# Chapter 2

# The need for speed – rapid evolution of microbiological testing in drinking water

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#### 2.1 INTRODUCTION

The detection of microorganisms in water has occurred for millennia. Initially, it happened incidentally – ancient cultures observed that when water was boiled prior to consumption, the incidence of sickness was dramatically reduced. They did not fully understand why this occurred, but the practice has continued to this very day as a reliable method for the disinfection of water. In the 19th century, the roots of modern water microbiology were laid through the work of John Snow in the City of London. Snow's work enabled others to conclusively link the discharge of sewage from overloaded cesspools into the Thames river to outbreaks of cholera and typhoid fever, dispelling the popular belief that these outbreaks were rooted in clouds of sickness descending upon the city, otherwise known as Miasmatic Theory (Halliday, 2001). Simultaneously, cities around the world were installing water filters to remove sediment from water sources prior to its delivery for consumption, mainly to improve esthetic qualities (primarily suspended sediment, taste and odor) but also indirectly removing at least some microbiological contaminants. At the same time, scientists including Robert Koch and Louis Pasteur were pioneering the field of microbiology via microscopes and culture tests – and thus the science of microbiological sensors

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doi: 10.2166/9781780408699\_0015

was born. Into the 20th century, the widespread adoption of microbiological testing, coupled with water filtration and later chlorination, resulted in the most significant decline in mortality rates in modern history (Cutler & Miller, 2005).

Despite early rapid advances, microbiological testing methods such as plategrowth methods remained largely unchanged until the mid-20th century, when biochemical and molecular-based test methods were first developed. The reasons for these developments were many: a desire for more rapid results, greater specificity, greater objectivity and ease-of-use by non-skilled workers, and the continual search for the "holy grail" - sensors embedded on-line in the water distribution system that can detect and characterize bacteriological targets in real-time, all the time. By the early 21st century, science had entered the golden age of microbiological test method development with a multitude of different technology options for achieving one or more of the above-mentioned goals. However, scientists have still not found the "perfect" sensor technology, and it is not obvious that a single technology is emerging as the leading contender for widespread deployment in public water supply networks. Furthermore, there is a wide gap between the available technology, end-user capability to deploy and manage sensor networks, and the nature of the regulatory compliance environment such that even if the perfect microbiological sensor existed, it would be a struggle to deploy rapidly across the industry.

This chapter will provide both a historical review of microbiological detection technologies as well as an overview of selected biosensor technologies for water quality monitoring, with a focus on the challenges that must be overcome to ensure the successful deployment of advanced microbiological sensor technologies in water supply networks.

# 2.2 ANCIENT AND MEDIEVAL TIMES – EARLY MICROBIOLOGICAL SENSING

Without water, life cannot not exist. It is therefore no surprise that human civilization developed close to sources of fresh water. Though water quantity was typically the deciding factor in where communities were founded, records indicate that our forerunners realized the benefits of a high-quality water supply thousands of years ago. For example, Sanskrit writings document the use of charcoal (now activated carbon) (Enzler, 2018) and Egyptian hieroglyphs mention the use of alum (USEPA, 2000), both of which are still in use today (Figure 2.1). Greek writings and even the Bible mention the use of filters to remove impurities (APEC Water, 2013). In general, these treatments were used to improve the aesthetic quality of water and led to the theory that if it is clean, it is safe to drink.

Unfortunately, that theory was not necessarily always true due to the hidden microbiological threats that lurked beyond view of the human eye. Indeed, other cultures such as the Chinese inadvertently discovered how to make water microbiologically safe through other means – that being, tea. By boiling water to make tea, they also disinfected the water. In Medieval Europe, there was at least an anecdotal awareness

of microbiological contamination risks in even clean-appearing water that led many to quench their thirst with wine or beer (Harris & Grigsby, 2009). In some ways, these actions were in response to results gleaned from the first microbiological sensors – gross observation of the impact contaminated water had on other people.



**Figure 2.1** Drawings on the walls of Egyptian rulers Amenophis II and Rameses II (APEC Water, 2013).

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Gross observation also led civilizations to seek sources of water elsewhere when waterborne illness occurred. Similarly, when water quantities became limited and began to restrict the growth of cities, methods were developed to bring in additional sources of safe water. In Rome a series of aqueducts (Figure 2.2) were developed over a 500-year period and allowed it to become the largest city of its time, far larger than the water resources within its periphery were able to sustain both from a perspective of quantity and quality (Enzler, 2018).

