View metadata, citation and similar papers at core.ac.uk
brought to you by CORE
provided by IntechOpen

We are IntechOpen, the world's leading publisher of Open Access books
Built by scientists, for scientists

4,800
Open access books available

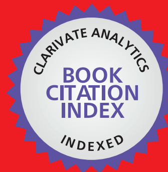
122,000
International authors and editors

135M
Downloads

154
Countries delivered to

Our authors are among the
TOP 1%
most cited scientists

12.2%
Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index in Web of Science™ Core Collection (BKCI)

Interested in publishing with us? Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

Chapter 2

Recent Developments in Monitoring of Microbiological Indicators of Water Quality Across a Range of Water Types

Sandra Mesquita and Rachel T. Noble

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/45847

1. Introduction

As human pressure increases, so does exploitation of natural water sources for production of drinking water, public use and appreciation of recreational waters. Subsequently, the demand for faster and reliable monitoring methods and approaches has also been intensified. Ensuring that water is safe, whether for bathing or consumption is a critical complex process that requires the participation of multiple stakeholders [1, 2] and also distinct degrees of knowledge with respect to water management.

For quantifying microbial contaminants that are dangerous to public health, fecal indicator bacteria (FIB) are used worldwide as indicators of the potential presence of dangerous pathogens that can be found in water used for bathing, drinking and harvesting of seafood. The "indicator" system relies on the assumption that FIB are present concomitant to the presence of human bacterial, viral and parasitic pathogens of concern. Furthermore, the indicator system simplifies management, because no single pathogen can be singularly attributed to the majority of waterborne disease. If pathogens were measured for management of water quality, the list of candidates to be quantified would be many. The FIB system relies on thresholds that have been established using epidemiological studies and risk assessment frameworks (see Annapolis Protocol and WHO bathing water guidelines).While the standards for drinking water, recreational water and shellfish harvesting waters and meats are based upon similar approaches [3], different types of waters can have different acceptable thresholds for the same FIB because of differences in either acceptable risk, the relative use of the water (i.e. for consumption versus recreation), and a range of other factors. Therefore,

INTECH
open science | open minds

© 2013 Mesquita and Noble; licensee InTech. This is an open access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/3.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

acceptable thresholds for FIB in drinking water, recreational water and shellfish harvesting waters and meats vary by continent, region, state and locality.

By definition an indicator (in this case, FIB) should be, 1) able to be enumerated using standardized tests that are relatively cost-effective and user-friendly, 2) present in high concentrations in human fecal contamination, matching the high concentrations of other respective pathogens, and 3) well studied in relation to human health, with epidemiological studies to link FIB concentrations and adverse health outcomes such as gastrointestinal and respiratory human diseases [4, 5, 6]. These assumptions can be tenuous at times for all types of waters and for all of the FIB groups currently utilized for water quality management. However, it is generally accepted that regulation using FIB to protect public health is successful. For example, total coliforms do not exactly fit these requirements since they are not exclusively fecal in origin; however they are often used in conjunction with other FIB enumeration approaches to protect public health in drinking water [7]. Even though some of the assumptions listed above are violated periodically for the use of FIB to protect public health in the regulation of drinking water, an alternative approach is often unattainable. Human pathogens are heterogeneous, can be highly seasonal, and can be highly infectious at low doses. The range of enteric viruses alone that could be monitored in drinking water is vast, and these pathogens are difficult to detect [8, 9, 11]. A discussion of the pathogens of concern is beyond the scope of this chapter, and the reader is referred to a range of quality publications on the topic (e.g. 11, 12).The "indicator" system can be used successfully, particularly if new research and methodologies in the water quality arena are incorporated into management. As a community, we can attain improved means of management of public health in water, if attention is paid to utilization and incorporation of new monitoring tools, new models and molecular tools. Current advances in science include rapid quantitative PCR assays (QPCR) developed to track distinct microorganisms in multiple water sources [12, 13, 14, 15, 16, 7].

In this chapter, we highlight recent advancements in the management of water, and in monitoring of microbial water quality in drinking water production and recreational waters. First, we highlight the current regulatory landscape for microbiological indicators. Second, we will describe the application and evolution of molecular tools for rapid quantification of FIB (E. coli and *Enterococcus* sp.), in a range of water types. We will include some of the limitations and advantages of different types of culture based methods as compared to the recently developed rapid QPCR-based methods. We will present a small case study conducted along high use beaches along the Pacific Ocean of California, USA. Third, we highlight recent developments in the field of Harmful Algal Blooms (HABs) and concerns over cyanobacterial toxins in surface water sources for drinking water. Following the presentation of information presented on HABs, and comparing *E. coli* and *Enterococcus*sp. concentrations using culture based and molecular (qPCR) rapid methods, the authors will consider the application of newly developed source tracking markers for quantification of fecal contamination fecal contamination. These microbial source tracking (MST) tools are currently being used and expanded for the management of a range of water types. The chapter is intended to highlight recent advancements, while promoting the consideration of other successful ap-

http://dx.doi.org/10.5772/45847

proaches for managing water resources well into the 21st century with an eye toward improved protection of public health.

2. Regulatory landscape across major water types: Highlighting the use of the fecal indicator bacteria as part of the "indicator" system

Traditionally, the application of FIB for water quality monitoring included enumeration of members of the total coliform group. This group was originally described on the basis of lactose fermentation detection instead of upon the principles of systematic bacteriology [3; 17-26]. Total coliforms are still used widely for management of drinking water, but their sole use in recreational water quality monitoring has decreased in favor of enumeration of FIB that are specific to fecal contamination from warm-blooded animals (i.e. *E. coli* and *Enterococcus* sp.). Total coliforms are defined by their respective characteristics, i.e. they are Gram negative facultative anaerobes that do not form spores are rod-shaped bacteria, with lactose fermentation occurring with acid production (24-48h, 36°C), and are indole-negative. Coliforms belong to the family Enterobacteriaceae which includes *Escherichia, Enterobacter, Klebsiella* and *Citrobacter, Kluyvera, Leclercia*, and some members of the genus *Serratia* [27].

In all of the culture-dependent methods, cultivation conditions are choose in order to improve the growth of the target microorganism while simultaneously inhibiting the growth of other microorganisms. Balance amongst sensitivity and selectivity is the reason for different methods for sample processing (drinking water *vs* highly contaminated waters) [28] and selection of different approaches for quantification using traditional culture based methods. The three most widely used culture based methods for quantification of FIB in any water type are Defined Substrate Technology (DST, e.g. IDEXX Colilert-18®), multiple tube fermentation (MTF), and membrane filtration (MF). The first edition of “Standard Methods for the Examination of Water and Wastewater” was released in 1905 and *E. coli* was selected as the most suitable indicator organism for raw drinking water [29]. Therefore multiple detection methods for *E. coli* were available early in the century. As scientific knowledge evolved a broader group of microorganisms (still including total coliforms, fecal coliforms, and *E. coli*) have been selected as surrogate measures in a wide range of water sources and treated water [30]. Two key factors have led to the trend of using *E. coli* as the ‘preferred’ indicator for the detection of fecal contamination, not only in drinking water but in other matrices as well: first, the finding that some ‘fecal coliforms’ were non-fecal in origin, and second, the development of improved testing methods for *E. coli* [1-19]. Membrane filtration is commonly selected for FIB quantification along the drinking water treatment process since it is flexible to the amount of sample filtered and since the method permits specific quantification. That is, both MTF and DST-based methods are either reported in a presence/absence format or in a “most probable number” format. Colilert® and Colilert- 18®, however, tend to be used at the “end of pipe” and are user friendly methods, such that their popularity has increased in the past decade.

