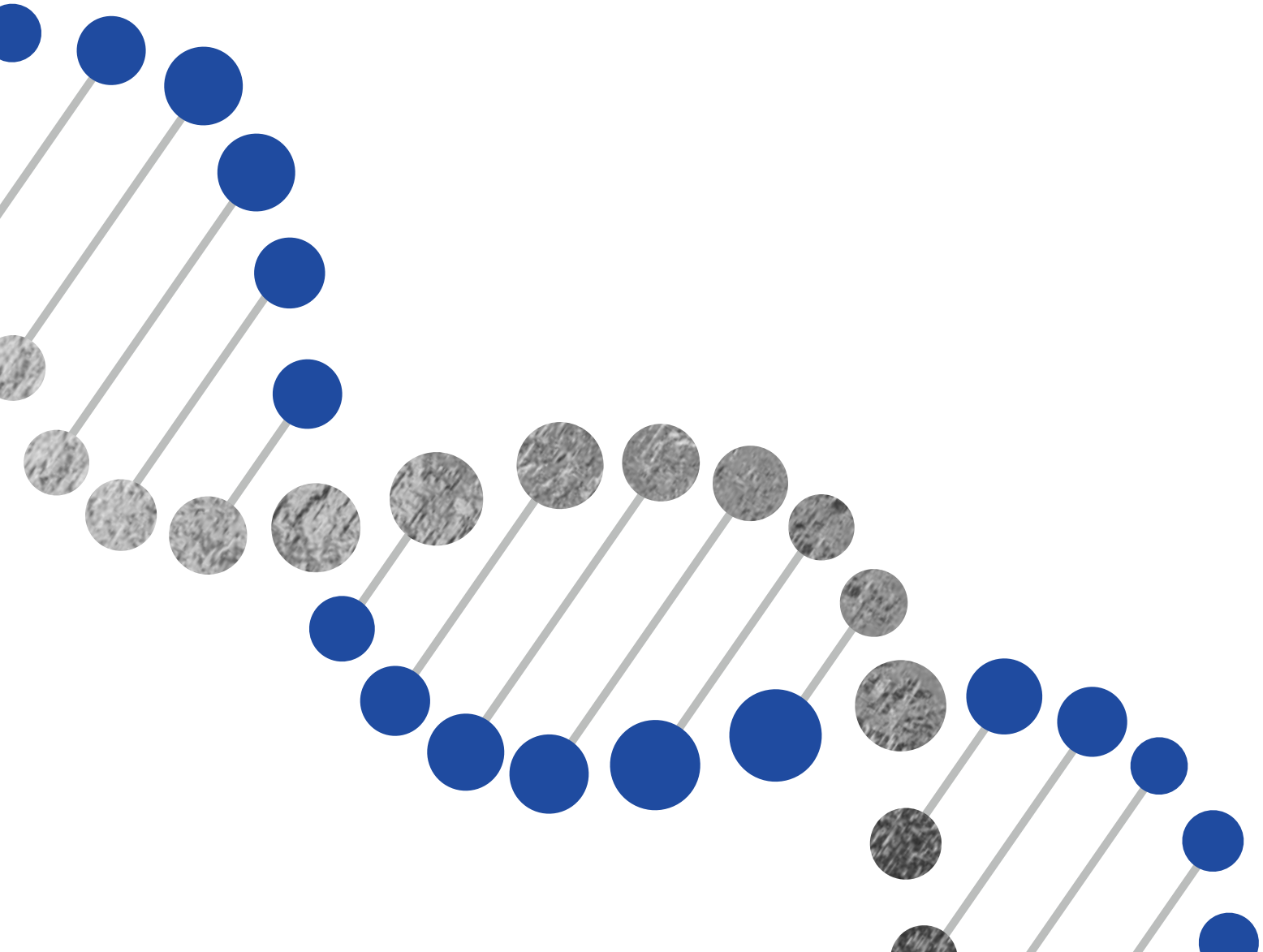
AWQC

THE ULTIMATE GUIDE TO MOLECULAR TESTING FOR WATERBORNE PATHOGENS



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Introduction

Microbial quality is the most important factor in determining the ongoing safety of water supplies for human consumption.

While millions of Australians turn on the tap every day without much thought about whether the water that will flow is safe to drink, waterborne disease remains a global concern which is estimated to cause more than 2.2 million deaths per year and higher cases of illness, which include diarrhoea and other illnesses[1].

Diarrhoeal disease is the second leading cause of death in children under five years old, killing around 525 000 children each year. It is both preventable and treatable.

Waterborne pathogens and related diseases are a major public health concern worldwide, not only for the illness and death they cause, but for the high cost of their prevention and treatment.