During the industrial revolution, western civilization grew at an exponential rate – especially in urban centres. This put pressure on these major population centres to supply sufficient quantities of clean water to sustain that population growth. Some cities resorted to building extensive water supply networks to bring water from far away locations, similar to the Roman aqueducts. Others began experimenting with methods that could convert contaminated water into clean water, such as slow sand filters which were first deployed in Scotland in the early 1800s (Blake, 1956). Neither of these solutions, however, brought forth a direct and impactful public awareness to the risks of microorganisms in drinking water supplies and their linkage to the water cycle. Ironically, it was the birth of the water closet that did exactly that.



Figure 2.2 Roman Aqueducts (Cartwright, 2012).

# 2.3 19TH CENTURY – LINKING THE WATER CYCLE TO HUMAN HEALTH

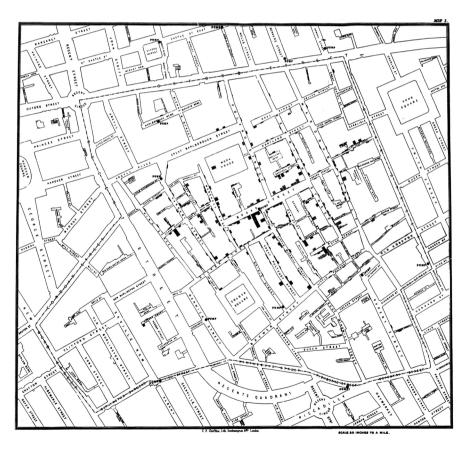
In 19th century England, the cesspit was a ubiquitous piece of infrastructure designed to capture and store human waste underground (Morris, 2009). The next phase of waste-handling technology, the water closet – invented in the late 18th century (Hardy, 1984) – was becoming more popular. To handle the elevated water flows from this new apparatus, city planners and engineers began to install modern sewer systems to allow these cesspits to drain more rapidly and not overflow. The drainage point of these systems were natural bodies of water, such as the Thames river.

Starting in the 1830s and continuing through to the 1860s, large swathes of London were overcome by outbreaks of cholera (Morris, 2009). The leading theory of the day was that a cloud of sickness had descended upon the city. This Miasmatic Theory (Halliday, 2001) had been used to explain such epidemics in the past, which seemed to come and go with the changing of the seasons. This time, however, the outbreaks did not stop despite seasonal change.

The physician John Snow began to explore the situation with great interest. His research began in 1849 and came to a head with the 1854 Soho epidemic (Morris, 2009). He found that most instances of illness and resulting fatalities occurred

near certain cisterns. He further found that fatalities not in that specific area often correlated with families that obtained water from the same cisterns, mainly on the basis that they preferred the taste of that water. He thus concluded that this was not a case of miasma outbreaks; rather, it was a waterborne outbreak.

After several months of investigation, two sources of contamination were identified. First, the integrity of some cesspits had failed, with their contents leaching into cisterns within their proximity. Second, the water source of some cisterns (namely, those being fed by draw points from the Thames river used by the Southwark and Vauxhall Waterworks Company) were located downstream of sewer system discharge points. It was thus concluded that the source of these outbreaks was human faecal material. Snow's study (Figure 2.3) was a major turning point in the history of public health and is regarded as the founding event of the science of epidemiology (Morris, 2009).



**Figure 2.3** Original map of cholera cases in the Soho epidemic of 1854 (Barton, **EDQ1** 2018).

Snow's revolutionary work instigated significant political controversy, and his theories were not widely accepted by the time of his death in 1858. However, further debate and study resulted in full acceptance even by his most prominent opponents such as William Farr in 1866. In the following years, work done by other prominent researchers including Louis Pasteur (France) and Robert Koch (Germany) led to the birth of today's most common microbiological sensors: microscopes and growth-based culture tests. It came to be in the 1880s that the Germ Theory of disease overtook Miasmatic Theory as the leading explanation for human infection (USEPA, 2000). A "golden era" of bacteriology ensued, in which the theory quickly led to the identification of the actual microorganisms that cause many diseases, waterborne or otherwise.

