Literature Digest

Monitoring Viable Bacteria in the Environment with PMA and PMAxx™

Viability PCR (v-PCR) with PMA and PMAxx™ has been validated in hundreds of peer-reviewed publications and numerous microbial strains for sensitive, accurate, and rapid detection of viable microbes. In this digest we have provided summaries and takeaways for three publications on the use of v-PCR with PMA and PMAxx™ for monitoring bacterial viability in the environment.

Download our full publication list for more validated applications using PMA and PMAxx™.

Evaluation of Microbial Viability Techniques on Monitoring Ascaris Suum in Wastewater

Ravindran, V. B., Shahsavari, E., Soni, S. K., & Ball, A. S. (2019). Viability determination of Ascaris ova in raw wastewater: a comparative evaluation of culture-based, BacLight Live/Dead staining and PMA-qPCR methods. Water Science and Technology, 80(5), 817–826. https://doi.org/10.2166/WST.2019.286

Summary

Raw wastewater acts as a reservoir for microbial pathogens that pose a significant public health risk. To evaluate these risks, microbial presence in wastewater needs to be properly assessed by accurate and sensitive methods. Currently, microbial pathogens in wastewater are evaluated by culture-based methods, LIVE/DEAD BacLight™ viability staining, and v-PCR with PMA. Culture-based methods and subsequent microscopy analysis can be time consuming and challenging to optimize for different target organisms and sample sources. In contrast, v-PCR relies on selective PCR amplification from live organisms, which allows rapid and highly sensitive microbial detection without culturing of samples. The *Ascaris* species of parasitic worms are prevalent in contaminated wastewater and are used as indicators to evaluate the effectiveness of water treatment processes. Ravindran et al. compared the efficacy of v-PCR with PMA, LIVE/DEAD® BacLight™ staining, or the standard culture-based method for monitoring viable *A. suum* ova in wastewater. All three methods gave comparable results for percent viability of A. *suum* in raw wastewater, suggesting that v-PCR and microscopy are valid alternatives to culture-based detection. In addition, the authors note significant drawbacks of microscopy-based viability methods due to their inherent variability and time consumption. In contrast, v-PCR offers comparable assessment with greater speed and specificity than the other two methods when evaluating *A. suum* ova viability in raw wastewater.

How PMA was used

v-PCR with PMA was compared with culture-based methods and microscopy-based LIVE/DEAD BacLight™ viability staining as a method for assessing the presence of viable *A. suum* ova parasitic nematode in raw wastewater.

Takeaways

- v-PCR with PMA offered comparable results to both culture-based and microscopy-based detection methods for monitoring viable *A. suum* in raw wastewater.
- v-PCR with PMA provided faster and more specific results compared to culture-based and microscopy-based methods.
- The PMA-Lite™ LED Photolysis Device was used to improve reproducibility and consistency of photoactivation across all samples.

Methods (viability determination)	Viable	Non-viable	Uncertain	Total
Culture-based	824 ± 24 (82%)	176 ± 24 (18%)	0 (0)	1,000
BacLight™ staining	397± 6 (79%)	84 ± 9 (19%)	9 (2%)	500
BacLight™ staining (wastewater)	174 ± 5 (87%)	21 ± 3 (11%)	4 (2%)	200
PMA-qPCR	424 ± 17 (85%)	76 ± 17 (15%)	0	500

Viability of *A. suum* ova in raw wastewater evaluated by culture-based, BacLight™ Live/Dead staining and PMA-qPCR. Adapted from Ravindran, et al. https://doi.org/10.2166/WST.2019.286 reproduced under the Creative Commons license.

Products Used

Product	Cat. No.	Unit Size
PMA Dye	40013	1 mg
PMA-Lite™ LED Photolysis Device	E90002	1 each

v-PCR with PMA Successfully Applied to Monitor VBNC Pathogens in Drinking Water Treatment Plants

Guo, L., Wan, K., Zhu, J., Ye, C., Chabi, K., & Yu, X. (2021). Detection and distribution of vbnc/viable pathogenic bacteria in full-scale drinking water treatment plants. Journal of Hazardous Materials, 406, 124335. https://doi.org/10.1016/J.JHAZMAT.2020.124335

Summary

Culture-based methods are commonly employed to monitor the presence of pathogenic bacteria in drinking water treatment plants (DWTPs). However, drinking water treatment processes are known to cause some bacteria to enter a viable but non-culturable (VBNC) state. These VBNC bacteria avoid culture-based detection methods yet still pose a significant health risk due to their ability to resuscitate to a pathogenic state after ingestion. Quantitative PCR (qPCR) can be used to detect microbial DNA, but is unable to discriminate between viable and dead microorganisms. v-PCR with PMA resolves this issue by offering a method to selectively amplify DNA from viable cells with intact membrane integrity, and therefore may be used to quantify VBNC bacteria.

