

Watercare Laboratory Services

MICROBIAL SOURCE TRACKING



Introduction

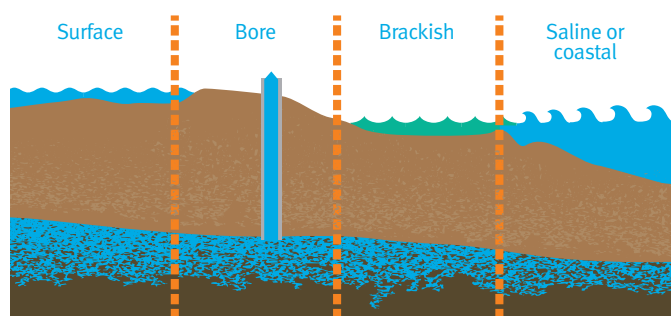
Identifying bacterial sources is an important step in controlling and managing faecal contamination, and mitigating risks associated with it. However, identifying contamination is generally difficult and over the past few years, molecular and chemical techniques have been developed to address these shortfalls. The aim is to provide regulators and stakeholders necessary information to assist them in preventing, controlling and remediating faecal contamination in the waterways and recreational swimming areas.

Watercare Laboratory Services has a molecular technique for this purpose that is referred to as Microbial Source Tracking (MST). In urban areas, faecal contamination arising from humans is likely to be the most dominant source. To provide support to the MST test results, we have also developed a chemistry test method to detect a suite of chemical determinands of human sewage at very low levels. These determinands are called pharmaceutical and personal care products (PPCPs).

What Matrices do we cover?

The matrices covered by our MST and PPCP test methods are:

- Surface water
- Bore water
- Saline or coastal water
- Brackish water.



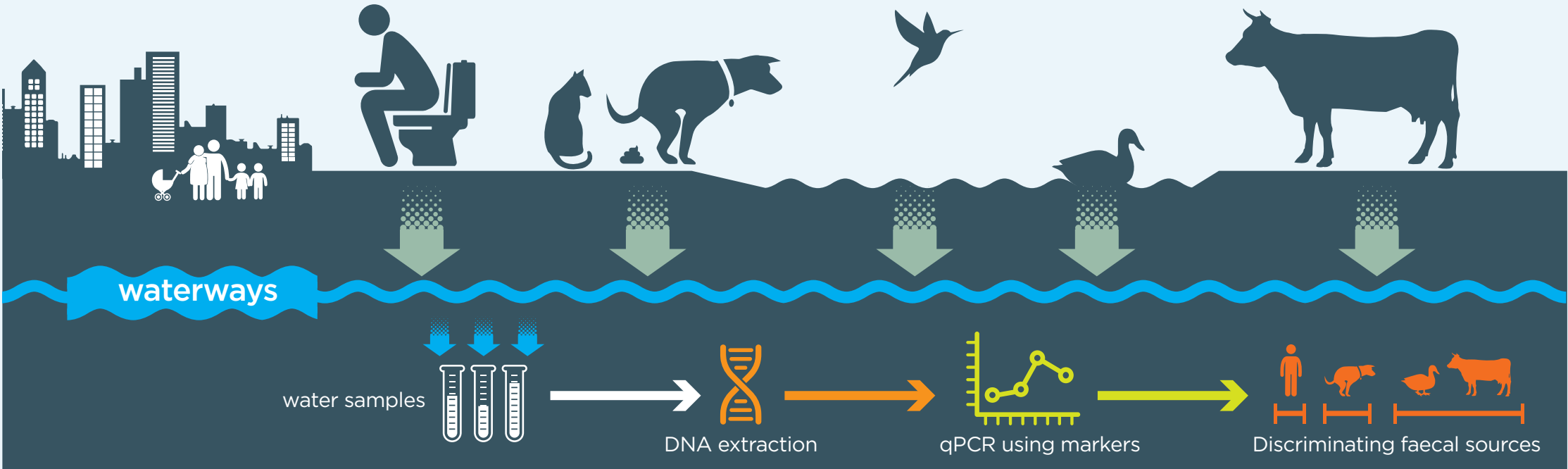
Watercare Test methods

Watercare Laboratory Services test methods are different because of the following:

- We are IANZ accredited for this method
- Proven MST markers (including the latest used by USEPA are used to indicate possible sources of faecal contamination;
- The selected suite of chemical markers targets human-specific determinands with high accuracy and precision;
- The combination of MST and PPCPs had been used successfully in finding the source of faecal contamination;
- Processing and testing of samples commences as soon as they arrive at the laboratory to prevent degradation of deoxyribonucleic acid (DNA) and hence faster turnaround of test results.

Accredited Laboratory Services

What is Microbial Source tracking? The process from taking samples through to MST testing



List of MST markers used in Watercare testing

Target	Marker	Marker target	Reference
General Faecal Marker	GenBac3	16S rRNA from Bacteroidales species present in a broad range of hosts.	<i>USEPA Method B: 2010. Bacteroidales in Water by TaqMan® Quantitative Polymerase Chain Reaction (qPCR) Assay June</i>
Human	HumM2	Cell surface-associated gene from Bacteroidales species associated with humans	<i>USEPA Method 1697. 2019. Characterization of Human Fecal Pollution in Water by HumM2 TaqMan® Quantitative Polymerase Chain Reaction (qPCR) Assay</i>
Human	HF183	16S rRNA gene from Bacteroides species associated with humans	<i>USEPA Method 1696: 2019 Characterization of Human Fecal Pollution in Water by HF183/BacR287 TaqMan Quantitative Polymerase Chain Reaction (qPCR) Assay</i>
Ruminant	BacR	16S RNA gene from Bacteroidales species associated with ruminants.	<i>Reischer G. H., D. C. Kasper, R. Steinborn, R. L. Mach and A. H. Farnleitner. 2006. Quantitative PCR method for sensitive detection of ruminant fecal pollution in freshwater and evaluation of the method in alpine karstic regions. Appl. Environ. Microbiol. 72:5610-5614.</i>
Canine	DG72	Non-ribosomal gene from canine-associated Bacteroids species	<i>Green HC, White KM, Keltly CA, Shanks OC 2014. Development of rapid canine fecalsource identification PCR-based assays. Environ Sci Technol. 7;48(19):11453-61.</i>
Avian	GFD	16S RNA gene from unclassified Helicobacter species	<i>Green H. C., L. K. Dick, B. Gilpin, M. Samadpour and K. G. Field. 2012. Genetic markers for rapid PCR-based identification of gull, Canada goose, duck, and chicken fecal contamination in water. Appl. Environ. Microbiol. 78:503-510.</i>
Gull	Gull 4	16S rRNA gene from Catellicoccus marimammalium	<i>Ryu, H., Griffith, J. F., Khan, I. U., Hill, S., Edge, T. A., Toledo-Hernandez, C., ... Santo Domingo, J. (2012). Comparison of gull feces-specific assays targeting the 16S rRNA genes of Catellicoccus marimammalium and Streptococcus spp. Applied and environmental microbiology, 78(6), 1909–1916. doi:10.1128/AEM.07192-11</i>

Pharmaceutical and personal care products (PPCP)

What are they?

Humans take drugs for therapeutic purposes. These chemicals and their associated metabolised by-products end up in sewage and are commonly found in the discharge from wastewater treatment plants. These are very good indicators of human source contamination of waterways.

Why test?

The presence of PPCP compounds is a strong indicator of the presence of human source sewage and thus human faecal contamination. The test used can see levels at 1 ppt which is like a drop in an Olympic-size swimming pool so is very sensitive. It also gives strong corroborative evidence for the MST markers to confirm the presence of human source contamination. The levels of the PPCP compounds found increase as they get closer to the source of contamination and thus may be used to track backwards to where the source is.