An example of current regulations for protection of public health in Europe, USA and other countries is summarized in Table 1 [20]. Note that many currently used methods both total coliform and *E. coli* enumeration together, as IDEXX DST kits such as Colilert-18® permit the simultaneous quantification of both groups. Furthermore, while guidelines have been issued in different countries, as highlighted in Table 1, the interpretation and implementation of monitoring typically falls in the hands of member states and provinces; this creates a wide range of monitoring approaches that are currently employed for management of drinking, recreational, and shellfish harvesting water quality. It is for this reason that only a table for drinking water is presented, as drinking water regulations are the most stringent and consistent across nations.

Parameter	Canada	United States	United Kingdom	EU directive	WHO*	Australia
Total Coliforms	0/100mL in 90%	0/100mL in 95%	0/100mL	0/100mL	*	*
Thermotolerant coliforms (fecal coliforms) or *E. coli*	*	0/100mL	*	*	0/100mL	*
E. coli	0/100mL	0/100mL	0/100mL	0/100mL	0/100mL	*
Enterococci	*	*	0/100mL	0/100mL	*	*
Cryptosporidium parvum	*	99% removal or inactivation	<1oocyte/ 10L	*	*	*
Cryptosporidium perfringens (including spores)	*	*	0/100mL	a	*	*
Pseudomonas aeruginosa	*	*	*	0/250mL	*	*
Colony count 22°C	*	*	No abnormal change	No abnormal change	*	*
Colony count 37°C	<500CFU/mL [b]	<500CFU/mL [b]	No abnormal change	20/mL [c]	*	*
Microcystins _LR	1,5µg/L	*	*		1,0 µg/L (provisional)	Lifetime exposure 1,3µg/L

[*] spaces left in blank indicate parameters not specified;[a] Necessary only if the water originates from or is influenced by surface water;

[b] HPC(35ºC for 48h) or <200 background coliforms on a total coliform membrane filter;[c] Necessary only in the case of water offered for sale in bottles or container

Table 1. International drinking water standards and guidelines from the World Health Organization (WHO) Modified from [31, 32 and 33]

http://dx.doi.org/10.5772/45847

The World Health Organization (WHO) Guidelines for Drinking-water Quality [32] represent an overall international scientific consensus, based on a wide range of participants, of the health risks presented by microbes and chemicals in drinking water. The existence of international guidelines for cyanobacteria microcystins (1 μg/L microcystins –LR) in drinking water has been established by WHO (1998). Most countries rely upon this value and developed specific systems adapted for their own reality. For instance, in Brazil federal legislation requires more intensive monitoring programs including toxin analyses or toxicity testing if cyanobacteria exceed 10,000 cells/ml or 1 mm^3 biovolume in a given water sample. This includes a mandatory standard of 1 μg/L applied for microcystins (variants not specified), and recommendations limiting to saxitoxin 3μg/L.

Evaluation of water quality for drinking water production requires sampling collection at specific temporal intervals to ensure safety for the public. Usually it is recommended to sample across the process of drinking water treatment, permitting an analysis of bacteriological water quality throughout processing and distribution [6,7]. This permits rapid identification of a process or component in the distribution system that is failing, and rather than troubleshooting across the entire process, subsequent attention can be focused on specific components. This approach simultaneously prevents local contamination from developing undetected at cross-connections or breaks in the distribution lines or due to a drop in positive pressure [1, 34, 35].

The definition of a recreational water body represents a wide range of environments that include the ocean, hot springs, lakes, reservoirs, streams and rivers [34]. Similarly, the diversity of potential human pathogenic microorganisms includes all viruses, protozoans, and bacteria that could potentially be present in natural fresh and marine recreational waters particularly those contaminated by wastewater [9, 36]. In recreational water quality monitoring, monitoring approaches are often guided by use. Beaches that are used intensely by the public (such as those in Santa Monica Bay, California, USA) are monitored daily throughout the summer bathing season. In some states or areas with lower beach usage, during specific "off seasons", or areas that are used only for secondary contact recreation such as kayaking and sailing, monitoring for FIB might be conducted only once per month. Although a broad discussion is beyond the scope of this document, the use of FIB for monitoring shellfish harvesting waters also relies heavily on the use of traditional culture-based methods such as MF and MTF, but management differs by country. In some EU states, management of shellfish is by quantities of *E. coli* in shellfish meats (e.g. oysters), whereas, quantification in the United States is based upon fecal coliforms in the water column surrounding the shellfish beds.

Advantages connected to the application of culture-based methods include low equipment requirements, user-friendliness, and low cost, which makes it the most used approach for quantifying FIB in any water type. On the other hand, the enrichment stage takes time (typically from 18-24 hours) causing the notification of the public to be delayed during a contamination event [37]. This delay also causes vital revenue to be lost because contaminated waters are often still restricted or "closed" for use (in the case of beaches) after the contamination event has already passed. Incubation methods also suffer from the fact that they can promote the growth of false positive organisms [38]. Rapid molecular methods have recent-

ly been developed for application to protection of public health in recreational waters [39]. These methods require 3 hours from sample processing to results, and they are qPCR based. They target specific organisms (*Enterococcus* sp. or *E. coli*). The use of such methods permits rapid public notification (e.g. [40]) resulting in improved protection of public health. One other advantage of such methods is the high specificity [28X]. Other rapid methods using qPCR have already been developed for quantification of microbial markers that are specific to sources of fecal contamination [44, 45].

3. Newly developed rapid molecular methods for recreational water quality monitoring

Millions of dollars are expended each year to measure FIB to and assess whether swimming at recreational beaches posed a risk to public health. The monitoring programs are typically conducted using culture-based methods that require an 18 to 96 hour incubation period. The lengthy incubation step results in situations where beaches are managed inaccurately, by keeping beaches open that are contaminated and closing beaches that are potentially clean again [40]. Newer, more rapid methods, such as qPCR have been developed by EPA and others for use in recreational water quality monitoring [39, 41]. Several of these new rapid PCR-based methods for quantification of *Enterococcus* sp. have been found to be significantly related to human health outcomes in epidemiology studies [42, 43]. In some cases, the association between rapid methods and adverse human health outcomes has been stronger than that observed between traditional culture-based methods and adverse human health outcomes [43].

Quantitative PCR is a novel primer-based molecular technique that combines the specificity of traditional PCR with the quantitative measurement of fluorescence for quantification of presence of specific types of nucleic acid in environmental samples. Many of the recently developed methods are actually quite similar to one another; differing only in the type of qPCR fluorophore "chemistry" used [39,41]. Even though epidemiological studies to assess relationships between rapid FIB quantification methods and human health outcomes have been successful, the implementation or widespread use of the rapid qPCR-based methods has not been without hurdles. Noble et al., 2010 [41] successfully demonstrated the use of rapid molecular methods by water quality personnel that had no previous experience with molecular techniques. Griffith and Weisberg, 2011 [40] built upon this success, by training three water quality laboratories to conduct the rapid methods, and implementing the use of the rapid methods for active beach management decisions (posting and closing of beaches). They also demonstrated the use of real time notification technology at the beaches to rapidly convey real-time water quality results through the assistance of a non-profit organization called "MiOcean". Griffith and Weisberg (2011) reported excellent management agreement (>96%, i.e. the agreement in whether a beach would be posted or remain open) among the rapid QPCR based enumeration of *Enterococcus* sp. [41] and culture based methods such as EPA Method 1600 (membrane filtration based quantification for *Enterococcus* sp.). Lavander and co-workers [46] have shown similar successes with quantification of *E. coli* at freshwater

Great Lakes beaches, reporting management agreement of 98% between the rapid qPCR based quantification of *E. coli* and traditional culture based methods such as Colilert-18.