On the back of these discoveries, several advances in water and wastewater treatment gained increasing adoption. Filtration was identified as a means to improve water quality prior to human consumption. Slow sand filtration was developed in the United Kingdom in the early 1800s and improved upon in the United States as rapid sand filtration in the late 1800s (Melosi, 2000). At the same time, mechanisms to contain human waste and prevent contamination of drinking water sources were developed, such as the activated sludge process in the early 1900s (Beychok, 1967). These factors combined to provide a multi-barrier approach against the threat of microbiological contamination in drinking water supplies, but they alone were not enough to mitigate the risk to acceptable levels.

Chlorination was first proposed in 1902 and was met with heavy resistance from the public, which feared the concept of 'doping' their drinking water (Enzler, 2018). Resistance to this was all but overcome in 1916 when the city of Milwaukee lost chlorination for a period of 7 hours due to operator error, resulting in 60 fatalities and over 100,000 illnesses (Becker, 1974). The resulting widespread adoption of chlorination by villages, towns and cities in the United States reduced the incidence of cholera and typhoid from 100 to 0.1 cases per 100,000 inhabitants over the first half of the 20th century, which is often referred to as the single greatest achievement in public health improvement in the 20th century (CDC, 2012; Cutler & Miller, 2005).

# 2.4 20TH CENTURY – ESTABLISHMENT OF REGULATORY FRAMEWORKS

By the early 1900s, public awareness about the risks associated with microorganisms in drinking water supplies had been established, as had the understanding of the water cycle and its impact on water quality. The major causes of waterborne disease had been identified and robust solutions for their prevention had been developed along with the means to confirm their effectiveness. All the tools were therefore available to solve the problem; it was now a matter of ensuring wide adoption through laws and regulations. The first example of such regulations was laid in the mid-19th century when the City of London passed the Metropolis Water Act of 1852 to ensure that all water supplied to the city would be filtered (City of London, 1852).

In 1914, the Congress of the United States passed the Clean Water Act, which mandated certain minimum provisions around water treatment and management (USEPA, 2000). In the 1960s, these regulations were strengthened primarily in response to deteriorating source water quality caused by waste discharged from rapidly growing industrial production. This led to a major overhaul of water regulations in the form of the Safe Drinking Water Act in 1974 (USEPA, 2000). At this point microbiological quality issues had given way to mostly chemical issues as the primary concern, such as industrial wastewater discharge causing contamination in the form of eutrophication. Later it was recognized that an over-reliance on chlorination to address microbiological concerns had created another problem in the form of disinfection by-products, which are created by the interaction of disinfectants such as chlorine with organic matter present in source water.

In general, modern regulations prescribe the following preventative measures and testing methods to guard against microbiological contamination in most countries around the world.

#### 2.4.1 Pressure

Water authorities are required to verify the maintenance of a positive pressure within their water supply at all times to prevent the infiltration of external contamination. However, it is recognized that maintenance of a positive pressure at all points within a water distribution system at all times is an impossible objective; hence, the quality of water within the distribution system must also be monitored on a constant basis (Snoeyink, 2006).

# 2.4.2 Turbidity

Turbidity is used as a general indication of contamination from soil or other debris that may imply the introduction of microorganisms. Though this technique can be deployed online to deliver results continuously and in real-time, it is not economically possible to cover all parts of the distribution system, nor is it possible to distinguish microbiological constituents from non-biological constituents (Schilz, 2018).

#### 2.4.3 Disinfectant residual

Many operators carry out their duties under the assumption that if one maintains a measurable disinfectant residual, then microorganisms cannot exist. While there is logic to this thought, it is not a panacea – owing to the nature of microorganisms, it is possible to have both a measurable disinfectant residual and living microorganisms in a given sample. Furthermore, depending on the disinfectant used, water chemistry may have a substantial impact on its efficacy, such as the mechanisms by which chlorine is impacted by pH (Hydro Instruments, 2010). Finally, not all disinfectants provide a residual (e.g. ultraviolet radiation), nor can all residuals be easily measured (e.g. non-oxidizing biocides), and this philosophy cannot cover situations where

disinfectants are not used in the water system (e.g. private wells, small towns, and certain countries).

