Microbial detection

Microbial detection from natural water sources using the MagMAX Wastewater Ultra Nucleic Acid Isolation Kit

Highlight

- The MagMAX Wastewater Ultra Nucleic Acid Isolation Kit provides a simplified solution for microbial detection from natural freshwater and ocean saltwater

Introduction

Indicators of microbial water quality are useful to determine whether a body of water is safe for recreational purposes. The wide variety of microorganisms that can be present in water samples, as well as the specific methods needed to quantify them, have presented a significant challenge to devising simple but informative approaches to standardizing water quality monitoring. The evaluation of coliphages as indicator organisms, using a culture-based method, is generally the standard method for water quality measurements [1]. However, these traditional methods are both time-consuming and labor-intensive. Rapid testing methods using qPCR can provide faster results. However, the need to concentrate the sample prior to nucleic acid isolation and the lack of automation have hindered the adoption of these methods. To overcome these challenges and help obtain results faster, we have utilized existing technology from the Applied Biosystems™ MagMAX™ Wastewater Ultra Nucleic Acid Isolation Kit. Here we demonstrate a simplified workflow for accurate, high-throughput, and sensitive molecular testing for the identification of microorganisms in marine and freshwater samples.

Materials and methods

Water samples

Freshwater samples were collected at three different locations in the Austin, Texas area (Hamilton Pool, Roy G. Guerrero River, and Lady Bird Lake). Saltwater samples were collected from the Atlantic Ocean (Garden City, South Carolina) and the Pacific Ocean (Del Mar, California). Grab samples were collected using high-density polyethylene containers and transferred to the lab in Austin, Texas. Upon arrival, all water samples were stored at 4°C and heat-inactivated at 65ºC for 1 hour prior to processing.

Processing 10 mL samples using magnetic beads for microbial enrichment

To prepare 10 mL contrived samples for processing, 15 mL of each water sample was centrifuged for 10 minutes at 10,000 x *g* at 4°C. From each clarified supernatant, 10 mL was transferred to a new tube and spiked with 10 µL of ZymoBIOMICS™ Microbial Community Standard (Zymo Research) that was diluted 100-fold. The clarified supernatants with the spiked-in microbial standard were processed in duplicate using the 10 mL wastewater workflow. Briefly, samples were transferred in 5 mL aliquots to two 24 deep-well plates, one of which contained 100 µL of Invitrogen™ Dynabeads™ Wastewater Virus Enrichment Beads per well to concentrate the samples. The samples were then processed on the Thermo Scientific™ KingFisher™ Flex Purification System (Figure 1). The concentrated microbial cells were eluted into a single plate that contained 500 µL of the lysis buffer from the MagMAX Wastewater Ultra Nucleic Acid Isolation Kit in each well. Proteinase K, binding buffer, and magnetic beads were added to the concentrated samples, and the extraction script was initiated. At the end of the run, the microbial nucleic acid was eluted in 100 µL of elution buffer.

qPCR analysis of microbial nucleic acid

Following nucleic acid isolation, the extraction efficiency was evaluated using quantitative real-time PCR (qPCR). The assays were performed using 2.5 µL of each extracted sample in a 384-well plate at a final volume of 10 µL, in duplicate, using the Applied Biosystems™ TaqMan™ Fast Virus 1-Step Master Mix and species-specific primers and probes. The cycling conditions used were: 1 cycle at 50°C for 5 min; 1 cycle at 95°C for 20 sec; 40 cycles of 95°C for 3 sec, 60°C for 30 sec.

Results

The number of microorganisms present in different water sources may vary depending on the source and type of water sample. Therefore, the microorganisms are normally concentrated prior to processing. To concentrate the microbial load, we utilized Dynabeads magnetic beads for microbial enrichment. These beads have been effectively utilized to concentrate viruses from wastewater samples [2]. Figure 2 shows the efficiency of recovery of gram-negative bacteria from different natural water types compared to a PBS control. The recovery of *Salmonella enterica* and *Escherichia coli* was significantly higher from ocean saltwater samples than from freshwater samples. *Pseudomonas aeruginosa* was recovered at similar levels from all water sources.

