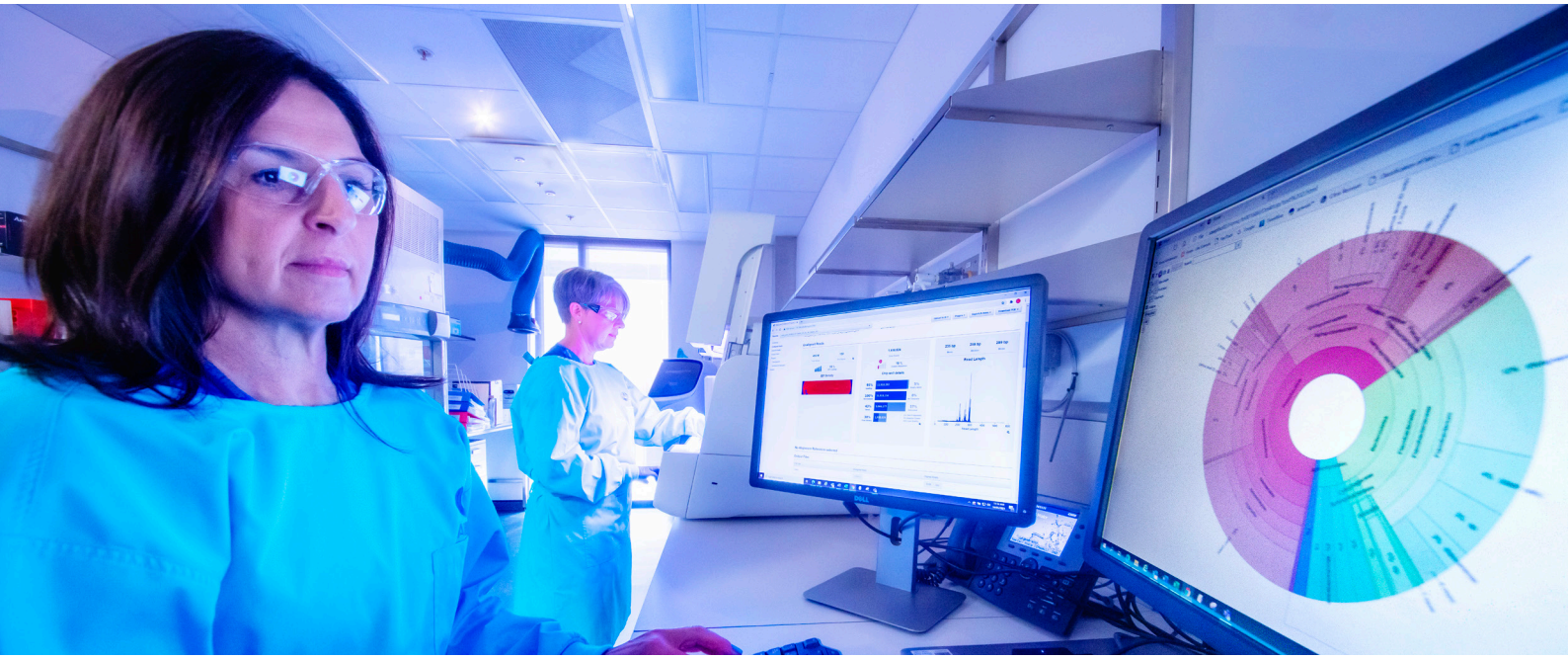
AWQC

ESCHERICHIA COLI SOURCE TRACKING

Escherichia coli confirmation, phylogrouping, bloom detection and whole genome sequencing



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

Routine monitoring for specific waterborne microbial or viral pathogens can be complex, expensive and time-consuming, and may fail to detect their presence. For these reasons, index bacteria have been selected as indicators or markers of the presence of faecal contamination and possible presence of numerous microbial pathogens. The bacterium *Escherichia coli* (*E. coli*) has been extensively used for routine water quality testing, as testing methods for this organism are relatively easy and inexpensive.

E. coli are normal inhabitants of the gastrointestinal tract of humans and animals. These bacteria are ecologically versatile and some strains have adapted to a variety of environmental conditions.

However, routine tests employed for *E. coli* detection (e.g. Colilert) do not provide information on the origin of *E. coli*. 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.