The most common and widespread health risk associated with drinking water is contamination, either directly or indirectly, by human or animal excreta and the microorganisms contained in faeces. If the contamination is recent, and those contributing to the contamination include carriers of communicable enteric diseases (diseases of the gut), some of the microorganisms that cause these diseases may be present in the water. Drinking such contaminated water or using it in food preparation may cause new cases of infection.

Pathogenic (disease-causing) organisms of concern include bacteria, viruses and protozoa. The diseases they cause vary in severity from mild gastroenteritis to severe and sometimes fatal diarrhoea, dysentery, hepatitis, cholera or typhoid fever.

The most common waterborne diseases are caused by organisms originating in the gut of humans or other animals. However, many organisms of environmental origin cause disease in humans. Such organisms include *Cryptosporidium*, the protozoan *Naegleria fowleri*, a number of bacteria, including *Salmonella*, *Shigella* and *Campylobacteria*, and *Legionella* spp., and some species of environmental mycobacteria.

Infection is the main, but not the only, problem associated with microorganisms in drinking water. For instance, certain cyanobacteria (blue-green algae) and bacteria can produce toxic algal blooms that affect humans and the toxins may remain in the water even when the organisms responsible have been removed.

For good reason, trust in the supply of safe, clean drinking water by Australian water utilities is rightly high, and an expectation for people living in developed countries like ours. Yet, the threat of contamination is ever-present.

Water quality scientists are on the frontline in the fight to safeguard public health as they monitor the effectiveness of the protective barriers that are designed to prevent the entry and transmission of pathogens into our drinking water systems.

Proper assessment of pathogens during water quality monitoring processes are key to decision-making regarding the choice of best water treatment, and prevention of waterborne disease outbreaks.

As leaders in the field of microbiological testing and analysis, the Australian Water Quality Centre (AWQC) has an unrivalled track record of developing and validating more effective testing methods for the detection of waterborne pathogens in the interests of protecting public health. An example being our use of advanced next generation sequencing (NGS) DNA testing methods to optimise water quality management.

We have curated the most extensive DNA database of waterborne pathogens in Australia and have developed molecular testing methods that provide unrivalled pathogen testing results for a broad range of applications. We can offer a full suite of molecular analyses for targeting input from animal sources into water, from source water to treated water. These include:

- *E. coli* host confirmation and phylogrouping
- environmental *E. coli* bloom and capsule detection
- faecal source tracking for human and animal sources at a forensic level
- rapid toxin gene detection
- complete bacterial diversity profiling
- whole genome sequencing.

In the interests of sharing information that will enhance water quality management practices and protect public health, we have developed this guide to provide insight into how the latest DNA testing innovations are key to understanding water contamination risks, resolving water quality issues, and making informed decisions to prevent waterborne disease outbreaks.

[1] World Health Organization (WHO) Water Sanitation and Health. 2015. http://www.who.int/water_sanitation_health/diseases.

Traditional microscopy testing techniques

Challenges with the current water quality testing methods

Assuring the safety of public health requires rapid and accurate water quality assessment for pathogenic microorganisms and toxic cyanobacteria (blue-green algae) blooms. The results of these tests inform important water quality management and operational treatment decisions.

Microbiologists have created a suite of powerful, sensitive and dynamic testing methods to monitor pathogen contamination in water. These tests are used to detect cultivable pathogens and the occurence of viable but non-culturable microbes, as well as the presence of pathogens on biofilms.

However, until recently there was no unified method to encompass the collection and analysis of a water sample for all pathogenic microorganisms of interest.

Routine monitoring for specific waterborne microbial or viral pathogens can be complex, expensive and time-consuming, and may fail to detect their presence.

The challenges of most testing and detection methods include the physical differences between the major pathogen groups; low concentration of pathogens in a large volume of water which usually requires enrichment and concentration of the samples before detection processing; the presence of inhibitors from the sample (especially if it comes from polluted water); culture-dependent detection methods; and detecting the host origin of pathogens.

Traditional microscopy testing methods are time-consuming and do not provide definitive diagnosis required for complex microorganisms.

Although culture-dependent methods are extensively used for pathogen detection in water, these methods are limited by their lack of specificity, low sensitivity, the excessive time needed to obtain reliable results, and risks posed when handling pathogens.

Index bacteria have been selected as indicators or markers of the presence of faecal contamination and possible presence of numerous microbial pathogens. Among these bacterium, *Escherichia coli (E. coli)* has been extensively used for routine water quality testing as these methods are relatively easy and inexpensive.