Even though success has been demonstrated in specific environments, it is clear that rapid qPCR-based methods for quantification of FIB are not appropriate for all places at all times. Three major issues to be resolved are ways to standardize assays, the approach used for quantification of the target organism, and inhibition of the qPCR.

Currently there are two modes of quantification being employed in the literature, both with advantages and disadvantages. One relies on quantifying the target cell, e.g. *Enterococcus* sp., where results are reported as cell equivalents/100 ml, where inhibition is assessed using a specimen processing control, but the final reported target concentrations are not modified quantitatively using inhibition data (see [40] for an example). The second approach reports target cell concentrations as calibrated cell equivalents/100 ml and relies on the use of cycle threshold ratios for both the target cell and the specimen processing control for final calculated quantities of the target cell concentration. The cell equivalent approach can, in certain circumstances, provide data that is more related to historical data reported for culture based methods [40,41, 46], but cannot be used at beaches were partial inhibition of the qPCR is common. On the other hand, the calibrated cell equivalent quantification approach can be more widely applied at a range of beaches, because inhibition is accounted for. However, the approach is imperfect because the specimen processing controls that are currently used are DNA-based, and they do not adequately predict inhibition of the amplification of FIB cells in real world samples [46].Dilution is another approach that can be used to effectively reduce inhibition, but this increases the limit of detection.

Figure 1 is a snapshot of results from a preliminary assessment of the performance of rapid qPCR-based methods as compared to membrane filtration (culture-based) methods for the quantification of Enterococcus sp. in water samples collected from an array of beach locations in Santa Monica Bay, California, USA. The rapid methods used here provide results within a few hours of sample collection. Quantification was conducted using the cell equivalent approach.

Inhibition of the qPCR has been discussed at length in other publications but is relevant to the implementation of rapid qPCR based methods for water quality assessment because of its unpredictable nature. Inhibition of qPCR can occur when high molecular weight compounds in the surface water (e.g., humic acids and other complex carbohydrates) combine with metal ions to sequester nucleic acids from polymerases and prevent amplification [47, 48, 49, 50]. Inhibition can sometimes be alleviated with the use of a commercial DNA extraction kit for sample purification. This requires extra time that is added to the total analysis time, and increases variability in the final results due to analyst error and variable DNA binding characteristics. Others have tried to deal with inhibition in analysis of surface and other water samples with the addition of adjuvants (e.g. bovine serum albumin, [49]. In recreational waters, many use salmon testes DNA to assess inhibition (e.g. 39). Cao et al. 2012 [10] assessed the use of dilution, salmon testes DNA, and internal controls for the assessment of inhibition in a range of water sample types, and no one approach clearly emerged as superior over the others. In fact, the internal controls and salmon testes DNA did not

agree in their assessment of inhibition of the target qPCR [52]. It may be that implementation of the rapid methods, at least at first, follows the approach developed in southern California by [40]. They utilized the cell equivalent quantification approach, and for beaches that demonstrated inhibition of the QPCR, traditional culture based methods were utilized to quantify FIB concentrations for beach management purposes.

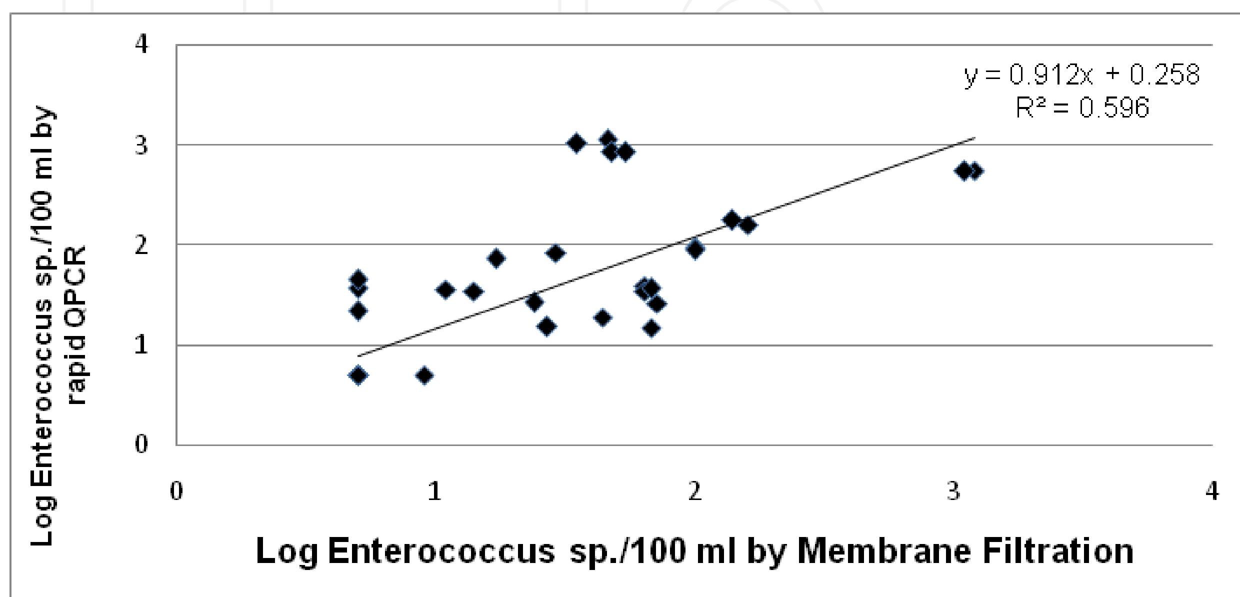


Figure 1. Example of rapid qPCR-based methods compared to membrane filtration (culture based) methods for the quantification of Enterococcus sp. in water samples collected from an array of beach locations in Santa Monica Bay, California, USA.

In the past years another new, rapid and reliable methodological approach has been tested to monitor microbial contamination, particularly in recreational marine and freshwater sources [53, 54]: the immunomagnetic separation / adenosine triphosphate (IMS / ATP) method. IMS-ATP was first developed by [55] and consists of the use of immune magnetic beads that selectively capture targeted bacteria using cell specific antibodies. A magnet is then used to separate the target bacteria from the rest of the sample with concentration determined by quantification of the ATP concentration in the sample using a bioluminescent assay [53]. While IMS/ATP has not been the subject of potential recommendation by the USEPA in 2012, there are advantages to its use. These advantages include the detection of only viable bacteria, therefore making it more directly related to quantification based upon other currently used culture based methods. Rapid quantification using IMS/ATP might be beneficial in areas receiving treated, disinfected wastewater for example, where the concentration of FIB-specific DNA is likely high, but the concentration of viable FIB cells would be lower. The capital costs of IMS/ATP are lower than those of the molecular methods. Another positive aspect of this technique is that it requires less than 4 hours. The major criticism of IMS/ATP concerns the availability of appropriate antibodies for quantification, particularly for the *Enterococcus* genus, which cannot be adequately captured using a single set of anti-

http://dx.doi.org/10.5772/45847

bodies. However, improvements are being made in the development of cocktails of antibodies combined with the development of new bead based capture systems [e.g. 54].

In the coming years, there is the potential to dramatically improve the sensitivity, accuracy, precision, and speed of the QPCR assays used for water quality applications, while dramatically reducing the cost associated. One key step forward will be in the area of standardization, as standardized kits and cell standards and controls become available in ready-made kits, the analyst error and variability associated with the assays will decrease dramatically. The idea is to develop an elegant and powerful, yet simple, tool that can be applied to a wide variety of water sample types, to simultaneously determine the FIB concentration and enumerate bacterial tracers or pathogen types, necessary for preliminary source identification and/or protection of public health. Many key groups around the globe are working collaboratively to ensure this success.