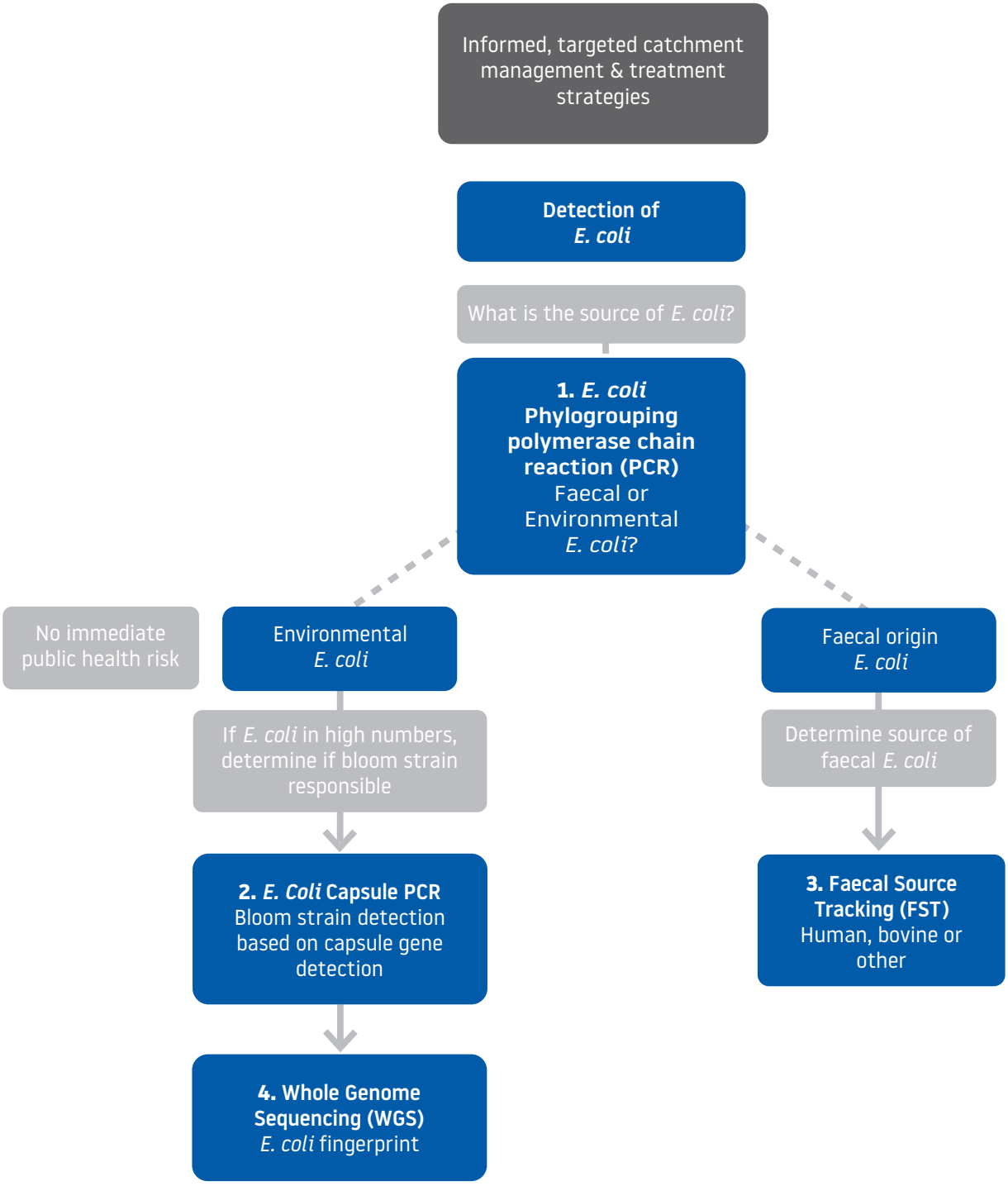
The Australian Water Quality Centre's (AWQC) advanced DNA based testing methods, including *E. coli* phylogrouping, capsule detection and whole genome sequencing, enable us to identify specific *E. coli* characteristics.