Guo et al. performed a comprehensive study on the application of v-PCR with PMA for detection of VBNC pathogenic bacteria in full-scale DWTPs. They studied the occurrence of five VBNC opportunistic pathogens in water samples taken from two DWTPs at each stage of treatment: *E.coli*, *E. faecalis*, *P. aeruginosa*, *Salmonella sp.*, *Shigella sp.*, and *L. pneumophila*. The number of v-PCR amplified gene copies for each strain was then converted to viable cell numbers using a standard curve from serially diluted bacterial cultures. Results revealed the presence of VBNC pathogens within the final treated water, suggesting greater microbial risk than results obtained by culture-based detection methods. Combining v-PCR with 16s rRNA sequencing, the authors also found that some opportunistic pathogens including *Mycobacterium* and *Sphingomonas* increased in abundance following disinfection treatment, which the authors attributed to the resistance of these organisms to chlorine. Overall, the study recommends the application of v-PCR with PMA or similar technologies to more accurately assess microbial risks in drinking water.

How PMA was used

v-PCR with PMA was used in a comprehensive study for the detection of VBNC bacteria from DWTPs. Quantifiable cell numbers of five bacterial strains were obtained from DWTP samples using v-PCR and a standard curve from serially diluted bacterial cultures. v-PCR was also combined with 16s rRNA sequencing to profile bacterial communities after each stage of the water treatment processes.

Takeaways

- v-PCR was successfully applied for detection and profiling of VBNC pathogenic bacteria in full-scale DWTPs.
- Significant quantities of VBNC opportunistic pathogens were detected after final water treatment, suggesting culture-based detection methods are insufficient for detecting VBNC bacteria.
- v-PCR with PMA or similar methods are needed to more accurately assess microbial risks in drinking water that are underestimated by traditional culture assays.

Products Used

Product	Cat. No.	Unit Size
PMA Dye	40013	1 mg

A Highly Sensitive v-PCR Assay with PMAxx™ for Detection of Potentially Viable Leptospira in Fresh Water Samples

Richard, E., Bourhy, P., Picardeau, M., Moulin, L., & Wurtzer, S. (2021). Effect of disinfection agents and quantification of potentially viable Leptospira in fresh water samples using a highly sensitive integrity-qPCR assay. PLOS ONE, 16(5), e0251901. https://doi.org/10.1371/JOURNAL.PONE.0251901

Summary

Leptospira is a pathogenic bacteria genus responsible for leptospirosis in humans as well as wild and domestic animals. Leptospirosis occurs worldwide and is responsible for at least 60,000 deaths a year. The pathogen spreads by direct or indirect contact with contaminated urine or water. However, because *Leptospira* are slow growing bacteria, culture-based methods of detection are difficult due to the likely presence of faster growing flora. In addition, there are limited studies on the survivability and persistence of *Leptospira* in the environment and their response to disinfection treatments. Molecular methods for detection such as qPCR are generally more sensitive and specific than culture-based methods. However, qPCR alone will often overestimate the true risk of infection because the bacteria genome is amplified regardless of viability.

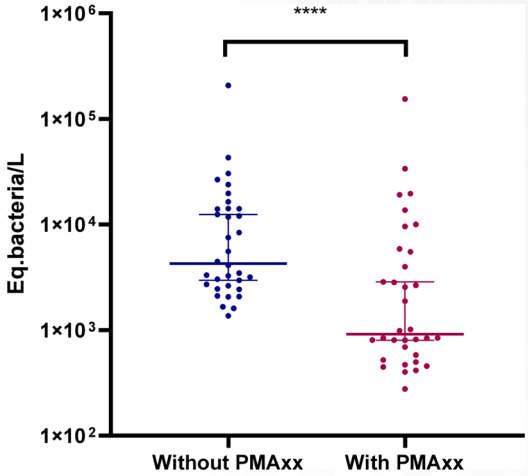
Richard et al. developed a rapid and sensitive method for quantifying potentially viable *Leptospira* in water samples. The assay involves PMAxx™ treatment to exclude DNA from non-viable cells, followed by qPCR amplification. The authors then applied the assay to evaluate the efficacy of UV, heat, and sodium hypochlorite disinfection treatments on *Leptospira*. The PMAxx™-qPCR assay was also compared against culture-based microscopy and plating methods. Results show PMAxx™-qPCR correlated well with culture-based detection methods when evaluating disinfection by heat. Yet, PMAxx™-qPCR displayed only a small decrease in signal after sodium hypochlorite treatment and no significant decrease after UV treatment, confirming these disinfection methods preserve membrane integrity. Lastly, the authors evaluated the application of PMAxx™ on environmental monitoring by performing qPCR on 32 surface water samples with and without PMAxx™ treatment. Results show a significant decrease in detectable *Leptospira* in PMAxx™ treated samples, suggesting the addition of PMAxx™ to qPCR-based environmental monitoring will reduce overestimation of viable bacteria.