Nonetheless, they may have the disadvantage of not providing information on their host origin and, sometimes, the presence of *E. coli* does not always correlate with other pathogens present in the water, such as the viruses and protozoa. Thus, water characterised as pathogen-free by monitoring *E. coli*, for example, may be contaminated with viruses or protozoa.

Summary

Monitoring for specific microbial pathogens is usually complex, expensive and time consuming.

Most tests take days to identify issues and are still unable to discriminate between pathogens of human-health concern and those of environmental origin that pose no risk to public health.

Conventional methods for detecting bacterial contamination require days for culturing, and they only target specific bacteria. Identification of protozoa often involves scientists spending hours and even days performing microscopy to identify and count parasites such as *Giardia* or *Cryptosporidium*, or cyanobacteria. In many cases, species are difficult to distinguish based on morphological characteristics alone.

Redefining water quality testing with DNA sequencing technology

Innovating for optimal public health outcomes

AWQC plays a critical role in the supply of safe, reliable water and wastewater services to the individuals and communities who rely on them.

As leaders in the field of microbiological testing and analysis, we were the first laboratory in Australia to apply molecular testing technology specifically to optimise water quality management.

Molecular testing techniques improve the characterisation of pathogens by identifying causative agents more accurately and quickly. Detecting viable microorganisms and characterising them according to microbial communities enables the creation of accessible data that enhances knowledge of waterborne pathogens and the possibilities to predict pathogen contamination and protect public health.

Our microbiology, molecular and biology services teams are supported by an internationally recognised and awarded research team that has an unrivalled track record of developing and validating more effective microbial and pathogen testing methods by harnessing innovative cutting-edge technologies.

An example being our use of a more effective method of DNA sequencing that enables water utilities to proactively manage water quality and to have well-informed, targeted catchment management and water treatment strategies.

Next-generation sequencing (NGS), also known as high-throughput sequencing, allows for sequencing of DNA and RNA much more quickly and cheaply than the previously used molecular testing methods, revolutionising the study of genomics and molecular biology.

Both NGS and polymerase chain reaction (PCR) DNA testing techniques are rapid and definitive. For example, our 16S rRNA (bDNA) test will identify 95 per cent of the bacteria in a single sample compared with traditional methodologies where less than 2 per cent of bacteria can be cultured and identified. This latter point represents a massive difference between the old and the new technologies using AWQC's fully validated and curated DNA database.

Using NGS technology, we can take a one litre water sample or a one gram soil/sludge sample and identify exactly what organisms, including bacteria, protozoa and vertebrates have been in contact with that water or soil/sludge. This combined information provides a more comprehensive picture of all possible contamination inputs.

Having harnessed and optimised high-throughput DNA sequencing technology, we comprehensively analyse water samples for common biological contaminants, such as *E. coli* and cyanobacteria, and provide reliable and targeted results.

DNA sequencing reduces the time to perform the water analysis and has far greater accuracy than traditional testing methods. This enables water utilities to significantly reduce the number of potential impacts by implementing a rapid, targeted response to contaminants, and, when needed, quickly notifying the public.

We have take DNA testing technologies and developed testing methodologies that enable us to characterise entire microbial communities and reveal a diversity of microorganisms not seen before due to their inability to be isolated by culture.

This has significant implications for improving public health, research, conservation efforts and conditions within water treatment plants.

Advancing the frontiers of molecular testing in microbiology

The need to provide of faster results and ever better outcomes in water quality testing has resulted in our migration from many traditional molecular biology techniques such as PCR, and culture assays to targeted bacterial species profiling and pathogen identification using next-generation sequencing DNA testing methods.

NGS is a high-throughput molecular testing methodology that enables rapid sequencing of the base pairs in DNA or RNA samples. It is more efficient and cost-effective than traditional DNA testing techniques, as it involves few manual processes, and has far greater accuracy than traditional methods.

Two NGS instruments are used to perform DNA analysis.

DNA extraction, purification, fragmentation and amplification are first performed using an instrument named the ION Chef™. The amplified DNA fragments tether to a small bead, which sits in one of millions of wells on a semiconducting chip, and are combined to generate complete sequences for each organism.

The chip is then placed in the ION Torrent™ GeneStudio™ S5 Prime, which reads the DNA using NGS methods and compares them against a reference library of DNA sequences available in its database.

The Ion Torrent™ is the first turnkey NGS solution that automates the specimen-to-report workflow and enables us to deliver many results in a single day.

The evolution of next-generation sequencing technology has enabled researchers to take advantage of increased throughput, higher accuracy, and longer reads to produce rapid and accurate sequencing of microbes with streamlined sample preparation and a simple and optimised data analysis workflow. Data visualisation tools enable results to be easily analysed.