4. Harmful Algae Blooms (HABs) in drinking water treatment plants and recreational waters

In general, harmful algal blooms (HABs) are a cause of great concerns amongst stakeholders involved in water quality regulation and management. Since this chapter is specifically devoted to the problems caused by microbial contamination, we are specifically focusing our treatment of the subject on the closely related cyanobacteria, and their ability to form toxic blooms (referred to herein as CYANOHABs). There is an array of reports on the increased incidence of toxic blooms caused by Cyanobacteria. Their cyanotoxins are a serious concern for raw water sources that are exploited for drinking water production and recreational purposes [33]. Most countries around the world rely upon the exploration of surface freshwater sources for drinking water production. Efficient surface water treatment systems usually combine a series of treatment stages. Commonly employed water supply treatment includes multiple steps, generally including in order: pre-chlorination, flocculation and coagulation (addition of salts of iron or aluminium), sedimentation and post-chlorination [R]. One specific problem associated with toxic blooms of cyanobacteria is that specific stages of drinking water treatment are needed to remove the toxins. Not all of the drinking water treatment plants have these stages available or possess a flexible system [56]. The goal is to guarantee the production of drinking water according to national water quality guidelines, including microbial and chemical parameters that could potentially promote human disease and/or fatalities. In Table 2 some of the international guidelines for the presence of cyanobacteria toxins is presented. Cyanotoxins can be classified from to a toxicological perspective: hepatotoxins, cytotoxins, neurotoxins, irritant compounds as well as dermatotoxins [33]. Common methods of detecting and quantifying cyanotoxins include Enzyme-linked immunosorbent assay (ELISA), and chromatographic analysis [33, 57].

The main concern for public health associated with cyanobacterial blooms is exposure to cyanotoxins which can cause central nervous system or liver damage. There are multiple modes of exposure to cyanotoxins but most common are: ingestion of contaminated water,

accidental ingestion by swimming on contaminated waters, and consumption of vegetables irrigated by contaminated waters or even inhalation (Table 3) [33,57]. A CYNOHAB's cause adverse effects to drinking water production plants. For instance there are physical impacts and the release of hazard by-products from the treatment stages such as chemical treatment. Other hazards come from oxygen depletion or pipe corrosion due to microbial activity within the pipes coming from bloom collapse. It is fundamental to have knowledge of source waters such as lakes that are prone to the development of toxic and non-toxic cyanobacterial blooms so that the overall population can be informed about public health risks. Several statistical approaches have been used to create models that could explain phytoplankton feed-back to nutrient enrichment and oxygen reduction, and these models may be adapted to other locations [56,58].

Whilst WHO's guidelines are based on total of enumerated cells (using microscopy techniques) and/or determination of chla (chlorophyll a) concentrations related to the amount of cells, there is a clear desire to develop faster quantification of cyanotoxins in environmental samples, especially large population areas. It is important to have rapid, reliable and accurate analysis i.e. high throughput molecular detection methods. In order to develop molecular assays, pure cultures from toxin producers need to be utilized to focus on genes specifically involved in the synthesis of a given toxin [AS].In Table 2, the guidelines from WHO for cyanobacterial levels and expected health effects due to exposure of contaminated recreational waters are shown.

Guidance level or situation	How guidance level derived	Health risks
20000 cyanobacterial cells/mL 10μg chlorophyll-a/L Low impacts in human health	From human bathing epidemiological study	Skin irritations and gastrointestinal illness
100000 cyanobacterial cells/mL or 50μg chlorophyll-a/L moderate chances of affecting human health	From provisional drinking water guideline value and data concerning other cyanotoxins	Long-term illness and/or Short-term problems such as skin irritations and gastrointestinal illness
High probability of adverse effects Scum formation in areas where whole-body contact and/or risk of ingestion/aspiration occur	Inference from oral animal lethal poisonings	Potential for acute poisoning
	Actual human illness case histories	Potential for long-term illness with Adverse health problems such as skin irritations and gastrointestinal illness

Table 2. WHO guidance values for recreational waters with respect to cyanobacteria contaminates waters.(modified from [33]).

Recently published molecular approaches for detection and quantification of toxigenic and non-toxigenic cyanobacteria are based on the application of multiplex approaches for target-

ing the genus (16S rDNA), and subsequently using other nested assays for sequences found in the non-ribosomal polypeptide synthetize complex (55KB in size) responsible for toxin production [56,57, 58]. Al-Tabrineh et al. (2012) recently developed and validated a quadruple target qPCR assay specifically designed for the detection and quantification of genes involved in the production of microcystins, nodularins, cylindrospermopsins and saxitoxins. There is a multiplicity of qPCR protocols published recently; however they typically focus on the quantification and detection of *Microcystis* spp. as a target due to the fact that it represents the most recurrent blooming cyanobacterial group in multiple water sources around the globe [59].

CYANOHAB related problems begin at the raw water source. Therefore, international guidelines such as the Water Frame Directive (Water Frame Directive, WFD; Directive 2000/60/CE of 23 October 2000) require a good ecological state in freshwater or highly modified freshwater sources (example water dams). Therefore, if a water body doesn't comply with these requirements it should be considered contaminated treated accordingly adding necessary steps to guarantee good finished water quality [60]. Thus, regular monitoring of freshwater sources for both recreational and drinking water purposes are necessary. Current modes of quantification starts with chla, but can extend to molecular quantification of specific phytoplankton groups, quantification of toxins (typically ELISA based), or pigment based quantification of phytoplankton groups [61].

Cyanotoxins	**Mechanism of toxicity**	**Taxa toxin producer**	**LD_{50}(mouse) pure toxin**
Protein-phosphatase blockers			
General microcystins	Blocks protein phosphatases by covalent binding promoting liver haemorrhage damage could occur	Microcystis, Planktothrix, Anabaena, Oscillatoria, Nostoc, Anabaenopsis, Hapalosiphon, Nodularin	45->1000µg/Kg
Microcystin-LR			60(25-125)µg/Kg
Microcystin-YR			70 µg/Kg
Microcystin-RR			300-600 µg/Kg
Nodularin			30-50 µg/Kg
Neurotoxins			
Anatoxin-a	Blocks postsynaptic depolarization	*Aphanizomenon, Oscillatoria, Anabaena, Cylindrospermum*	250 µg/Kg
Saxitoxins	Blocks sodium channels	*Aphanizomenon, Anabaena, Lyngbya, Cylindrospermopsis raciborskii*	10-30 µg/Kg
Cytotoxin			
Cylindrospermopsin	Blocks protein synthesis; substantial cumulative toxicity	*Cylindrospermopsis raciborskii*	200 µg/Kg/d

Table 3. Examples of cyanobacterial toxins and their acute toxicity modified from [33]

5. The application of microbial source tracking in water

5.1. Microbial source tracking in water

Microbial source tracking (MST) is the use of microbial markers (including bacteriophage, bacteria, viruses, protozoans, etc.) to determine the source of fecal pollution present in an aquatic system (i.e., human, pet, livestock). Some refer to the scientific discipline as bacterial source tracking (BST), but this term is older and has given way to MST, acknowledging the wider array of information that can be gleaned from targets other than bacteria. The MST field continues to advance rapidly and much that has been published ten years ago is now obsolete and so it is generally better to refer to studies conducted since 2005 for the most updated information. For preliminary information on the evolution of the MST field, the reader should refer to the US Environmental Protection Agency's MST Guide (EPA 2005), article by [62]. MST methods were once categorized into two types — library dependent approaches and library independent approaches. Library dependent methods relied on matching the known source "fingerprints" or isolate patterns generated by molecular or phenotypic methods to unknowns collected from the environment. The most commonly used library dependent methods include, antibiotic resistant analysis (ARA), carbon utilization profiles (CUP), pulse field gel electrophoresis (PFGE), restriction- or amplified- fragment length polymorphism (RFLP or AFLP), random amplified polymorphic DNA (RAPD), repetitive extragenic palindromic PCR (rep-PCR) and ribotyping.