#### 2.4.4 Faecal indicators

Once it became known that waste was the primary source of waterborne illness, it became easier to design microbiological tests that would detect the presence of microorganisms associated with faecal matter. The work of Koch, Pasteur, and others served as the basis for early test methods, including the Heterotrophic Plate Count (Bartram *et al.*, 2003). As use became more widespread, however, it became apparent that the rate of false positives in indicating faecal contamination was unacceptably high. This was due to the fact that this category of microorganism is one of the more prevalent in the natural environment and hence in water in general. Therefore, researchers developed more specific test methods such as tests for only coliform bacteria or even more specific still, tests for only *E. coli* (which is most closely linked to faecal matter). These tests are the primary two relied upon today by water consumers around the world to indicate safe water and are embodied in easy-to-use methods from a variety of manufacturers that are readily commercially available. One example is the IDEXX Colilert-18 test that can detect and quantify *E. coli* and coliform bacteria after incubation for 18 hours (IDEXX, 2018).

Despite the robust evolution of water cycle management, treatment processes, monitoring programs and regulatory frameworks, access to clean drinking water is not yet universal – and where it is not readily available, the impact on public health can be devastating. The World Health Organization estimates that 2.1 billion people lack access to safe drinking water, twice that many lack adequate sanitation, and that contaminated drinking water causes over 500,000 diarrhoeal deaths each year (WHO, 2017). Clearly, civilization still has significant progress to make in terms of achieving 100% coverage in clean water and sanitation services – and not just in bringing it to those parts of the world where it does not currently exist.

#### 2.5 21ST CENTURY - A NEW PARADIGM

Much has changed since the days of John Snow. Most of the world's population has access to clean and safe drinking water, with a significant proportion paying very affordable rates to have that clean water delivered directly to their home. Treatment processes are more affordable and accessible, and the basic monitoring tools are simple enough to be used by almost anyone. However, even where sophisticated drinking water infrastructure and management programs exist, there are some fundamental problems rapidly approaching that are forcing the sector to re-evaluate priorities.

# 2.5.1 Aging infrastructure and shifting demand

Water infrastructure in the western world is aging and subsequently deteriorating. This is leading to greater and greater rates of water loss in modern cities. Not only

is this a waste of water and therefore money, it also represents a significant hazard. If water can leak out of the distribution network, contaminant-laden water can also potentially enter. Increasing ingress will invariably lead to a deterioration in water quality, which will lead to an increased rate of biofilm formation, the inevitable consequences of which will include microbiologically influenced corrosion, aesthetic (i.e. taste and odor) issues, or worse, human health impacts (Qureshi & Shah, 2014).

Furthermore, population growth is slowing down in some areas of the world while speeding up in others. Not only does this mean that water distribution infrastructure is being put under different and sometimes conflicting pressures, but utilities are also being forced to seek alternative water sources of questionable microbiological quality, such as direct potable reuse (Gale, 2018) or seawater desalination (Gleick, 2018). Put simply, systems that were designed for one purpose in the past may not be fit for their required purpose at present, not to mention that new infrastructure will be required in new major population centres. These factors all combine to create major operating and capital budgetary challenges (Qureshi *et al.*, 2014).

### 2.5.2 Changing workforce

The demographics of the water sector are changing rapidly (Brueck *et al.*, 2010). Many experienced water operators and engineers are retiring and taking with them substantial institutional relating to operation and data interpretation best practices. This leaves water utilities in a difficult situation given ever-constrained operating budgets as to how they secure equivalent knowledge moving forward.

#### 2.5.3 Consumer awareness

Consumers are becoming more aware of and informed about the products they use and consume, including the water they use and consume. There is however no established educational resource to teach the public about what they should expect from their water supply outside of the obvious (i.e. water should be available when needed and it should be safe to use). Consumers can also connect with one another instantly via social media. This means that if a customer perceives a failure in water quality – whether they are correct or not – this information or misinformation can be disseminated widely nearly instantaneously.

Thus, improved engagement with and education of the public is of paramount importance. This problem will only become more pressing as sensor technology becomes less and less expensive and becomes integrated with point-of-use filtration systems and appliances, as many consumers will not be sufficiently informed in how to understand the data. Given these facts, it is inevitable that elevated consumer awareness will influence the evolution of regulatory frameworks in terms of the quantity, frequency and subsequent reporting of measurements made, which will add to water utility operating costs.