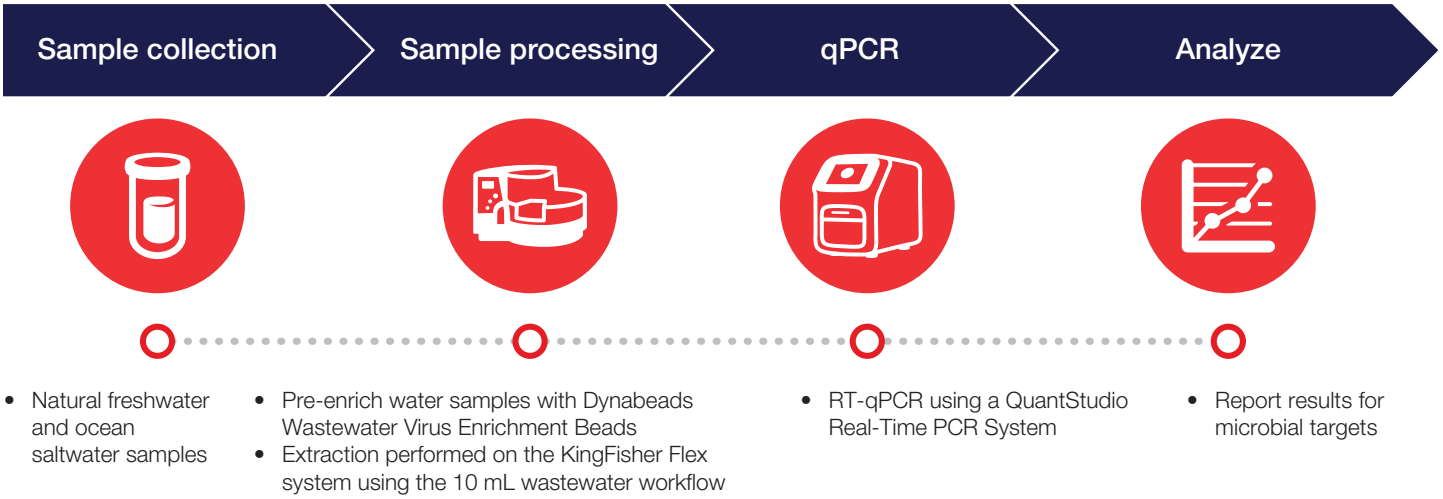

Figure 1. Simplified workflow for processing 10 mL of freshwater and saltwater samples for microbial detection using the MagMAX Wastewater Ultra Nucleic Acid Isolation Kit.

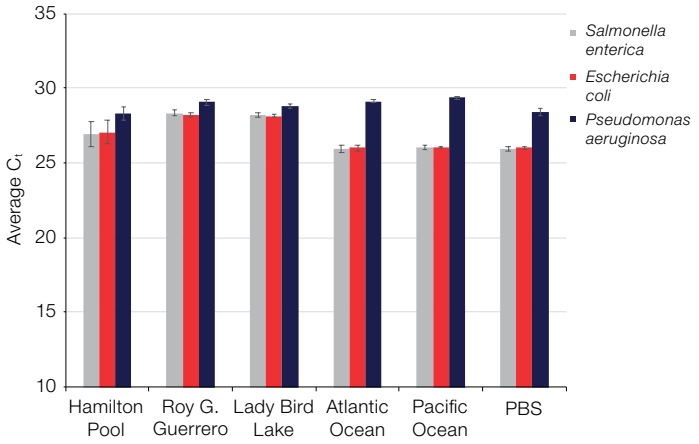

Figure 2. Detection of gram-negative bacteria from contrived natural freshwater and ocean saltwater samples.

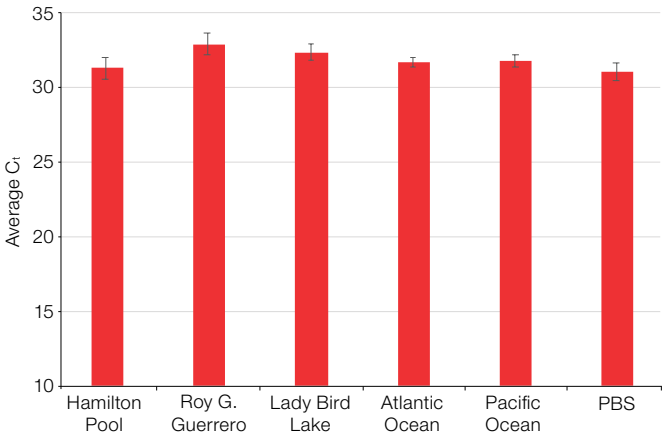

Figure 4. Detection of a representative fungus (*Cryptococcus neoformans*) from contrived natural freshwater and ocean saltwater samples.