Library independent approaches are based upon detection (often presence/absence) or quantification of specific markers of fecal contamination, and are not dependent upon any sort of classification into categories. Library independent methods include F-specific and somatic coli phage serotyping and genotyping, and host-specific and marker-specific PCR and qPCR for a wide range of targets (see Table 3). Many older approaches utilized conventional PCR, but those have generally given way to the increased use of qPCR. Conventional PCR results are typically reported as presence/absence, or at best by using a serial dilution technique, can produce semi-quantitative information [63]. As opposed to conventional PCR, which is limited to a presence/absence result, qPCR provides for quantification over a wide dynamic range. The platforms currently being used for qPCR include hardware with proven rapid cycling, sensitive optics, and multiplex capabilities.

5.2. Bacteroides based microbial source tracking

One of the most fruitful organisms of focus in MST has been the Bacteroidales family. The Bacteroidales family has yielded a range of quantitative markers for human, dog, cow, horse, and other types of fecal contamination (e.g. 23, 71) The Bacteroides genus was suggested as early as 1967 as an alternative indicator due to the fact that *Bacteroides* spp. levels present in human and animal intestinal tracts are about 10 to 100 times above the other FIB [14, 16]. The high degree of difficulty for cultivation of *Bacteroides* spp. reduced its use in the late decades of the 20th century, but the development of molecular tools allowed the detection and quantification of members of the family Bacteroidales, without cultivation [64].

Consequently, there has been a dramatic increase in publications detailing development of qPCR assays for the quantification of Bacteroidales in recreational waters as indicators of specific types of fecal contamination [14,65]. While several studies have demonstrated the value of the family Bacteroidales, some have also highlighted problems associated with cross-reactivity for certain markers, making testing and validation of assays particularly important [64, 65]. One of the most important advancements has been the development of the HF183 human specific markers that has been shown in a range of studies to be specific and relatively sensitive [66,67,68]. Acknowledging that no one marker can be used everywhere and in all circumstances, the HF183 marker has been atypical is that it has been successfully utilized more than any other single marker in application for detection and quantification of human fecal contamination in recreational waters. Recent work [69] using the HF183 marker has shown that data generated can be suitable for providing stakeholders and decision markers with valuable information for remediation projects [31].

Recently, another qPCR-based method has been developed by [70] to quantify fecal *Bacteroides* spp. The assay was specifically designed to select for the *Bacteroides* species that are most human-associated, but it also amplifies Bacteroides spp. from animals. The focus of the assay is on a cohort of *Bacteroides* species that are most prominent in the human gut. This method has been successfully used as a screening tool, with more specific methods used to quantify specific types of fecal contamination [71]. Another successful approach is reported by [71], where the authors developed an array of fecal *Bacteroides* based markers for a range of types of fecal contamination. They reported a quantitative universal *Bacteroidales* assay called BacUni-UCD that detected universal fecal *Bacteroidales*in all of the test samples (n=73) including human, cat, seagull, horse, dog and cow feces. The human assay BacHum-UCD presented by [68], successfully discriminated fully between human and cow stool samples and had slight cross-reactivity with dog stool, however, the method is highly quantitative when used in combination with human specific approaches outlined by [68]. All of the wastewater samples tested during the [68], study were positive for the BacHum-UCD marker, showing it to be 100 percent sensitive for human source identification. In 2009, Ahmed and co-workers [73] published a test of the host specificity of the five published sewage-associated *Bacteroides* markers. They tested 186 fecal samples from 11 animal species including humans. All of the human fecal samples were positive for all five markers indicating 100 percent sensitivity of the above referenced markers of [71], [68] and [72]. The HF183 marker has been found to have the capability to differentiate between human and animal feces with 99 percent accuracy [73]. It has been used in several mid-Atlantic stormwater studies to indicate the presence of human fecal contamination [74].

5.3. Virus based methods

Pathogen-based research for MST has focused almost solely upon pathogens that are carried via the fecal-oral route. The work that has been accomplished regarding enteric viruses has relied on the fact that high concentrations of these viral pathogens can be found in human sewage. High concentrations are a vital characteristic, given the limitations with concentration and isolation of pathogens in wastewater and complex aquatic matrices. In 2005, in a

review written by Fong and Lipp [75], the authors recount the major developments in the past decades, including applications of human and animal viruses to source tracking. The authors detail the disadvantages and advantages of virus-based detection and quantification methods. In particular, they present a summary of information of the myriad of animal viruses applied to source tracking studies for human fecal contamination, namely human enteroviruses (echovirus and Coxsackie virus for example) and adenoviruses (Ad5, 7, 40, and 41). The review is completed by iterating the need for strong study designs with the use of virus detection and quantification methods for microbial source tracking applications, due to the limitations with the ability to detect viral targets, which can often occur in aquatic systems at low concentrations and high patchiness.

Rajal and co-workers [76] presented work on the quantification of human and animal pathogenic viruses in real world water samples. They used a known sample volume and tracer addition of surrogate viruses to calculate the limit of detection. This work was one of the first to detail carefully the quantification and recovery in a tightly designed study. For any type of human pathogenic virus quantification, recovery estimations are vital. This can typically be done through the use of spiked samples or by adding surrogate DNA or RNA to the sample, or with the use of a competitive internal positive control (CIPC). In 2006, Gregory et al.[78] reported the first use of a CIPC for full quantification of RNA viruses for water quality applications. The point of this internal control was to permit assessment of the efficiency of the combined reverse-transcriptase and PCR steps to enterovirus quantification and to provide a scenario for CIPC design approaches for other virus types. Similar approaches were developed by [79] and have resulted in successful quantification of important human pathogenic enteroviruses in recent years.

While not all viruses will be infectious in a water sample, accurate quantification of viruses in a water sample along with flow measurements can permit loading estimates of potentially pathogenic material, specific to source, thereby permitting partitioning specific sources of fecal contamination in complex environments. Also, the accurate quantification of the human pathogenic viruses is an important parameter in the successful determination of the potential public health risk and can be a key component to Quantitative Microbial Risk Assessment (QMRA).

Recently, human polyomaviruses (HPyV) have emerged as a useful human-specific viral marker. There has been a series of studies conducted that report the presence and quantification of these viruses in raw sewage samples and/or environmental waters in sites ranging from Florida [82], California [82, 83], Australia [66], Spain [84], Germany [85] and Japan [86]. These viruses are double-stranded DNA viruses frequently isolated from urine, and in some cases feces, of both healthy and immunocompromised individuals. In a recent study conducted by [87], both nested PCR and qPCR were used to assess the presence of HPyV in raw and treated sewage in Rio de Janeiro, Brazil. The study interestingly demonstrated the clustering of different types of the HPyV coming from distinct African and European lineages. While this study was focused on samples from a sewage treatment plant, it demonstrates the specificity of information that can be gleaned from viral pathogen-based investigations.

http://dx.doi.org/10.5772/45847

A very recent publication by [87] has demonstrated the use of a range of MST approaches at a beach in southern California that has been the site of a range of water quality investigations (e.g. [43, 88]). Doheny State Beach, in Orange County, California, USA is a high priority, intensely utilized beach location that has suffered from a range of beach water quality problems, In the past decade, for example, the beach has been given a poor grade several times on the "Heal the Bay Beach Report Card" (www.healthebay.org). This study demonstrated the utility of the HF183 human specific marker, adenovirus, HPyV, and *Methanobrevibacter smithii* as markers of fecal contamination. They found distinct correlations among the highly human specific molecular markers, i.e. adenovirus at Doheny State Beach was correlated to both HPyVs and the HF183 molecular marker. This study dramatically presents the benefits of application of a range of microbial source tracking approaches (i.e. the use of the toolbox approach), as opposed to a single marker. It also demonstrates the complexity of real-world water quality investigations and the importance of conducting studies over appropriate time and sampling scales.