# 2.5.4 Evolving science

Over the past several decades, scientists, engineers and regulators have recognized that there is a myriad of waterborne threats to human health in addition to coliforms and *E. coli* (Krewski *et al.*, 2004). These include organisms such as cryptosporidium, giardia, enterococci, algae, amoeba, legionella and more. Additionally, there are several nuisance microorganisms that can affect water quality and aesthetics, such as nitrifying bacteria, corrosion-causing bacteria, and those contributing to odoriferous compounds such as geosmin and 2-methylisoborneol. Owing to the rapid advances in genomic technologies, this list is growing exponentially, and it is inevitable that new threats will be discovered that need to be monitored in the future, again adding to the costs of water utility operation.

While the incidence of waterborne disease has been dramatically reduced in many parts of the world through basic water cycle management, treatment, and monitoring, there are fundamental shifts in infrastructure availability, demographics, and awareness that create new challenges. Evolving science and consumer awareness will require the sector to advance its monitoring capabilities to cover a broader spectrum of contaminants more frequently, but this must be done in the context of limited budgets and workforce turnover all while continuing to drive adoption of the fundamentals in underrepresented parts of the world. While difficult, this is not an impossible task given the great many technology advancements made in recent times to enable civilization to increase productivity – in other words, to accomplish more given less time.

#### 2.6 THE ECONOMICS OF TIME IN DRINKING WATER

There are many economic challenges facing the water sector in the early 21st century, including both capital and operating cost constraints. Given these pressures and recognizing that regulatory framework modifications will take time, the advancement and adoption of microbiological sensors requires a firm economic argument. In that vein, there is perhaps no more obvious area that the sector can benefit from the adoption of new microbiological sensors than in improving productivity and thus available time.

Current practices for microbiological problem recognition and resolution are reactive in nature. This is in large part due to the industry standard microbiological test being the culture test (WHO, 2006), which requires microorganisms to grow from presumptive single cells to entire colonies so that they can be counted, either through colorimetric indication or counted by the human eye. At best, the timeframe to obtain results from such tests is 1 day, but in many cases can be as much as a week. Furthermore, these tests tend to be designed for specific threats (e.g. faecal contamination) and will subsequently ignore most microorganisms.

Waiting at least a day to obtain results creates a few several side effects. Firstly, microbiological contamination is unlikely to remain static while waiting for results – contamination may grow, relocate or even dissipate while waiting for

results. This means that the results once obtained may not reflect present state in the process. Secondly, the delay in obtaining results may lead to an ineffective use of resources such as with operators being prevented from doing further work or with water supplies remaining shut during the delay, such as in the case of line breaks and repairs. The following sections outline several benefits that water utilities would attain by having more rapid microbiological testing results.

#### 2.6.1 Water and chemical conservation

Knowing sooner as to the location and extent of contamination could help to ensure that operators apply just the right amount of remedial treatment, for instance additional chemical disinfectant or flushing or both. This approach can save in the consumption of chemicals and the wasting of water, both of which have a real cost for the utility. For example, optimization of annual flushing activities to reduce their duration to when the objective (i.e. scouring of biofilm) has been achieved – rather than running for a prescribed period as done in most cases – has been shown to equate to \$54,000 in annual savings for a utility serving 20,000 customers (Whalen *et al.*, 2014).

#### 2.6.2 Time and travel conservation

Being able to have results on-site and in near-real-time could save on operator time and travel expense costs for water operators to carry out investigations and/or apply remedial treatment, such as in instances of customer complaints about aesthetic issues. A shorter time-to-result could also enable shorter time-to-action, thus avoiding having to go back-and-forth to the location of contamination. Furthermore, performing testing on-site would eliminate concerns around changes in microbiological characteristics of samples during transportation and storage. This could provide real reductions in operating costs through primarily less operator time and transportation fuel costs being used in problem-solving exercises. One reference has identified the potential for annualized savings of \$50,000 for a utility serving an average-size municipality serving 10,000 customers (Whalen, 2016).

