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RT-qPCR Method for Four-Hour Negative Reporting of *E. coli* in Water

Announcing the introduction of Method MM336, a cutting-edge approach for the rapid detection and enumeration of *E. coli* in water using RT-qPCR technology. This innovative method helps significantly reduce traditional *E. coli* testing turnaround times – from a full day to just a few hours – enabling faster responses during contamination events. By helping minimise service disruptions, Method MM336 can enhance operational resilience and support water utilities in delivering safe, reliable water services with confidence.

Why faster detection matters

In Victoria, routine monitoring for *Escherichia coli* (*E. coli*) is essential to verify that preventative measures such as disinfection and filtration are functioning effectively. Under the Safe Drinking Water Regulations (SDWR) and Australian Drinking Water Guidelines (ADWG), *E. coli* must not be detected in any 100 mL sample of drinking water (NHMRC & NRMMC, 2011; NHMRC, 2011). Any detection requires immediate investigation and corrective action.

Traditionally, compliance relies on culture-based methods, which typically take 18–27 hours to deliver results. While suitable for routine monitoring, this timeframe is inadequate during contamination events when rapid decision-making is critical. The need for rapid results has grown significantly since false-positive allowances were removed under the SDWR, meaning any *E. coli* detection is now treated as a regulatory breach.

The solution

Method MM336 reduces turnaround time to approximately 4 hours, enabling faster public health responses during contamination events and helping minimise operational risk.

Key benefits offered

- Rapid confirmation of results to inform mobilisation requirements
- Fast response during contamination investigations and incidents.
- Reduced costly delays and improved public health protection
- Fast reporting to the Victorian Department of Health

Specifications

Table 1: Method specifications

Method code	MM336
Analytes	All water matrices
Sample volume	1 L minimum
Holding time	26 hours
Turn around time	~4 hours
Limit of reporting	1 cfu/100 ml (estimated)
Reporting format	Qualitative and Quantitative

PCR: A brief history

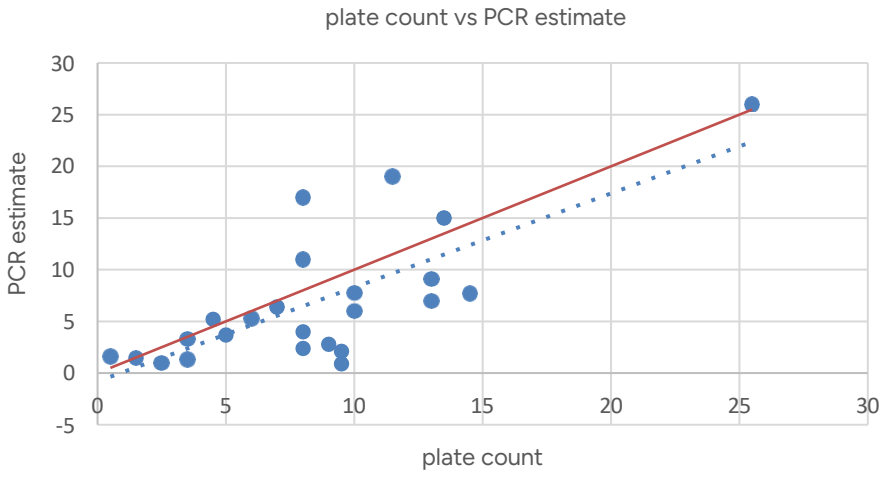
Polymerase Chain Reaction (PCR) has transformed water microbiology by enabling rapid, sensitive detection where traditional culture-based methods are slow and labour-intensive.

This evolution began with the introduction of real-time quantitative PCR (qPCR) in the 1990s (Heid et al., 1996), which allowed amplification to be monitored in real time and microbial targets to be quantified with high precision.

Regulatory acceptance followed over the next two decades. In 2012–2013, the U.S. Environmental Protection Agency (EPA) incorporated qPCR into recreational water monitoring through Methods 1609 (US EPA, 2013) and 1611 (US EPA, 2012) for Enterococci, later extending this approach with Draft Method C for *E. coli*. These protocols established rigorous quality criteria and standardised workflows, cementing PCR as a regulatory-approved tool and fundamentally changing water quality assessment.

Today, PCR is embedded in international frameworks such as ISO/TS 12869:2019 and supported by initiatives under the EU Bathing Water Directive. As a result, the focus has shifted from proving feasibility to optimising performance and expanding applications.

Figure 1: Correlation between plate counts and PCR estimates for E. coli in spiked samples.



The new PCR method from ALS combines two *E. coli* markers with proven sensitivity and specificity, supported by extensive research. EC16S, first described by Huijsdens et al. (2002), offers broad inclusivity, while EC23S857 – validated under U.S. EPA Draft Method C (Sivaganesan et al., 2019) and widely adopted for regulatory applications – delivers superior sensitivity and quantitative performance.

Together, these markers provide complementary strengths, forming the basis of a dual-target strategy that helps reduce false negatives and build confidence in results. This approach is integrated into a PCR workflow aligned with international best-practice principles for molecular water testing, helping ensure reliable detection across diverse water matrices.