6. Conclusions

Over the past decades, the use of culture-based FIB based quantification approaches has dominated the management of microbial contaminants for drinking water, recreational water, and shellfish harvesting water quality. While these approaches are useful and have resulted in a dramatic improvement in water quality management over that observed prior to the 1950's, there is an array of new molecular approaches that can be used to bolster water quality management and the process of public notification (i.e. water advisories, beach posting and closings, and shellfish harvesting water closures).

Here, highlights have been presented of three areas for immediate advancement of the science. Rapid qPCR based methods are poised for future use to improve the accuracy of beach water quality postings and beach closures. Molecular assays specific for toxin producing strains of cyanobacteria, and other HAB species can be utilized to improve the management of our vital raw surface waters. Microbial source tracking techniques are poised to improve all facets of water quality management; from site specific investigations of the predominant fecal sources to assessment of the efficiency of wastewater treatment systems.

This is an exciting time for researchers given the fast evolution of molecular approaches. The design of appropriate methods, that are versatile, fast and reliable, can permit information to be transmitted to managers in shorter periods of time. The molecular methods will only improve in their cost effectiveness and user-friendliness in the next few years. Finally, the successful application of predictive multivariate models and formalization of QMRA approaches will improve dramatically with the infusion of the scale appropriate molecular information on microbial contaminants, sources of contamination and rates of discharge and loading.

Author details

Sandra Mesquita[1] and Rachel T. Noble[2]

1 Center Centre for Marine Sciences from Algarve (CCMAR), Campus de Gambelas, Portugal

2 Institute of Marine Sciences, University of Chapel Hill, North Carolina, Morehead City, USA

References

[1] Tallon P, Magajna B, Lofranco C and Leung KT. Microbial indicators of fecal contamination in water: a current perspective. Water, Air, and Soil Pollution, 2005; 166:139–166

[2] Bláha L, Babica P, Blahoslav. Toxins produced in cyanobacterial water blooms – toxicity and risks Interdisc Toxicol, 2009; 2(2): 36–41

[3] Standard methods for the examination of water and wastewater. Greenberg, A.E, Clesceri, L.S. and Eaton, A.D. (Eds). 18th edition; 1992;

[4] Balarajan R, Raleigh VS, Yuen P, Wheeler D, Machin D, Cartwright R.(1991) Health risks associated with bathing in seawater. British Medical Journal, 1991; 303: 1444

[5] Haile RW, Witte JS, Gold M, Cressey R, McGee C, Millikan RC, Glasser A, Harawa N, Ervin C, Harmon P, Harper J, Dermand J, Alamillo J, Barrett K, Nides M, Wang GY. The health effects of swimming in ocean water contaminated by storm drain runoff. Epidemiology, 1999; 10: 355-363

[6] Dufour AP, Ballentine P. Ambient water quality criteria for bacteria. Technical Report EPAA440/5-84-002. U.S. Environmental Protection Agency, Washington DC, 18, 1986

[7] Izbicki JA, Swarzenski PW, Reich CD, Carole Rollins C and Holden, PA. Sources of fecal indicator bacteria in urban streams and ocean beaches, Santa Barbara, California. Annals of Environmental Science, 2009; 3:139-178

[8] World Health Organization. Report of WHO Scientific Group of human viruses in water, wastewater and soil. Technical Report Series, no. 639. World Health Organization, Geneva, Switzerland, 1979

[9] Ikner LA, Gerba CP and Bright KR,. Concentration and Recovery of Viruses from Water: A Comprehensive Review on Food Environmental Virology, 2012;4:41–67

[10] Cao Y, John F Griffith JF, Samuel Dorevitch S and Stephen B Weisberg SB. Effectiveness of qPCR permutations, internal controls and dilution as means for minimizing

the impact of inhibition while measuring Enterococcus in environmental waters. Journal of Applied Microbiology 2012; 113(1):66-75

[11] World Health Organization. Report of WHO Guidelines for drinking water quality, I-recommendations. World Health Organization, Geneva, Switzerland, 2004;

[12] Figueras MJ and Borrego JJ.. New Perspectives in Monitoring Drinking Water Microbial Quality. Int. J. Environ. Res. Public Health, 2010; 7:4179-4202

[13] U.S. ENVIRONMENTAL PROTECTION AGENCY. Ambient Water Quality Criteria for Bacteria. EPA-440/5-84-002, U.S. Environmental Protection Agency, Washington, D.C., 1986

[14] Griffith JF, Cao Y, McGee CD, Stephen B. and Weisberg B. Evaluation of rapid methods and novel indicators for assessing microbiological beach water quality. Water Research, 2009; 43:4900 – 4907

[15] Harmsen, H J. M, Raangs GC, He T, Degener JE, and Welling GW. Extensive set of 16S rRNA-based probes for detection of bacteria in human feces. Applied and Environmental Microbiology, 2002; 68:2982–2990

[16] Leila Kahlisch l, Henne K, and Gröbe, L.. Assessing the Viability of Bacterial Species in Drinking Water by Combined Cellular and Molecular Analyses. Microb Ecol 2012; 63:383–397

[17] Eichler S, Christen R, Höltje C, Westphal P, Bötel J, BrettarI,Mehling A. and Höfle MG. Composition and dynamics of bacterial communities of a drinking water supply system as assessed by RNA- and DNA-based 16S rRNA gene fingerprinting. Applied and Environmental Microbiology, 2006; 72:1858–1872

[18] Paavola, J., Institutions and environmental governance: a reconceptualization. Ecological Economics 2007; 6, 1:93–103

[19] Orin C. Shanks, O C, Kelty,CA, Sivaganesan, M, Varma M and Richard A. Haugland, RA Quantitative PCR for Genetic Markers of Human Fecal Pollution, Applied and Environmental Microbiology, 2009;.75, 17:5507–5513

[20] U.S. ENVIRONMENTAL PROTECTION AGENCY, Ambient Water Quality Criteria for Bacteria. EPA-440/5-84-002, U.S. Environmental Protection Agency, Washington, D.C., 1986

[21] Dafour, AP. Health Effects Criteria for Fresh Recreational Waters. EPA-600/1-84-004, U.S. Environmental Protection Agency, Research Triangle Park, N.C., 1984

[22] Davies, CM, Long, JA, Donald, M and N. J. Ashbolt, NJ, Survival of fecal microorganisms in marine and freshwater sediments. Applied and Environmental Microbiology 1995; 61:1888–1896

[23] Layton, A., McKay L,Williams D, Garrett V, Gentry, R and Sayler. G. Development of Bacteroides 16S rRNA gene TaqMan-based real-time PCR assays for estimation of to-

tal, human, and bovine fecal pollution in water. Applied Environ Microbiology 2006; 72:4214–4224

[24] Matsuki T, Watanabe K, Fujimoto J, Takada T, and Tanaka, R. Use of 16S rRNA gene-targeted group-specific primers for real-time PCR analysis of predominant bacteria in human feces. Applied and Environmental Microbiology, 2004; 70:7220–7228

[25] Boehm, AB, Ashbolt, NJ. A sea change ahead for recreational water quality criteria. Journal of Water Health 2009; 7, 1: 9-20