# 2.6.3 Boil Water advisory mitigation

Adopting a more proactive approach to drinking water management could improve the ability to mitigate waterborne outbreaks such as those in Walkerton, Ontario in 2000 (Salvadori *et al.*, 2009) and Milwaukee, Wisconsin in 1993 (Gradus, 2014) through reducing their duration and thus their impact, or by their outright prevention. Boil water advisories can result in a massive expense to water utilities directly through the cost of remedial actions and substitute supplies as well as indirectly through reputation impacts or worse actual outbreaks of disease. Presuming a significant boil water advisory to be a 1-in-20-year event, at a cost of \$1.3 per person per day in substitute supply costs for 3 days, and \$418 per illness at a 10% illness rate (Wagner *et al.*, 2005; all figures adjusted to 2018), preventing

boil water advisory for an average (10,000 customer) municipality represents a \$23,000 annual savings to the utility.

## 2.6.4 Infrastructure preservation

Staying ahead of microbiological contamination could minimize impact on infrastructure from issues like corrosion. This would lead to longer infrastructure life thus delaying capital cost expenditures. In the USA alone, forecast drinking water infrastructure investment requirements are \$370B over 20 years across the country (New England USEPA, 2008). Based on 35,000 operating drinking water utilities, this equates to approximately \$530,000 in annual capital expenditure requirements, on which conservative interest payments would be at least \$20,000 per year, assuming a 4% interest rate).

Bringing these opportunity costs together for an average municipal operator serving 10,000 customers results in substantial available savings, as shown in Table 2.1.

Table 2.1 Savings opportunities from advanced
microbiological sensors for a utility serving 10,000 customers.

Aspect	Annual Savings (\$USD)
Water & chemical conservation	\$27,000
Time & travel conservation	\$50,000
Boil water advisory mitigation	\$23,000
Infrastructure financing	\$20,000
TOTAL OPEX	\$120,000
Infrastructure deferral	\$530,000
TOTAL CAPEX	\$530,000

Clearly, having faster results leading to faster and more targeted action without delay results in significant economic efficiency and benefits to society. Over the past 150 years, the water sector has gone from taking years to days to solve faecal-based microbiological contamination in drinking water processes. Available technology now affords the sector the opportunity to further reduce this from days to minutes, in addition to offering the opportunity to expand beyond faecal contamination to additional health risks and operational concerns.

#### 2.7 UPGRADING THE TOOLBOX

Looking to other sectors for inspiration, there are several technologies that could be leveraged for enhanced detection spectrum and faster response time. Sectors like food, personal care product, and pharmaceutical manufacturing leverage technologies such cell counting, optical sensing, rapid methods, and genomics to improve product quality and reduce inventory holding times to streamline the supply chain and reduce costs. The water sector has the same opportunity to leverage these newer technologies to improve product quality, reduce disruptions, and save time. The following sections discuss three categories of sensors that could improve detection spectrum and reduce response times.

# 2.7.1 Online cell counting and sensing

As mentioned in Section 2.4.2, the water sector routinely makes use of turbidity to detect intrusion of particles in drinking water processes. This technique is broad spectrum, but perhaps too broad in that it does not differentiate between biological and non-biological material. In the pharmaceutical sector for example, instrumentation such as laser-refraction and in-line optical technology has been used for many years to provide a 24/7/365 warning against specific microbiological incursions in raw, process, and final product water streams.

Recently, such tools have been introduced into the drinking water sector. One example is BACMON (Grundfos, 2017), an in-line camera-based microscope technology that photographs particles in cells and compares against a reference database to confirm the presence of microorganisms. Another is flow cytometry (Van Nevel *et al.*, 2017), a cell counting and sorting technique that has long been a mainstay in the medical sector, though tends to interrogate a small sample size and may require staining to increase specificity and to distinguish live cells from dead cells.

Such techniques can be very beneficial in that they may be always on and are sensitive to low levels of microbiological contamination, though depending on the technology they may not be particularly precise and can be quite expensive. Deployment of such technology would be ideal at strategic locations within a water distribution system such as at reservoirs and booster stations and are an ideal first line of defence when placed alongside other online sensors such as disinfectant residual and turbidity. These sensors would save utilities considerable time in providing alerts to contamination as quickly as possible so that operators could be dispatched to investigate.

# 2.7.2 Portable and rapid microbiological methods

Rapid, portable methods have been a mainstay of the food processing sector for over 30 years, where having a fast and on-the-spot indicator of biological contamination to verify surface cleanliness at any point in the manufacturing process to ensure no accumulation of biological matter that could lead to the manifestation of pathogenic microorganisms. These methods have completely changed the economics of the food sector in terms of risk avoidance and inventory management (IMMR-4, 2014).