Unrivalled microbial DNA testing capabilities

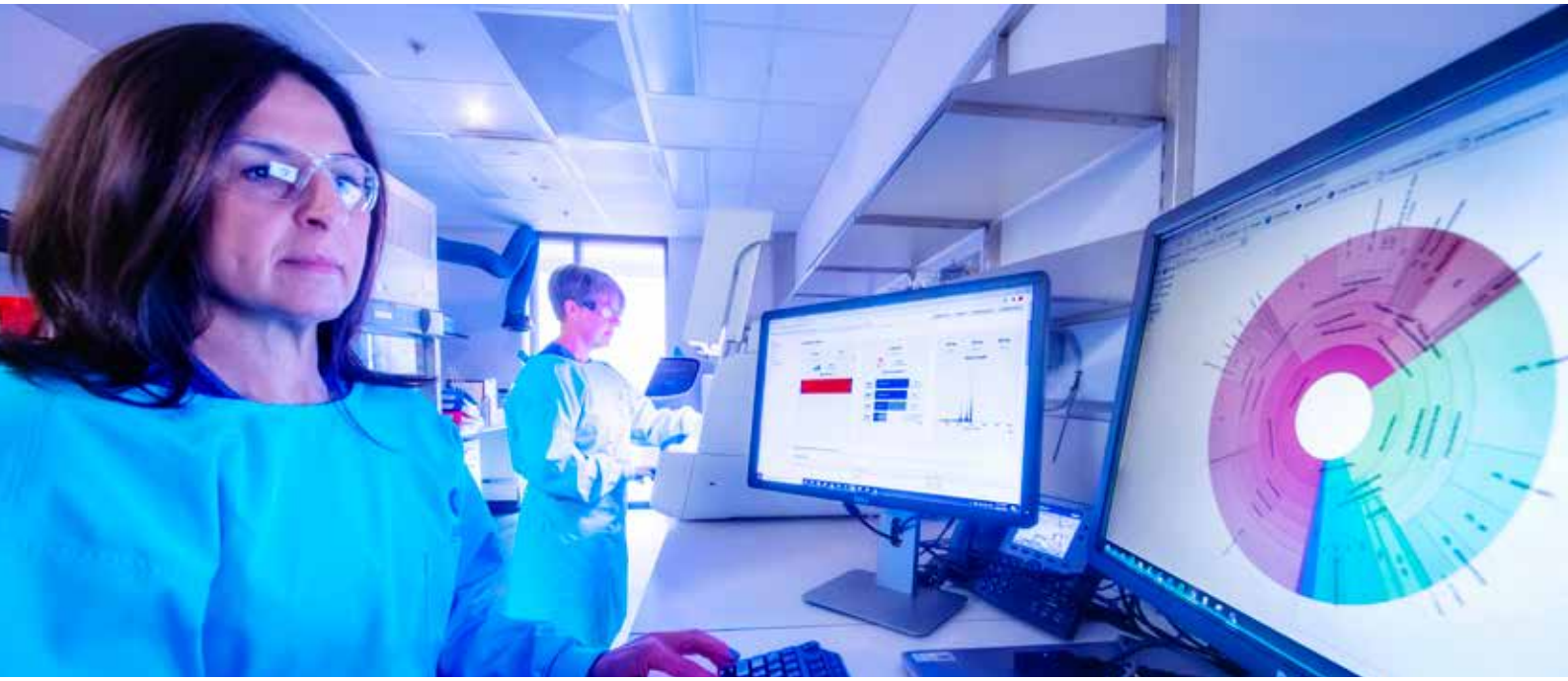
AWQC microbiologists and testing method development experts have configured these powerful DNA sequencing instruments to provide reproducible tests that are unrivalled by other water quality testing laboratories in Australia.

Our databases are curated and refined using validated species, with specific primers giving consistent data across areas and locations both temporally and spatially dispersed.

Our capabilities in generating unique databases, bioinformatic pipelines and molecular services research are unparalleled. The method validation is so advanced that it has resulted in the use of our NGS testing methodologies in forensic applications and resolving unique problems for the water industry across Australia. Our highly skilled professionals have expertise in all areas of microbiological investigation as well as connections with universities, including through doctoral and undergraduate students.

Quick and accurate discrimination between closely related bacterial species to the strain level is our specialty, including determining any toxin producing pathogens.

In some cases, we can also provide higher taxonomic resolution, especially in cases where species cannot be distinguished based on morphological characteristics.



Applications

Let's get testing

Our molecular testing technology provides data that enables drinking water suppliers to better understand water contamination and water quality risks, and make timely operational decisions.

Supporting a broad range of applications, we offer a full suite of rapid turnaround DNA molecular analyses.