[26] Gentry, R W, Layton AC, McKay LD, McCarthy JF, Williams DE, Koirala SR, and Sayler GS. Efficacy of Bacteroides measurements for reducing the statistical uncertainty associated with hydrologic flow and fecal loads in a mixed use watershed. Journal of Environmental Quality, 2007; 36:1324–1330

[27] Figueras MJ, Robertson W, Pike E and Borrego JJ. Sanitary inspection and microbiological water quality. In: Monitoring Bathing Waters. A Practical Guide to the Design and Implementation of Assessments and Monitoring Programs; Bartram J. and Rees G (Eds), E & FN Spon: London, UK, 2000; pp. 113-126

[28] Fielda KG and Samadpourb M. Fecal source tracking, the indicator paradigm, and managing water quality. Water Research, 2007; 41: 3517-3538

[29] Environmental Health Directorate – Health Protection Branch. Recreational Water Quality-Published by Authority of the Minister of National Health and Welfare, 1978;

[30] Kornacki JL and Johnson JL. Enterobacteriaceae, Coliforms and *Escherichia coli* as quality and safety indicators'. In: F. Downs (Eds), Compendium of Methods for the Microbiological Examination of Foods, APHA. Washington DC, 2001;

[31] World Health Organization. Guidelines for drinking-water quality, 3rd ed., vol. 1. World Health Organization, Geneva, Switzerland; 2008

[32] WHO, Guidelines for Drinking-Water Quality, 2nd edition Geneva, 1993; ISBN 92 4 154460(Albinana-Gimenez et al. 2009), Germany (Hamza et al. 2009) and Japan (Haramoto et al. 2010)

[33] Chorus I and Bartram J (Eds.). Toxic cyanobacteria in water: a guide to their public health consequences, monitoring and management. E&FN Spon, ISBN 0-419-23930-8, London, 1999;

[34] Izbicki JA, Swarzenski PW, Reich CD, Carole Rollins C and Holden, PA. Sources of fecal indicator bacteria in urban streams and ocean beaches, Santa Barbara, California. Annals of Environmental Science, 2009; 3:139-178

[35] Whitman RL, Przzybyla-Kelly K,Shivley DA and Byappanhali MN. Incidence of the enterococcal surface protein (esp) gene in human and animal fecal sources. Environ SciTechnol 2007; 41(17):6090–6095

[36] Dafour, AP. Health Effects Criteria for Fresh Recreational Waters. EPA-600/1-84-004, U.S. Environmental Protection Agency, Research Triangle Park, N.C., 1984

[37] Leecaster MK and Weisberg SB. Effect of Sampling Frequency on Shoreline Microbiology Assessments. Marine Pollution Bulletin, 2003;42(11): 1150-1154.

[38] Pisciotta J, et al. The role of neutral lipid nanospheres in *Plasmodium falciparum* crystallization. Biochem J, 2007, 402:197–204.

[39] Haugland RA, Siefring SC, Wymer LJ, Brenner KP and Dufour AP. Comparison of Enterococcus density measurements by quantitative polymerase chain reaction and membrane filter culture analysis at two freshwater recreational beaches. Water Research, 2005; 39:559-568.

[40] Griffith JF and Weisberg SB. Challenges in Implementing New Technology for Beach Water Quality Monitoring: Lessons from a California Demonstration Project. Marine Technology Society Journal 2011; 45:65–73

[41] Noble RT, Blackwood AD, Griffith JF, McGee CD and Weisberg SB. Comparison of Rapid Quantitative PCR-Based and Conventional Culture-Based Methods for enumeration of *Enterococcus* spp. and *Escherichia coli* in Recreational Waters. Applied and Environmental Microbiology, 2010; 76(22):7437-7443

[42] Wade TJ, Calderon RL, Brenner KP, Sams E, Beach M, Haugland R, Wymer L and A. P. Dufour AP. High Sensitivity of Children to Swimming-Associated Gastrointestinal Illness Results Using a Rapid Assay of Recreational Water Quality. Epidemiology, 2008; 19: 375-383

[43] Colford JM, Schiff K, Griffith JF, Yau V, Arnold BF, Wright C, Gruber J, Wade T, Burns S, Hayes S, McGee C, Gold M, Noble RT and Weisberg, SB. Using rapid indicators for Enterococcus to assess the risk of illness after exposure to urban runoff contaminated marine water. Water Research, 2012; 1-11.

[44] Scott TM, Jenkins TM,, Lukasik J and Rose JB. Potential use of a host-associated molecular in *Enterococcus faecium* as an index of human fecal pollution. Environmental Science and Technology, 2005; 39 (1):283–287.

[45] McQuaig SM, Scott TM, Harwood VJ, Farrah SR and Lukasik JO. Detection of human-derived fecal pollution in environmental waters by use of a PCR-based human polyomavirus assay. Applied and Environmental Microbiology, 2006; 72(12):7567–7574

[46] Lavender JS and Kinzelman JL. A cross comparison of QPCR to agar-based or defined substrate test methods for the determination of *Escherichia coli* and enterococci in municipal water quality monitoring programs. Water Research 2009; 43(19): 4967-4979

[47] Tsai YL and. Olson BH. Rapid method for separation of bacterial DNA from humic substances in sediments for polymerase chain reaction. Applied and Environmental Microbiology, 1992; 479(58):2292-2295

[48] De Boer SH, L.J. Ward LJ, X. Li X and S. Chittaranjan. S. Attenuation of PCR in- hibition in the 411 presence of plant compounds by addition of BLOTTO. Nucleic Acids Research, 1995; 23:2567-2568

[49] Kreader CA. Relief of amplification inhibition in PCR with bovine serum albumin or T4gene 32 protein. Applied and Environmental Microbiology, 1996; 62:1102-1106

[50] Watson RJ and B. Blackwell. Purification and characterization of a common soil component which inhibits polymerase chain reaction. Canadian Journal of Microbiology, 2000; 46:633-642

[51] Thurman EM, Aiken GR, Ewald M, Fischer WR, Forstner U, Hack AH, Mantoura RFC, Parsons JW, Pocklington R, Stevenson FJ, Swift RS and SzpakowskaB.Isolation of soil and aquatic humic substances. pp. 31-43 in: F.H. Frimmel and R.F. Christman (ed.), Humic Substances and Their Role in the Environment. John Wiley and Sons, Ltd. New York, NY, 1988

[52] Cao Y, Griffith JF and Weisberg SB. Evaluation of optical brightener photo decay characteristics for detection of human fecal contamination. Water Research, 2009; 49 (8):2273-227

[53] Bushon RN, Brady AM, Likirdopulos CA and Cireddu JV. Rapid detection of *Escherichia coli* and enterococci in recreational water using an immunomagnetic separation⁄ adenosine triphosphate technique Journal of Applied Microbiology, 2009;106: 432–441

[54] Lee CM, Griffith JF, Kaiser W and Jay, JA. Covalently linked immune magnetic separation⁄ adenosine triphosphate technique (Cov-IMS⁄ATP) enables rapid, in-field detection and quantification of *Escherichia coli* and Enterococcus spp. in freshwater and marine environments. Journal of Applied Microbiology, 2009; 109:324–333

[55] Lee JY. and Deininger RA. Detection of *E-coli* in beach water within 1 hour using immunomagnetic separation and ATP bioluminescence. Luminescence 2004; 19:31–36

[56] Wiegand C and Pflugmacher S. Ecotoxicological effects of selected cyanobacterial metabolites a short review. Toxicology and Applied Pharmacology, Vol. 203, (March 2005); pp. 201-218, ISSN 0041-008X

[57] Al-Tebrineh J, Leanne A. Pearson LA, Serhat A. Yasar SA and Brett A. Neilan BA. A multiplex qPCR targeting hepato- and neurotoxigenic cyanobacteria of global significance. Harmful Algae, 2012; 15:19–25