One example of such a technique is the 1st Generation ATP test method, which has also been applied to limited success in certain water applications such as in commercial and industrial cooling systems. These methods however were never designed for water systems, where greater robustness and a quantitative cell count result was required. Over the past decade, a 2nd Generation ATP test method has become commercially available and is designed specifically for water systems. The ATP test is useful in that it detects all living cells and the results are easy to interpret. However, this method is also non-specific and must be used in the context of understanding that water systems are not sterile and there is an underlying background level of microbiological contamination.

Portable tools such as these provide operators with the ability to take the test into the field and identify where and how much microbiological contamination exists at any point in the treatment and distribution system as fast as they can sample, which can lead to a near-real-time problem recognition and correction cycle. They also enable on-site re-testing when suspect results are obtained, which is not possible with laboratory testing due to the process environment having changed by the time results are returned. These methods are useful for guiding and confirming remedial actions given that these actions are typically broad-spectrum and serve as an ideal complement to the on-line sensors mentioned in 2.7.1 and in that regard are unlikely to ever be replaced. However, these methods do not fulfil the requirement for specificity in the types of microorganisms detected.

# 2.7.3 Microorganism identification

Since its original discovery, deoxyribonucleic acid (DNA) has been the most widely studied biological cell component. This is not surprising, given that it represents the code for all life. Genomics technology provides the most specific indication possible of the type of biological cell present in the sample – even more specific than a culture test. Indeed, whereas there are only several thousand developed culture media formulations for specific categories of microorganisms, the development of genomics technologies has led the sector to estimate that in total there are over one trillion species of microorganisms on Earth, only 0.001% of which have been identified to date (Bakalar, 2016).

At present, genomics-based methods are mainly focused on DNA measurement, and generally come in two types: quantification (via polymerase chain reaction, or PCR), and identification (for example via next-generation sequencing). In the former, a 'primer' is used to provide a template to locate DNA of the same sequence in the sample, hence one must know what is being looked for before starting the test. In the latter, all individual DNA strands are read and mapped against a reference database to translate into actual microbiological species (a process called bioinformatics). Due to its sensitivity to the target microorganisms, great care must be taken in testing execution to not provide erroneous results.

In present form and compared to current industry standard test methods, these tools are extremely powerful, but can be expensive and time-consuming. In many cases, knowing the specific microorganisms present in the sample can assist with troubleshooting the source of process failures, but may not necessarily change the immediate remedial action. The field of DNA measurement is evolving rapidly, and while these methods are currently best executed in the laboratory, portable and on-line versions are under development and likely to come to market soon (Juhler *et al.*, 2012). Furthermore, one of the longest-standing deficiencies of genomics-based tests has been an inability to distinguish living from dead cells – and methods to overcome this limitation are being rapidly developed. This increases the likelihood that genomic technology will eventually replace culturing as the go-to mechanism of identifying specific microorganisms. For now, performing genomics-based tests in the laboratory are a powerful tool for utilities to use for troubleshooting complex problems or optimizing unit operations.

These technologies can provide real, tangible benefits for the water sector, all centred on saving time – that is, in minimizing the time to realize, interrogate, and correct contamination events. Each is fit for a different purpose, and none provide the same information as regulatory-approved culture-based methods – hence, they would be complementary. They also all require different skills to execute and to interpret data. All these points require a different way of thinking to achieve adoption in the water sector.

#### 2.8 THE FUTURE AND THE HOLY GRAIL

For years, the water sector has been waiting for the perfect biological sensor to come along and advance its abilities to determine when and where microbiological contamination occurs (Tatari *et al.*, 2016). In contemplating what constitutes the perfect sensor, the following factors may be considered:

- Instantaneous time-to-result the sensor should provide near-immediate results.
- 2. **Continuous monitoring** the sensor should be able to monitor 24/7/365.
- 3. **Sensitive to incursions** the sensor should be sensitive enough to detect an incursion at its earliest possible stages.
- 4. **Specific to known microbiological threats** the sensor should be specific enough to look for all the threats known at the time.
- 5. **Goes beyond health-based targets** providing the capability to measure more than just a few specific microorganisms.
- 6. **Accuracy** the sensor should provide valid results across the wide range with no false positives or negatives, including the ability to distinguish live cells from those that have been killed even if the cells are just recently dead.