Our molecular tests include:

- *E. coli* confirmation and phylogrouping
- environmental *E. coli* bloom detection
- faecal source tracking (FST) for human and animal sources at a forensic level
- complete bacterial diversity profiling (bDNA)
- complete vertebrate diversity profiling (vDNA)
- rapid cyanobacterial toxin gene detection
- whole genome sequencing (WGS).

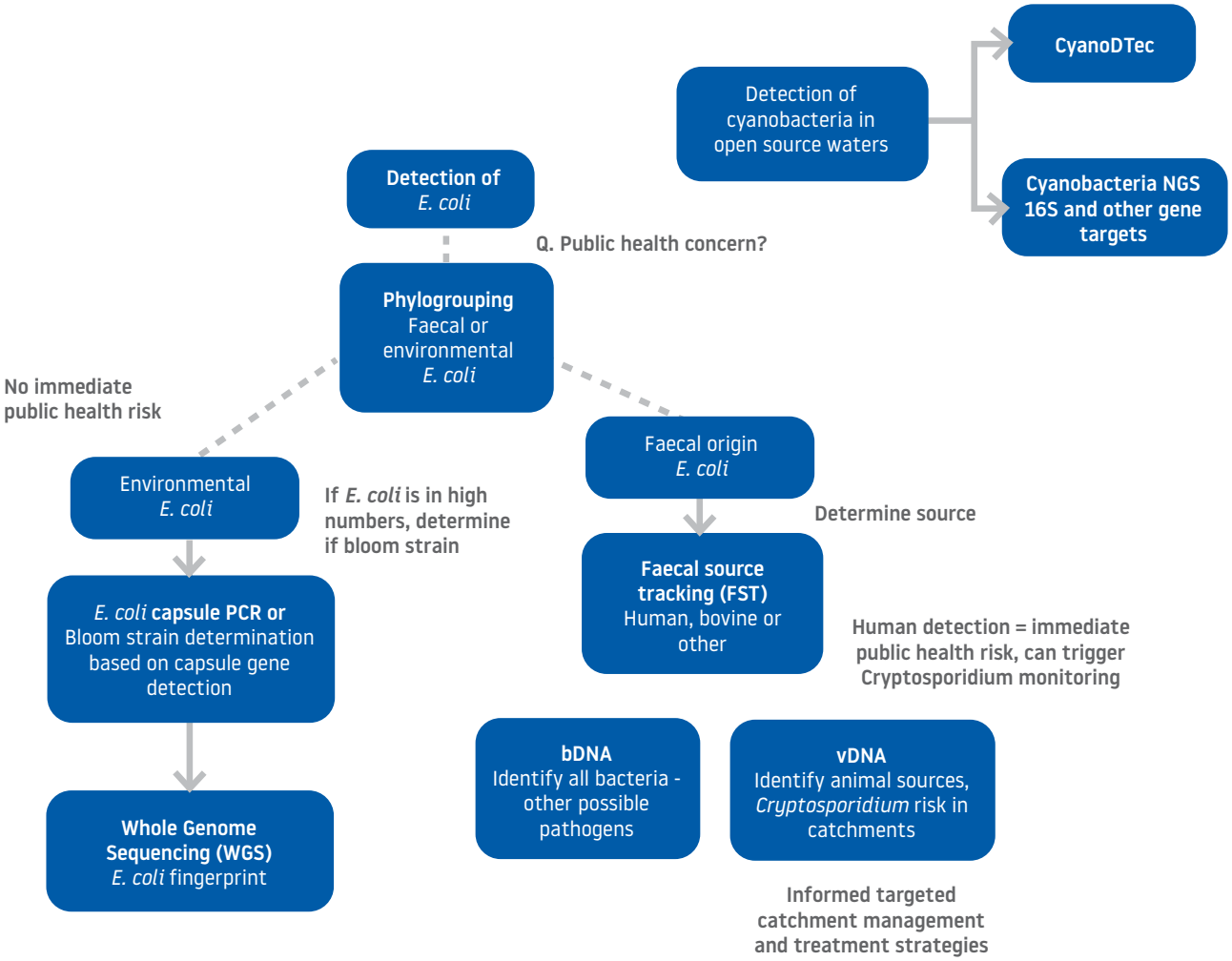


Figure 1. *E. coli*, cyanobacteria and *Cryptosporidium* DNA testing applications flowchart

E. coli confirmation and phylogrouping

- Powerful screening technique, early warning of an *E. coli* bloom.
- Rapid and cost effective 24 hour TAT for Emergency issues with blooms or incidents.

Most waterborne pathogens are introduced into drinking water supplies via contamination with human or animal faeces. These pathogens cause a range of conditions from mild to severe gastroenteritis, diarrhoea, dysentery, hepatitis and cholera.