The identification of *E. coli* phylogroups/capsule types can be used to determine the ecological niche and significance of any *E. coli* detected in source waters or distribution networks, leading to fully informed risk management responses.

Specialist water services

Ensuring public health

Figure 1. *E. coli* confirmation, phlyogrouping, bloom detection and whole genome sequencing flow chart



1. *Escherichia coli* confirmation and phylogrouping

Phylogrouping enables us to determine if the source of *E. coli* contamination is of animal or environmental origin, which will inform the risk assessment process.

E.coli strains can be separated into a phylogroup structure, which at present includes the following eight phylo-groups: A, B1, B2, C, D, E, F and cryptic clade I (Clermont *et al.*, 2013).

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 the human gut flora.

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

The A0 phylogroup is carried by birds, reptiles, fish and some mammals. The B1 phylogroup is predominantly environmental.

- The risk of these types 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 (this can include humans and other animals like pigs). The E phylogroup is associated with herbivores and cattle.

- The risk of these types of *E. coli* to human health is medium, there is a possibility that human infective pathogens may also be present.

2. 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 at these levels 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).

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.

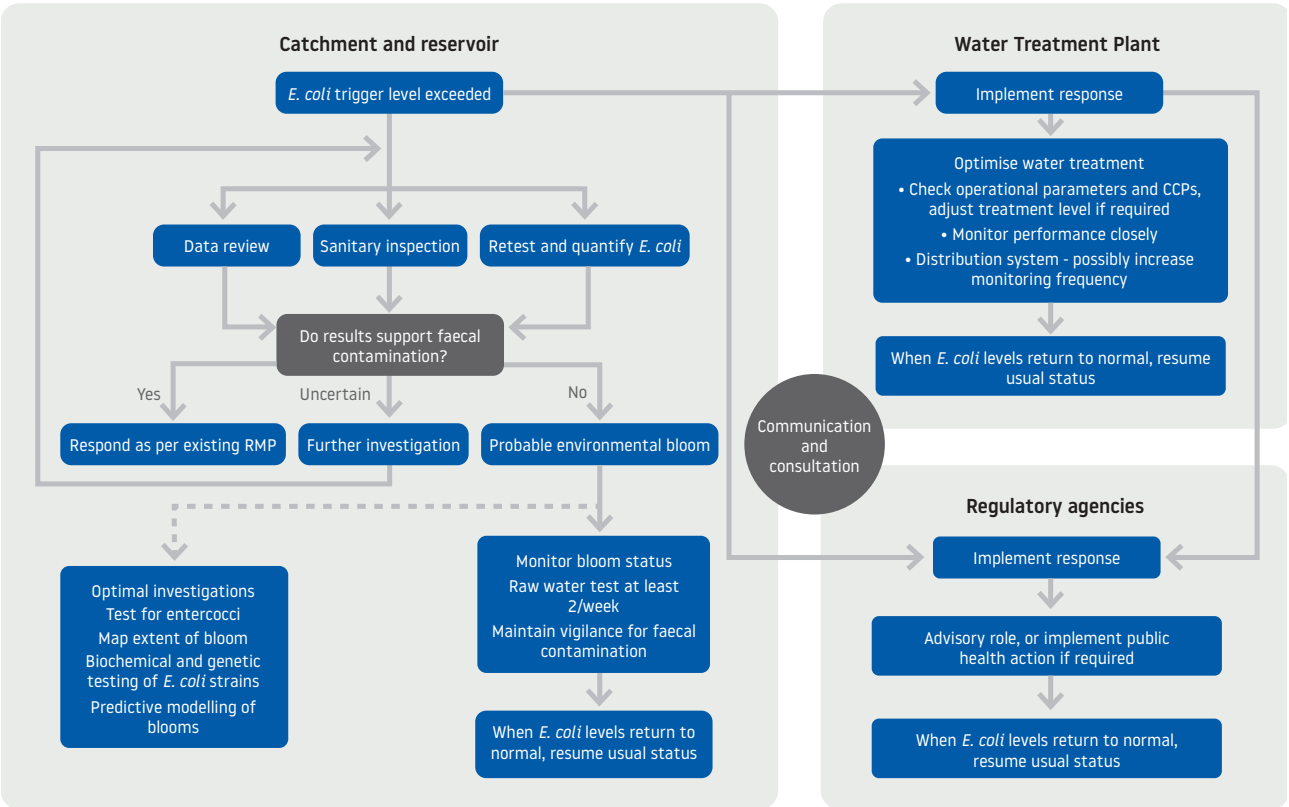
Typically, environmental *E. coli* strains do not have pathogenic tendencies and are therefore less of a health risk than faecal-borne *E. coli*.