- 7. **Reliable and low maintenance** the sensor should be as reliable as possible and require no more maintenance than commonly used sensors.
- 8. **Wide coverage and/or portability** the sensor should be able to be deployed anywhere and everywhere throughout the water management cycle.
- 9. **Easy to interpret** the sensor should output results and insights that are easily understood by water process stakeholders.
- 10. **Affordability** the sensor should be operationally cost effective and offer a reasonable return on investment of capital.

The above checklist is a very challenging set of criteria for any one technology to meet. This will require the sector to think differently about how to achieve its goals – such as looking at whether a combination of sensors could give the same benefits. This approach would require operators to be educated to think that all microbiological tests measure different characteristics, and that they need to seek multiple perspectives. Table 2.2 shows how the three technologies mentioned in the previous section perform against these goals:

**Table 2.2** A comparison of three advanced molecular microbiology techniques against all three under a combined approach to incumbent culture methods.

Technology	Cell Sensing	Portable & Rapid Tests	Genomics	Combined	Culture
Instantaneous	Χ	X		Х	
Continuous	Χ			X	
Sensitive	Χ	X	X	X	Χ
Specific			X	X	X
Inclusive	Χ	X	X	X	
Accurate		X	Χ	X	Χ
Low maintenance	Х	X		X	
Wide coverage		X	X	X	X
Interpretation	Χ	X		X	Χ
Affordability	Χ	X		Χ	X

As seen in Table 2.2, an approach where these three types of sensors are deployed together in the drinking water system can provide all the attributes sought. However, all this new data poses a challenge to acceptance by the water community. Fortunately, one of the major advancements of the 21st century is the growth of cloud-based computing and intelligent computer-based decision support systems. All the data from these platforms could be integrated into a

single smart system (Figure 2.4) that through basic provision of metadata by the operator could provide them with insights and recommended actions, rather than just random data points.

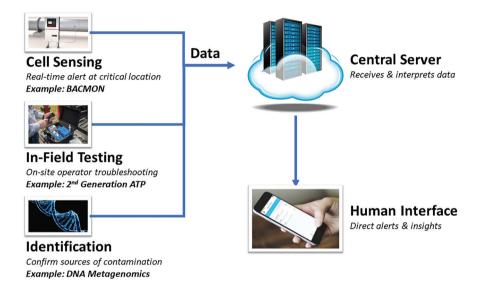


Figure 2.4 Combining three technologies to provide value to the water sector.

Taking the approach outlined here would flip microbiological management from a reactive to a proactive stance, thus enabling the savings of time, resources, and ultimately, money. As such, it is not necessary to wait for the 'perfect' biological sensor – the sector only need apply the tools at its disposal and integrate them into smart decision support systems to reap the benefits.

Achieving acceptance in this regard would require market education. Current training provided to water operators in microbiology is minimal – most are aware only of the required health-based testing and that if those targets are achieved, no further action is required. Taking the next step will require a change in how operators are educated on the risks and opportunities posed by a wider range of microorganisms, as well as adequate adoption drivers – whether regulatory or economic. As the former takes a long time to change, it is likely that adoption would occur faster by focusing on the latter – namely, the return on investment available through the adoption of more advanced monitoring tools such as those outlined in 2.6.

#### 2.9 CONCLUSION

Over the course of 150 years, the water sector went from having an anecdotal awareness of microbiology in water to having robust, regulatory-enforced water

management programs that were established help to ensure that drinking water is safe to drink. However, these regulatory frameworks have not kept up with societal changes nor the needs of utilities to stay ahead of emerging risks and address the operational challenges posed by microorganisms. The technology exists today to take a quantum leap in water microbiology management, and its adoption simply requires a change in approach by water utilities to go beyond what is required by regulations. Given the economic pressures these utilities are encountering and given the emergence of more and more unforeseen threats, it is imperative that the sector works to change the landscape in a proactive rather than a reactive way. The need for speed is borne from finding faster ways of detecting and addressing microbiological contamination, and this can be done through technology available today. It is not just about time to result – it is about time to action, and it is about incorporating economics into the traditional health-based conversation to provide a robust, sustainable framework that can overcome microbiological risks both today and tomorrow in sustainable ways.

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