Thermotolerant coliforms (faecal coliforms) are always present in high numbers in human and animal faeces. *E. coli* is the most common thermotolerant coliform present in faeces (typically >90 per cent) and is regarded as the most specific indication of recent faecal contamination. While most thermotolerant coliforms are non-pathogenic, there are some pathogenic subspecies of *E. coli* that can cause gastrointestinal illness.

E. coli (or thermotolerant coliforms) should not be detected in a minimum 100mL sample of drinking water. If detected, immediate action should be taken as it is an indication of faecal contamination as it suggests that water quality may have been seriously compromised.

However, routine water quality tests (for example Colilert) do not provide information on *E. coli* origin. This information is particularly important when the identification of specific *E. coli* strains will determine the risk profile and water treatment options used by a water utility.

Phylogrouping enables us to determine if the source of contamination is of animal or environmental origin, which will inform your risk assessment process and water quality management actions.

E. coli strains can be separated into a phylogroup structure, which at present includes eight phylo-groups: A, B1, B2, C, D, E, F and cryptic clade I.

Phylogroup B1 strains are the group most often detected in water samples and are considered environmental strains. By contrast B2 and D strains are usually detected in human gut flora.

The A0 phylogroup is carried by birds, reptiles, fish and some mammals.

The B1 phylogroup is predominantly environmental.

The risk of this type of *E. coli* to human health is low.

The B2-3 phylogroup is predominantly carried by humans.The risk of this type of *E. coli* to human health is high as it may indicate a human faecal contamination event and thus the potential for human infective pathogens to also be present.

The D1 phylogroup is associated with mammalian omnivores, which can include humans and other animals like pigs.

The E phylogroup is associated with herbivores and cattle. The risk of this type of *E. coli* to human health is medium, there is a possibility that human infective pathogens may also be present.

Table 1. *E. coli* risk ratings guide

	Risk rating		
Source	High	Medium	Low
A0 (birds, reptiles and some mammals)			X
A1 (mammalian omnivore/bloom)		X	
B1 (environmental/herbivore/bloom)			X
B2-1, B2-2 (mammalian omnivore)		X	
B2-3 (human)	X		
C (unknown/bloom strain)			X
D1 (mammalian omnivore)		X	
D2 (mammalian/mammalian omnivore)			X
E (herbivore/bovine)		X	
F (mammalian)			X
Clade 1 (extra-intestinal pathogenic, ExPEC)	X		
Clade 3, 4 & 5 (environmental)			X

Environmental *E. coli* bloom detection

E. coli bloom events in Australian reservoirs and recreational waters are not uncommon. During these events, elevated *E. coli* counts from 10,000 to 100,000 cells/100ml of water have been reported.

While these counts are well above the safe levels specified in the Australian Drinking Water Guidelines, research has shown that cell counts this high would require an unachievable level of faecal contamination.

Instead, the strains responsible may represent, free living *E. coli* of environmental origin (Power et al., 2005; Alm et al., 2011).

The discovery of non-faecal, environmental *E. coli* forming blooms in reservoirs has the potential to adversely affect risk assessment processes for water utilities. For example, a positive *E. coli* test result from a reservoir will likely result in its closure and the release of public health notice and increased water treatment costs, even though it may be a harmless environmental strain.

Relatively few strains have been found to be responsible for *E. coli* bloom events, and all strains isolated from bloom events in Australia carry a capsule originating from *Klebsiella*.

Typically, environmental *E. coli* strains do not have pathogenic tendencies and are therefore less of a health risk than faecal-borne *E. coli*.

These bloom strains typically belong to phylogroups A1, C or B1 and carry both the galF gene and a Klebsiella capsule gene. This results in an environmentally adapted organism which produces a mucoid capsule. The capsule then protects the organism from exposure to treatment (Nanayakkara et al., 2018).

Three bloom-forming strains (capsule types KL16, KL49 and KL53) have been associated with previously reported Australian east coast bloom strains. Five bloom forming strains (capsule types KL53, KL60, KL63 and KL101) have been associated with previously reported Australian west coast bloom strains.

We are the only Australian laboratory to offer a rapid DNA based analysis that can detect all known Australian encapsulated bloom strains.

Faecal source tracking

- Identifies human or bovine sources of contamination.
- Rapid same-day turnaround 4-6 hours.
- Cost effective.

Implementing pathogen management plans is not easy when contamination can come from so many potential sources and you are unable to identify the exact source.

While traditional culture-based methods only tell you if and when faecal contamination is present, our DNA fingerprinting methods will identify the exact animal species causing the pollution in a water source. Using next-generation sequencing, we can identify the source of faecal contamination in water sources ranging from reservoirs to bores.