[58] Sivonen K and Jones G. Cyanobacterial toxins. In: Toxic cyanobacteria in water: a guide to public health significance, monitoring and management. Chorus I and Bar-

tram (Eds).The World Health Organization, ISBN 0-419-23930-8, E and FN Spon, London, UK, 41-111pp, 1999

[59] Conradie KR and Barnard S. The dynamics of toxic *Microcystis* strains and microcystin production in two hypertrofic South African reservoirs. Harmful Algae 2012; in press

[60] Henderson, Chips M, Cornwell, Hitchins P, Holden B, Hurley S, Parsons SA, Wetherill A and Jefferson B. Experiences of algae in UK waters: a treatment perspective. Water and Environment Journal, 2008; 22:184–192

[61] Pinckney JL, Richardson TL, Millie DF, Paerl HW. Application of photopigment biomarkers for quantifying microalgal community composition and in situ growth rates. Organic Geochemistry, 2001; 32: 585–595

[62] Simpson JM, Santo Domingo JW and Reasoner DJ. Microbial source tracking: state of the science. Environmental Science and Technology 2002; 36:5279–5288

[63] Noble RT and J. A. Fuhrman, JA. Enteroviruses detected in the coastal waters of Santa Monica Bay, CA: Low correlation to bacterial indicators. Hydrobiologia, 2001;460:175-184

[64] Gentry, R W, Layton AC, McKay LD, McCarthy JF, Williams DE, Koirala SR, and Sayler GS. Efficacy of Bacteroides measurements for reducing the statistical uncertainty associated with hydrologic flow and fecal loads in a mixed use watershed. Journal of Environmental Quality, 2007; 36:1324–1330

[65] Boehm, AB, Ashbolt, NJ. A sea change ahead for recreational water quality criteria. Journal of Water Health, 2009; 71: 9-20

[66] Ahmed W, Stewart J, Powell D and Gardner T. Evaluation of Bacteroides markers for the detection of human faecal pollution. Letters in Applied Microbiology, 2008; 46(2): 237-242

[67] Ahmed W, Goonetilleke A, Powell D, Chauhan K and Gardner T. Comparison of molecular markers to detect fresh sewage in environmental waters. Water Research, 2009;43:4908-4917

[68] Seurinck S, Defoirdt T, Verstraete W and Siciliano SD.Detection and quantification of the human-specific HF183 Bacteroides 16S rRNA genetic marker with real-time PCR for assessment of human faecal pollution in freshwater. Environmental Microbiology, 2005; 7(2):249-59

[69] Mieszkin S, Furet JP, Corthier G, and M. Gourmelon M. Estimation of pig fecal contamination in a river catchment by real-time PCR using two pig-specific Bacteroidales 16S rRNA genetic markers. Applied and Environmental Microbiology 2009; 75:3045–3054

[70] Converse RR, Blackwood AD, Kirs M, Griffith JF and Noble RT, Rapid QPCR-based assay for fecal Bacteroides spp. as a tool for assessing fecal contamination in recreational waters. Water Resources, 2009; 43:4828–4837

[71] Kildare BJ, Leutenegger CM, McSwain SM, Bambic DG, Rajal VB and Wuertz S. 16S rRNA-based assays for quantitative detection of universal, human-, cow-, and dog-specific fecal Bacteroidales: A Bayesian approach. Water Research, 2007; 41:3701-3715

[72] Bernhard A and Field, KG. Identification of nonpoint sources of fecal pollution in coastal waters by using host-specific 16S ribosomal DNA genetic markers from fecal anaerobes. Applied and Environmental Microbiology, 2000; 66:1587–1594

[73] Ahmed W, Goonetilleke A, Powell D, Chauhan K and Gardner T. Comparison of molecular markers to detect fresh sewage in environmental waters. Water research, 2009; 43:4908-4917

[74] Parker JK, McIntyre D and Noble RT. Characterizing fecal contamination in stormwater runoff in coastal North Carolina, USA. Water Resources, 2010; 44(14):4186-4194

[75] Fong TT and Lipp EK. Enteric viruses of humans and animals in aquatic environments: health risks, detection, and potential water quality assessment tools. Micro Mol Biol Rev, 2005; 69(2):357-371

[76] Rajal, McSwain BS, Thompson DE, Leutenegger CM, Wuertz, S. Molecular quantitative analysis of human viruses in California stormwater. Water Research, 2007; 41(19):4287-4298

[77] Wong M, Kumar L, Jenkins TM, Xagoraraki I, Phanikumar MS and Rose JB, (2009) Evaluation of public health risks at recreational beaches in Lake Michigan via detection of enteric viruses and a human-specific bacteriological marker. Water re- sources, 2009; 43:1137-1149

[78] Gregory JB, Litaker RW and Noble RT. Rapid One-Step Quantitative Reverse Transcriptase PCR Assay with competitive internal positive control for detection of Enteroviruses in environmental samples. Applied and Environmental Microbiology, 2006; 72(6):3960-3967

[79] Fuhrman JA, Liang X and Noble RT. Rapid Detection of Enteroviruses in Small Volumes of Natural Waters by Real-Time Quantitative Reverse Transcriptase PCR Applied and Environmental Microbiology, 2005; 71(8): 4523–4530

[80] Baums L, Goodwin KD, Kiesling T, Wanless D, Diaz MR, Fell, JW. Luminex detection of fecal indicators in river samples, marine recreational water, and beach sand. Marine Pollution Bulletin, 2007; 54 (5):521-536

[81] Carson CA, Christiansen JM, Yampara-Iquise H, Benson VW, Baffaut C, Davis JV, Broz RR, Kurtz WB, Rogers WM and Fales WH. Specificity of *Bacteriodes thetaiotaomicron* marker for human feces. Applied and Environmental Microbiology, 2005; 71(8):4945-4949

[82] McQuaigSM , Scott TM , Lukasik JO , Paul JH , Harwood VJ . Quantification of human polyomaviruses JC virus and BK virus by TaqMan quantitative PCR and comparison to other water quality indicators in water and fecal samples .Applied and Environmental Microbiology 2009; 75: 3379-3388

[83] Rafiquea and Jiang, CS. Genetic diversity of human polyomavirus JCPvV in Southern California wastewater. Journal of Water Health, 2009; 6:533-538

[84] Albina-Gimenez N, Clemente-Casares P, Calgua B, Huguet, JM, Courtois S and Girones R. Comparison of methods for concentrating human adenoviruses, polyomavirus JC and noroviruses in source waters and drinking water using quantitative PCR. J Virol Methods, 2009; 158: 104-109

[85] Hamza IA, Jurzik L, Stang K, Uberla K and Wilhem M. Detection of human viruses of a densly-populated area in Germany using virus adsorption method optimized for PCR analyses. Water Resources, 2009; 43:2657-2668

[86] Haramoto E, Kitajima M, Katayama H and Ohgaki S. Real-time pCR detection of adenoviruses, polyomaviruses and torque viruses in river water in Japan. Water resources, 2010; 44:1747-1752

[87] Fumian TM, Guimarães FR, Pereira Vaz BJ, da Silva MT, Muylaert FF, Bofill-Mas S, Gironés R, Leite JP and Miagostovich MP. Molecular detection, quantification and characterization of human polyomavirus JC from waste water in Rio De Janeiro, Brazil, 2010; 8(3):438-45

[88] McQuaig S, Griffith J and Harwood VJ. The Association of Fecal Indicator Bacteria with Human Viruses and Microbial Source Tracking Markers at Coastal Beaches Impacted by Nonpoint Source Pollution Applied and Environmental Microbiology AEM.00024-12; published ahead of print 6 July 2012

IntechOpen

IntechOpen