Compounds tested for using PPCP

<i>Atenolol</i>	Beta blockers - primarily in cardiovascular diseases
<i>Benzophenone</i>	used as a photo initiator in UV curing applications such as inks, imaging and clear coatings in printing industry
<i>Caffeine</i>	stimulant drug
<i>Carbamazepine</i>	anticonvulsant and mood stabilizing drug - treatment for epilepsy and bipolar disorder.
<i>Ciprofloxacin</i>	antibiotic for bacterial infections treatment.
<i>Cotinine</i>	metabolite of nicotine - valuable indicator of tobacco smoke exposure
<i>Deet</i>	active ingredient in insect repellent
<i>Diltiazem</i>	calcium channel blocker - treatment of hypertension, angina pectoris and arrhythmia
<i>Diphenhydramine</i>	antihistamine possessing anticholinergic, antitussive, antiemetic and sedative
<i>Doxycycline</i>	antibiotic similar to tetracycline
<i>Fluoxetine</i>	antidepressant of the selective serotonin reuptake inhibitor - treatment for major depressive disorder
<i>Metoprolol</i>	beta blocker - treatment of cardiovascular system, especially hypertension
<i>Naproxen</i>	Non-steroidal anti-inflammatory drug - relief of variety of pain, fever, swelling and stiffness
<i>Paracetamol</i>	analgesic(pain reliever) and antipyretic (fever reducer)
<i>Sucralose</i>	artificial sweetener
<i>Sulfamethoxazole</i>	bacteriostatic antibiotic - used in conjunction with trimethoprim
<i>Trimethoprim</i>	bacteriostatic antibiotic - prevention and treatment of urinary tract infection
<i>Lamotrigine</i>	Anti-epileptic
<i>Acesulfame</i>	artificial sweetener
<i>Diclofenac</i>	Non-steroidal anti-inflammatory drug - reduce inflammation and as an analgesic reducing pain
<i>Gemfibrozil</i>	oral drug used to lower lipid level
<i>Ibuprofen</i>	Non-steroidal anti-inflammatory drug - relief of variety of pain, fever, swelling and stiffness
<i>Trichlorocarbanilide</i>	antibacterial agent common in personal care products - soaps and lotions
<i>Triclosan/Irgasan</i>	similar to triclocarban - antibacterial and antifungal agent, mouthwash

Accredited Laboratory Services

At Watercare we offer:

Speed

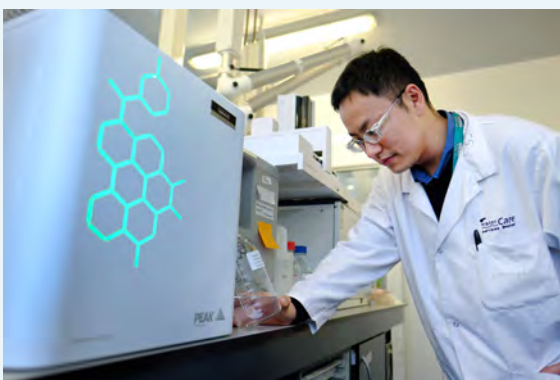
- Rapid turnaround time with MST PCR results being made available in ten days or less. This is critical when the data is intended to guide remediation efforts and public health decisions.

Accuracy

- Watercare uses both PCR markers selected for the detection of human faecal pollution by the USEPA.
- Watercare's PCR methods are based on the recently published and validated USEPA methods 1696 and 1697
- The MST and Pharmaceutical and Personal care products (PPCP) tests offer corroborating evidence from two entirely different technologies.
- Watercare offers a broad range of microbiological and chemical tests which can be used to complement MST studies.

Location & logistics

- Our Auckland laboratory is the only facility offering MST testing in the North Island
- Conveniently located near Auckland Airport allowing for rapid transfer of couriered samples to the testing facility.



Watercare Laboratory Services offer a full range of testing services comprising:

Online water monitoring

Ambient air quality monitoring

Stack emissions testing

Odour monitoring

Lab testing

- inorganic
- organic
- microbiology

Sampling

We test a range of matrices:

Water

Soil

Air



0800 522 365



labsales@water.co.nz



www.watercarelabs.co.nz