E. coli whole genome sequencing (WGS) allows us to fingerprint isolates for faecal source tracking, and to identify bloom-forming and potentially pathogenic *E. coli*.

WGS involves the examination of multiple *E. coli* genes. The method developed by the AWQC includes the Achtman loci in addition to a further 157 genomic regions, allowing the identification of more than 4,499 *E. coli* sequence types.

This unique identification of isolates (equivalent to a DNA fingerprint) allows *E. coli* WGS to be used for tracking and monitoring *E. coli* presence over time at a single location, or *E. coli* isolate movement through a water distribution network or water body.

In addition to fingerprinting isolates, the bioinformatics pipeline was developed to identify any genes associated with human health risk (for example, virulence or antimicrobial resistance genes) or the potential to form blooms (for example, particular capsule gene sequences).

Importantly, the benefits of WGS can be realised without the need for any complex, time-consuming traditional techniques whilst providing detailed and reliable information. This molecular-based monitoring technique has significant implications for public health, research, conservation efforts and optimising processes and conditions within water treatment plants.

Complete bacterial (bDNA) profiling

- Informs catchment and reservoir management practices.
- Two-week turnaround.
- Reliable, targeted results.
- Comprehensive water profile.

It is useful to know the diversity and abundance of aquatic life in water sources, as they can be indicators of a healthy water system and inform catchment management practices.

Conventional methods for detecting bacterial contamination are based on traditional culture techniques which only target specific bacteria, some of which require days of incubation.

Using next-generation sequencing (NGS), we're able to take a simple water sample and determine exactly what bacteria organisms are causing issues.

Bacterial diversity using NGS is becoming a favoured analysis in comparing bacterial profiles using the universal 16S rRNA gene amplicon which has regions that are both highly conserved and highly variable (hypervariable) among bacterial species. The analysis covered nine (V1-V9) of the ION Torrent hypervariable primer regions that are specifically method-validated at the AWQC using our curated and customised databases.

The alterations of the microbiome can define richness, diversity and composition between groups, as well as effects in complex water matrices, bores, wastewater treatment processes and treatment chains.

With three independent bacterial bioinformatics pipeline methods on offer (custom BLAST, QIIME2 and Kraken2), we can customise for any bacterial issue encountered, including complex biofilms.

Results are presented in an accessible, user-friendly report that includes a ranking of the organisms detected in order of relative abundance and percentage of total DNA detected.

Figure 2. Krona plot

The bacterial DNA data is used to make assessments over time, based on sample baseline data. The bacterial DNA detected in a sample can be monitored to determine if the community structure is shifting to favour different bacterial groups. The bacterial DNA diversity profile represents the current status of the sample and should remain relatively static, depending on the source, volume and (if applicable) disinfection protocols.

CyanoDTec

- Detects and quantifies potentially toxic cyanobacteria genes.
- Rapid same-day turnaround in 3-4 hours.
- Powerful screening technique, early warning of potential toxicity of bloom.
- Cost effective.

Sample and predict harmful algae blooms

In many aquatic ecosystems world-wide, including drinking water supplies, cyanobacteria and dinoflagellates can proliferate into algal blooms. Members of these microbial phyla can produce an unparalleled array of bioactive secondary metabolites, some of which are potent toxins.

Not all cyanobacteria species produce toxins, so the presence of an algal bloom does not immediately infer a risk of toxins is present. The CyanoDTec test quantitates both the amount of overall cyanobacteria present in a water sample and the number of genes responsible for the production of the toxins.

Toxins associated with blue-green algae are either hepatotoxins (which can cause liver damage) or neurotoxins (which can cause neurological damage).

The hepatotoxins include microcystin, nodularin and cylindrospermopsin while saxitoxin is the primary neurotoxin produced by cyanobacteria.

CyanoDTec is a PCR based DNA testing technology that detects and quantifies the presence of cyanobacteria, blue-green algae, and their toxin-producing genes in aquatic environments.

Genomic information related to toxin synthesis has indicated their environmental and cellular regulators, as well as associated transport mechanisms. The information gained from the discovery of these toxin biosynthetic pathways has enabled the genetic screening of various water matrices.

Cyanobacteria toxins are produced by many strains of cyanobacteria spanning multiple genera, yet as toxicity is not uniform among strains, conventional bacteriological classification methods are unable to accurately predict toxicity or analytical methods for the detection of the toxins. These often take days to perform and are not predictive.

Our validated, rapid molecular test detects cyanotoxin production in fresh, brackish and marine water environments that pose a direct threat to public health.

