- Used to generate asset specific targets / acceptable ranges (please state the assets for which specific range of values are used) (4)
- The data is currently not used by our company (e.g. still undertaking method validation, not using...
etc.) (6)

- Other (please provide details) (5) ________________________________

- Used to inform risk assessments (7)

- Asset planning (8)

- Asset management (9)

- Asset maintenance (10)

- Water Safety plans (11)

- Trend analysis (12)

With regards to your extended distribution system, do you use FCM for monitoring of any of the following?

- Service reservoirs (1)

- Distribution network (2)

- Customers taps (not for regulatory monitoring) (4)

- Other (5) ________________________________

Does your company have an agreed method for interpreting the results from FCM? (Please provide details)

________________________________________________________________
________________________________________________________________
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As the UK water sector moves towards Ofwat's "proportionate and targeted regulation - Ofwat's risk-based approach" what are the challenges and opportunities for the use of FCM for drinking water quality
monitoring?

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What are the challenges and opportunities for using FCM for regulatory compliance monitoring?

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Hypothetically, if FCM was implemented for routine compliance monitoring, who do you think would ensure quality control and assurance and standardisation between water quality labs?

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End of Block: Have/use FCM

Start of Block: No FCM

Approximately how many drinking water treatment works does your company operate (including surface, ground water and boreholes)?

________________________________________________________________

________________________________________________________________

End of Block: No FCM
Does your company have plans to undertake monitoring of drinking waters using FCM?

- Yes (please provide brief details) (1)
  ________________________________

- No (please state why) (2) ________________________________

- Prefer not to say (4)

Please rate the following with respect to your company's perspective of using FCM for monitoring drinking water.
<table>
<thead>
<tr>
<th>Statement</th>
<th>Strongly agree (1)</th>
<th>Agree (2)</th>
<th>Somewhat agree (3)</th>
<th>Neither agree nor disagree (4)</th>
<th>Somewhat disagree (5)</th>
<th>Disagree (6)</th>
<th>Strongly disagree (7)</th>
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<tbody>
<tr>
<td>FCM is a very reliable approach</td>
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<td>FCM is more uncertain than traditional cultivation methods</td>
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<tr>
<td>FCM will not be useful as a monitoring tool for compliance purposes</td>
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<td>FCM does not provide more information than total viable counts</td>
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<td>FCM data can be applied to setting control charts/operational ranges</td>
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<tr>
<td>FCM can be used to actively manage water treatment or supply assets (9)</td>
<td>Strongly agree (1)</td>
<td>Agree (2)</td>
<td>Somewhat agree (3)</td>
<td>Neither agree nor disagree (4)</td>
<td>Somewhat disagree (5)</td>
<td>Disagree (6)</td>
<td>Strongly disagree (7)</td>
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</table>

As the UK water sector moves towards Ofwat's "proportionate and targeted regulation - Ofwat's risk-based approach" what do you believe to be the challenges and opportunities for the use of FCM for drinking water quality monitoring?

________________________________________________________________
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What do you believe to be the challenges and opportunities for using FCM for regulatory compliance monitoring?

________________________________________________________________
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Hypothetically, if FCM was implemented for routine compliance monitoring, who would ensure quality control and assurance and standardisation between water quality labs?

________________________________________________________________
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Appendix C: Survey results not currently reported in main document.
Challenges and opportunities for drinking water quality

Table 9 Challenge and opportunities for drinking water quality

<table>
<thead>
<tr>
<th>Challenges</th>
<th>Opportunities</th>
</tr>
</thead>
<tbody>
<tr>
<td>Method standardisation</td>
<td>Easily monitor change in water quality</td>
</tr>
<tr>
<td>Data collection and interpretation</td>
<td>Speed and accuracy of method</td>
</tr>
<tr>
<td>Long term data collection for baselines (trends)</td>
<td>Potential of online monitoring</td>
</tr>
<tr>
<td>Infrastructure (e.g. updating IT systems)</td>
<td>Future potential to target specific bacteria</td>
</tr>
<tr>
<td>Instrumentation issues</td>
<td></td>
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<tr>
<td>Combining with other technologies</td>
<td></td>
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<tr>
<td>Cultural change</td>
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</tr>
</tbody>
</table>

Who will ensure quality control between water quality labs.

- SCA/UKAS/Independent verification/between water quality labs

Method of ‘selecting’ gates?

- Mostly user selected, one automatic gate (YW, but ALS undertake the analysis)

analysing sample

- 3 x no, 5 x yes (milliQ water, Evian, Highland Spring)

External standard, i.e. bead validation

- 3 x no, 5 x yes