How PMA was used

A PMAxx™-qPCR assay was evaluated against culture-based methods for the detection of viable *Leptospira* bacteria after heat, UV, and chlorine disinfection treatments. PMAxx™ was also tested for its application in environmental monitoring by testing for *Leptospira* in freshwater samples.

Takeaways

- PMAxx™-qPCR results correlated well with culture-based methods when evaluating heat disinfection of *Leptospira* bacteria.
- Sodium hypochlorite and UV-based disinfection methods preserve membrane integrity and are therefore difficult to evaluate using PMAxx™-qPCR.
- Applying PMAxx™ to qPCR-based environmental monitoring of *Leptospira* could be used to reduce overestimation of viable bacteria.



Distribution and median of *Leptospira* genome (16S gene) with (red) and without PMAxx™ (blue) on environmental samples. This analysis was based on 34 environmental freshwater samples collected in the Ourcq canal (Paris) in June-September 2018. Credit Richard et al. https://doi.org/10.1371/JOURNAL.PONE.0251901 reproduced under the Creative Commons license.

Products Used

Product	Cat. No.	Unit Size
PMAxx™, 20 mM in H2O	40069	100 uL

Interested in Learning More About Viability PCR with PMA & PMAxx™?

Check out these educational pages, references, and other resources available on our website.

Learn More

Learn more about Viability PCR

Viability PCR Application Note

Other Highlighted Citations & References

Comparison of Propidium Monoazide with Ethidium Monoazide for Differentiation of Live vs. Dead Bacteria

Viability PCR with PMA Validated for Determination of Infectious SARS-CoV-2 Viruses

Total and Viable Bacteria and Fungi in the International Space Station

Antibiotic Development - Tracking Compound Penetration into Gram-Negative Bacteria with Click Chemistry

PMAxx™ qPCR: An Effective Tool for Detection of Seed-Borne Pathogens

Combining Droplet Digital PCR (ddPCR) with the Viability Dye PMA to Measure Probiotic Success

Microbial Genomics in a Lunar/Mars Simulated Habitat

Evaluation of Dental Plaque and Halitosis in Dogs by PMA and qPCR

Viability PCR for Infectious Norovirus in Food and Water Samples

Click here for a full list of PMA & PMAxx™ publications

Other Helpful Resources

View Biotium's full catalog of Viability PCR Reagents

Full list of PMA & PMAxx™ Validated Bacteria Strains

Literature Digest: Viral Integrity with PMA & PMAxx™

Viability PCR Products

Cat. No.	Product name	Unit size
40069	PMAxx™ Dye, 20 mM in dH_2O	100 uL
40013	PMA Dye	1 mg
40019	PMA Dye, 20 mM in dH_2O	100 uL
E90002	PMA-Lite™ LED Photolysis Device	1 device
31038	PMA Enhancer for Gram-Negative Bacteria	16 mL
31075, 31076	Viability PCR Starter Kits	200 assays
31033	Real-Time Bacterial Viability Kit-Salmonella (InvA)	200 assays
31034	Real-Time Bacterial Viability Kit-M. tuberculosis (groEL2)	200 assays
31035	Real-Time Bacterial Viability Kit-Staph. aureus (nuc)	200 assays
31036	Real-Time Bacterial Viability Kit-MRSA (mecA)	200 assays
31050	Real-Time Bacterial Viability Kit-E. coli (uidA)	200 assays
31037	Real-Time Bacterial Viability Kit-E. coli O157:H7 (Z3276)	200 assays
31051	Real-Time Bacterial Viability Kit-Listeria monocytogenes (hly)	200 assays
31053	Real-Time Bacterial Viability Kit-Legionella pneumophila (mip)	200 assays