Legionella testing

- Isolates and enumerates *Legionella* species in water.
- Powerful screening technique, early detection of *Legionella*.
- Cost effective.
- Three-day turnaround.

Our method couples standard bacterial culture techniques with DNA technology enabling rapid detection. The method meets the current standard method (AS/NZS 3896) and has a superior advantage in speed, with confirmed *Legionella pneumophila* counts available in three days, compared to up to seven days by the standard method.

The delivery of rapid results enables remedial action to be taken much quicker than previously possible.

Additionally, because the assay tests the DNA of the bacteria, it is highly sensitive and specific and increases the accuracy of results.

Overview of applications for molecular analyses

Applications for molecular analyses	bDNA Bacterical diversity profiling	vDNA Vertebrate diversity profiling	CyanoDTec Cyanobacterial toxin genes plus total cyanobacterial load	*E. coli* WGS Whole genome sequencing	*E. coli* Phylogrouping	Faecal source tracking	Cyanobacterial geosim and MIB genes Taste and colour	*E. coli* capsules *E. coli* bloom strain detection
E. coli bloom detection				✓	✓			✓
E. coli capsule detection				✓				✓
E. coli typing and tracking				✓	✓	✓		
E. coli bloom masking faecal detections		Operational for vertebrate inputs		Optional for tracking		✓		✓
Toxic algal bloom detection			✓					
Human/bovine faecal contamination in water						✓		
Taste and odour			Optional				✓	
Sanitary surveys		✓				✓		
Bore investigations/ bore intrusion issues	✓	Optional				✓ (Facael intrusions)		
Catchment management/risk assessment		✓			✓	✓		
E. coli detection in drinking water systems				✓	✓			
Quantification of cyanobateria			✓					
Tracking faecal/ *E. coli* discharges/point sources				✓		✓		
Reservoir management	✓	✓	✓				✓	
Biofilms, bacteria screening	✓							
Health-based targets		✓	✓			✓	✓	
Biodiversity surveys	✓	✓						
Detect endangered/ elusive animals		✓						

DNA sampling protocol

The sampling protocol outlined below has been designed to enable collection of samples from aquatic environments for DNA analysis. Our scientists can help with any additional or project-specific questions about sampling for DNA analysis. Please contact us for further information about how we can work with you.

Sample contamination risks

When collecting samples for DNA analysis we adhere to a sound sampling protocol, with a particular focus on preventing sample contamination. Samples for DNA analysis can easily be contaminated and even minute amounts of sample contamination can significantly affect or invalidate results due to the high sensitivity of the analytical equipment used.

DNA analysis is dependent on organisms' genetic material persisting in the water (for example, faeces, sloughed-off cells and other material shed from organisms). For this reason, sampling must be conducted in a way that reduces the chances of any contamination with extraneous DNA, such as human DNA through direct contact with the water or sampling equipment. Sampling containers need to be sterile and free of DNA. We can supply a certified DNA-free bottle, along with applicable legal chain of custody paperwork for legal samples.

Sampling equipment must also be sterilised and free of DNA. Any equipment and materials are used over several sampling sites need to be thoroughly sterilised between sites. DNA removal can be achieved by using a DNA decontamination reagent such as DNAZap™ or 20 per cent hypochlorite (bleach). If bleach is used for disinfecting equipment, it must be followed by cleaning with alcohol to remove any remaining bleach and then thoroughly rinsed with distilled water.

Everyone collecting samples must be made aware of the potential sources of contamination. These include:

- DNA can potentially be carried from one sampling location to the next on boots, waders, nets, sample rods and other equipment. Samplers should not enter the water without thoroughly cleaning and decontaminating waders/rubber boots. Following general cleaning, waders/rubber boots need to be decontaminated using 20 per cent hypochlorite bleach, and then thoroughly rinsed with distilled water.
- Powder-free, single use disposable gloves must be worn at all times. Sample contamination occurs immediately should a sample be collected by dipping a bottle in the water without wearing sterile gloves.
- There should be no smoking when collecting samples.
- If sampling from flowing water, samples must be collected upstream of the sampler to reduce chances of contamination.

The importance of local aquatic ecosystem knowledge

DNA is not homogenously distributed throughout a water body. This is of particular importance when looking for specific organisms. Sampling sites should be selected according to expert knowledge of the habitat preference of any organisms of interest. Samples should be collected from as close to these known habitats as possible.

The persistence of DNA in the environment can vary depending on many factors. Mitochondrial DNA persistence in water samples (as used by our method) can range from one week up to one month depending on factors known to cause DNA degradation, such as UV exposure, temperature, salinity and low water levels. DNA in water can also spread over a large area away from an initial source point.

Locations

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