We also saw efficient recovery of gram-positive bacteria (Figure 3) from freshwater and saltwater samples compared to a PBS control. The average C_t values are slightly higher than those of gram-negative bacteria, likely caused by the substantially thicker peptidoglycan layer in gram-positive bacteria, resulting in less efficient lysis of the bacteria. However, we have seen that the recovery of gram-positive bacteria can be improved by bead-beating (data not shown). Bead-beating plates or tubes can be purchased separately or as part of the Thermo Scientific™ MagMAX™ Microbiome Ultra Nucleic Acid Isolation Kit. We also observed efficient recovery of the fungus *Cryptococcus neoformans* (Figure 4) using the MagMAX Wastewater Ultra Nucleic Acid Isolation Kit. The average C_t values for this fungus are higher than those of the bacteria. This is likely due to the lower abundance of fungi in the spike-in standard used for these experiments. Nevertheless, this workflow provides efficient enrichment and detection of bacterial and fungal species that are commonly present in different water sources.

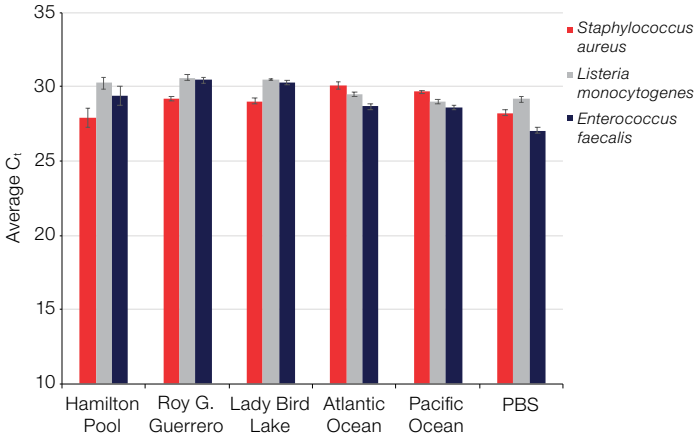

Figure 3. Detection of gram-positive bacteria from contrived natural freshwater and ocean saltwater samples.

Conclusion

We have successfully evaluated contrived microbial loads in natural freshwater and ocean saltwater samples using the MagMAX Wastewater Ultra Nucleic Acid Isolation Kit. We also presented an efficient workflow for assessing microbial loads in freshwater and saltwater samples that can be used to monitor wastewater treatment plant efficiency, drinking water quality, and recreational water safety. Although this workflow cannot distinguish between viable and dead microbial cells in water samples, RNA has relatively low stability and degrades relatively faster in dead cells. Therefore, the correlation of cell viability with the persistence of nucleic acids must be well-characterized to adopt this method as a surrogate to the more traditional culture-based technique [3]. The results accomplished two goals of public health relevance: the development of a robust workflow for identifying microbial contaminants in water samples, and the implementation of molecular assays for the identification of waterborne pathogens.

Ordering information

Product	Quantity	Cat. No.
MagMAX Wastewater Ultra Nucleic Acid Isolation Kit with Virus Enrichment	100 preps	A52610
MagMAX Microbiome Ultra Nucleic Acid Isolation Kit, with bead plate	100 preps	A42357
MagMAX Microbiome Ultra Nucleic Acid Isolation Kit, with bead tubes	100 preps	A42358
KingFisher Flex Purification System	1 instrument	Go to thermofisher.com/kingfisher
KingFisher Apex Purification System		
KingFisher Duo Prime Purification System		
QuantStudio Real-Time PCR Systems	1 instrument	Go to thermofisher.com/quantstudio
TaqMan Fast Virus 1-Step Master Mix	5 x 1 mL	4444434
	1 x 10 mL	4444436
	1 x 1 mL	4444432
TaqMan Gene Expression Assay	Multiple	Go to thermofisher.com/taqmangeneexpression

References

1. Holcomb DA, Stewart JR (2020) Microbial indicators of fecal pollution: recent progress and challenges in assessing water quality. *Curr Environ Health Rep.* 7(3):311-324. doi:10.1007/s40572-020-00278-1.
2. Thermo Fisher Scientific. Multiple workflow options for detection of SARS-CoV-2 in wastewater samples. Application Note (2021). **https://assets.thermofisher.com/TFS-Assets/BID/Application-Notes/detection-sars-cov-2-wastewater-samples-app-note.pdf**
3. Birch L, Dawson CE, Cornett JH, Keer JT (2001) A comparison of nucleic acid amplification techniques for the assessment of bacterial viability. *Lett Appl Microbiol.* 33(4):296-301. doi:10.1046/j.1472-765x.2001.00999.x.

For more information, visit **thermofisher.com/magmaxwastewater**