Verification outcomes

Comprehensive verification studies have shown that Method MM336 delivers high sensitivity, strong specificity, and consistent reliability in potable water.

These findings support using the method as a robust test to support culture-based enumeration for *E. coli* monitoring.

Sensitivity and specificity

PCR results demonstrated strong concordance with plate culture, with sensitivity and specificity both exceeding 95% (Table 2), meeting internationally recognised performance criteria for qPCR.

The method achieved 100% negative predictive value, offering exceptional confidence in negative results.

Estimated counts based on PCR correlated closely with plate culture counts in spiked samples, with Pearson correlation coefficients >0.95 (Figure 1). This confirms that the method can not only detect *E. coli* contamination reliably but also provide meaningful estimates of colony forming units (CFU).

While PCR specificity was slightly below 100% (Table 2), culture-based methods detect only cells capable of growth under laboratory conditions. They may miss viable but non-culturable (VBNC) cells or trace contamination, both of which PCR can identify thanks to its lower detection limit.

Although PCR can theoretically amplify DNA from non-viable cells, the strong correlation between PCR and culture counts across spiked samples indicates that interference from extraneous DNA is negligible and does not compromise quantitation. These findings underscore PCR's reliability as a rapid, sensitive alternative to culture for potable water monitoring.

Reliability in potable water

Performance was assessed exclusively in potable water samples, with trace amounts of effluent used only as a spike source to achieve target *E. coli* concentrations. Results confirmed consistent amplification efficiency and reproducibility, with replicate analyses showing <5% coefficient of variation (CV).

No significant matrix interference was observed.

Strategic marker selection

Table 2: Sensitivity and specificity of method MM336 (PCR), calculated against method MM698 (culture), for 44 potable water samples (positives spiked).

Metric	Sample type (n)	Concordance	95% CI	Counts (TP/FN/FP/TN)
Sensitivity	Culture positive (24)	100%	85.8%-100%	24/0/-/-
Specificity	Culture negative (20)	95%*	75.1%-99.9%	-/-/1/19*

* PCR detects VBNC cells and offers a lower detection limit, enabling identification of contamination that culture may miss. While PCR can theoretically amplify DNA from non-viable cells, performance metrics meet internationally recognised benchmarks for qPCR.

TP = true positive; FN = false negative; FP = false positive; TN = true negative

Procedure

Sample concentration

Potable water samples are collected in sterile bottles dosed with sodium thiosulfate, stored at 5 ± 3 °C, and processed within 26 hours. A 1 L sample is concentrated by vacuum filtration through a 0.45 µm membrane using aseptic technique.

The membrane is transferred to a bead tube for nucleic acid extraction. Each batch includes a process blank and positive to help verify workflow performance.

Nucleic acid purification and PCR

Total nucleic acid is extracted using the MagMAX™ CORE kit with bead-beating on an automated platform. Extracted eluate is used in a duplex RT-qPCR assay with an internal amplification control (IAC) included to monitor inhibition. Each PCR batch includes standards and a no-template control to help ensure quality.

Result analysis and reporting

Copies per reaction are converted to estimated CFU per 100 mL using an experimentally verified conversion factor and the effective volume. Results are reported as detected/not detected, alongside quantitative estimates. The method achieves sensitivity consistent with ADWG requirements (1 cfu/100 mL) as outlined by NHMRC and NMMRC (2011).

Compliance alignment

The method was developed to meet ADWG requirements for *E. coli* detection, helping ensure sensitivity consistent with potable water safety standards.

The workflow and verification process are structured to support NATA accreditation for potable water testing, reinforcing ALS' commitment to accredited, high-quality analytical services.

Key compliance features include:

- Dual-marker design for enhanced inclusivity and sensitivity
- Rigorous QA measures including multi-level process controls and internal controls for robust confidence in results.
- NATA accreditation pending
- Quantitative reporting with strong correlation to cfu using experimentally verified conversion factors

Special requirments

Method request

When submitting samples, clearly indicate method code MM336 on the chain of custody (CoC). Include any priority turnaround or out-of-hours requirements to ensure correct processing.

Sample submission

Samples are to be received by the ALS Scoresby lab within 20 hours of collection.

For same-day reporting, samples must be received by the ALS Scoresby lab by 6 pm.

ALS can supply a 2L sterile bottle for sample collection.

Parallel analysis

This PCR method is performed in parallel with the standard plate culture method (MM698).

Clients should collect sufficient sample volume to allow both analyses to be completed without compromise.

Out-of-hours reporting

For urgent or time-sensitive results, ALS offers reporting outside standard business hours. To avoid delays, clients must nominate a dedicated contact person on the CoC who will be available to receive data and respond promptly. Out-of-hours rates apply.

Given the critical nature of this method, we recommend contacting ALS' Client Services team by phone immediately after sample dispatch, so appropriate measures can be put in place and all arrangements confirmed.

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