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

The AWQC is the only Australian laboratory to offer a rapid DNA-based analysis that can detect all known Australian encapsulated *E. coli* bloom strains.

Figure 2. Utility Response Protocol taken from Water Research Australia Factsheet Project 1101: Management of Environmental *E. coli* Blooms



3. *E. coli* Whole Genome Sequencing (WGS)

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

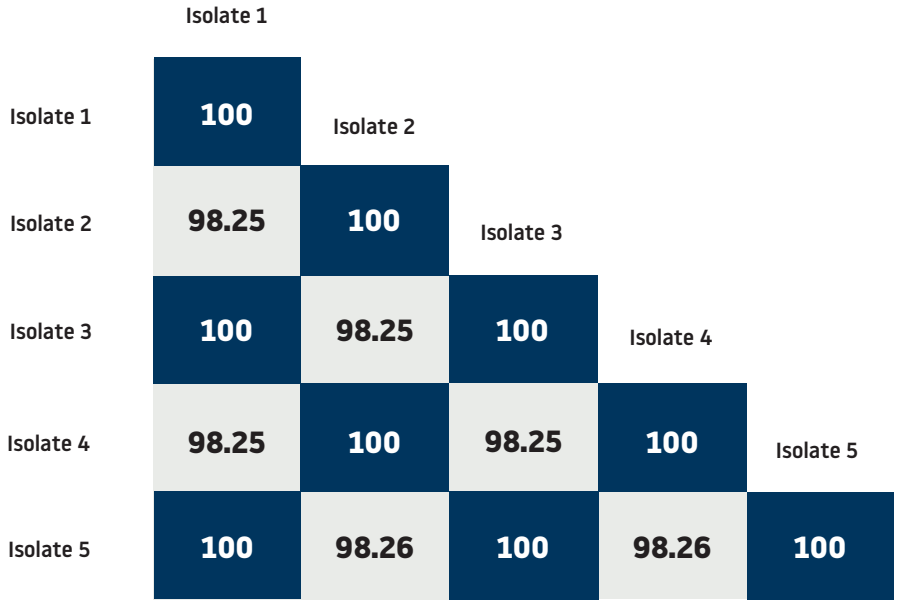
WGS involves the examination of multiple *E. coli* genes. The method developed by the AWQC includes the Achtman loci in addition to a further 157 genomic regions, allowing the identification of more than 4499 *E. coli* sequence types.

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.

Figure 3: Similarity matrix



Sampling requirements

Also refer to AWQC's DNA sampling fact sheet.

E. coli Phylogrouping

LIMS code: EC_GROUP

Sampling requirements

1 x 300ml standard bacto bottle or can take isolates from Colilert tray that triggered the exceedance.

Note: Requires Colilert analysis (ECOL_DST_X) to be added separately or can also be run on supplied *E. coli* isolates / existing Colilert tray in which case no ECOL_DST_X charge is applicable.

*Isolates can be sent as purified colonies on plates if required for identification.

Turnaround times (TAT)

Standard TAT: 5 business days
Fastest (emergency) TAT: 48hrs (excluding Colilert)

E. coli Capsule

LIMS code: EC_CAPSULE

Sampling requirements

1 x 300ml standard bacto bottle or can take isolates from Colilert tray that triggered the exceedance.

Note: Requires Colilert analysis (ECOL_DST_X) to be added separately or can also be run on supplied *E. coli* isolates / existing Colilert tray in which case no ECOL_DST_X charge is applicable .

*Isolates can be sent as purified colonies on plates if required for identification.

Turnaround times (TAT)

Standard TAT: 5 business days
Fastest (emergency) TAT: 48hrs (excluding Colilert)

E. coli WGS

LIMS code: TBA

Sampling requirements

1 x 1L DNA free bottle

Turnaround times (TAT)

Standard TAT: 10 business days