Literature Digest

Viral Integrity with PMA & PMAxx™

PMA or PMAxx™ combined with qPCR is a well-published method for sensitive, accurate, and rapid detection of intact viral capsids. In this digest we have selected three publications with summaries and takeaways on the use of this method for evaluating viral integrity.

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PMA/PMAxx™ Validated in Over 40 Studies for Assessing Integrity of Viral Capsids

Leifels, M., Cheng, D., Sozzi, E., Shoults, D. C., Wuertz, S., Mongkolsuk, S., & Sirikanchana, K. (2021). Capsid integrity quantitative PCR to determine virus infectivity in environmental and food applications – A systematic review. Water Research X, 11, 100080. https://doi.org/10.1016/J.WROA.2020.100080

Summary

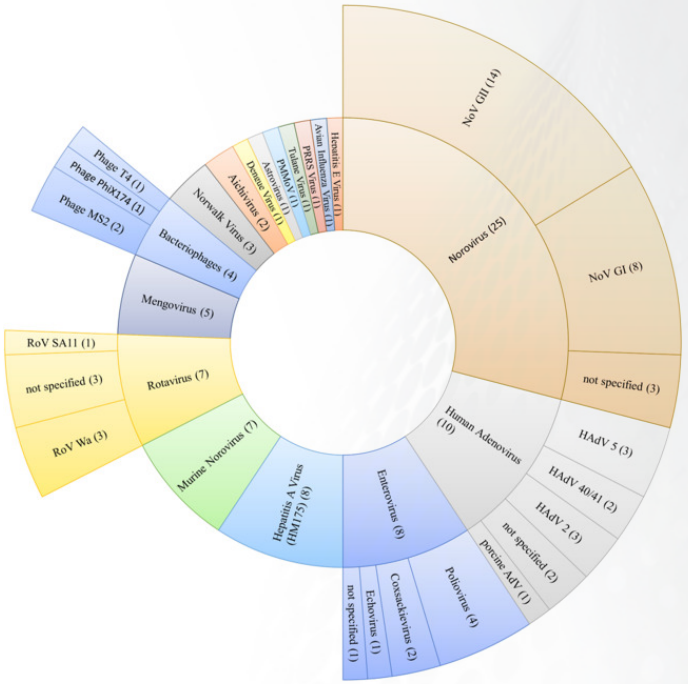
Capsid integrity quantitative PCR, a molecular detection method that measures exclusion of azo dyes by viral capsids as a correlate of integrity, is a widely used application in virology. Yet, standards for pretreatment conditions for various virus types remain limited. Leifels et al. conducted a comprehensive review of forty-one recent peer-reviewed studies employing capsid integrity qPCR for viruses in the fields of food safety and environmental virology, with the aim of establishing recommendations for the detection of intact viruses. This literature review provides a framework for general assay conditions including concentration ranges, temperature ranges, and photoactivation parameters for various azo dyes. The authors concluded that the application of capsid integrity qPCR can be implemented into existent qPCR workflows to support risk assessment and guide consumer safety.

How PMA & PMAxx™ were used

A systematic and comprehensive literature review was conducted to establish protocols and considerations for viral integrity qPCR methods. The study focused on publications using the azo dyes PMA, PMAxx™, PEMAX, and EMA for capsid integrity monitoring. The search was performed in relevant databases including Pubmed, Scopus, Ovid, Medline, and Web of Knowledge since the first introduction of the azo dye pretreatment in 2003.

Takeaways

- Cumulatively, PMA and PMAxx™ are used in 70% of capsid integrity qPCR studies.
- PMA and PMAxx™ show a higher efficiency in removing false positive signals from qPCR for both DNA and RNA viruses than EMA and PEMAX.
- Capsid integrity is strongly correlated with virus infectivity, allowing the application of PMA and PMAxx™-based assays to evaluate the infectivity of novel viruses during outbreaks.



Genus and strain number of viruses investigated and frequency of occurrence in case studies (in parentheses). Credit: M. Leifels, et al. https://doi.org/10.1016/j.wroa.2020.100080 reproduced under the Creative Commons license.

Products Used

Product	Cat. No.	Unit Size
PMAxx™, 20 mM in H_2O	40069	100 uL
PMA Dye	40013	1 mg
PMA Dye, 20 mM in H_2O	40019	100 uL

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RT-qPCR Assay with PMA Validated for Determination of Infectious SARS-CoV-2 Viruses

Hong, W., Xiong, J., Nyaruaba, R., Li, J., Muturi, E., Liu, H., Yu, J., Yang, H., & Wei, H. (2021). Rapid determination of infectious SARS-CoV-2 in PCR-positive samples by SDS-PMA assisted RT-qPCR. Science of The Total Environment, 797, 149085. https://doi.org/10.1016/J.SCITOTENV.2021.149085

Summary

The global health crisis caused by the COVID-19 pandemic has fueled the need to understand the mechanisms of transmission. To limit the spread of the virus, it is important to be able to distinguish intact versus inactivated SARS-CoV-2 viruses.

Hong et al. report an RT-qPCR assay assisted by SDS and propidium monoazide (PMA) for rapid detection of intact SARS-CoV-2 viruses in PCR-positive samples. Traditionally used methods, such as RT-qPCR of viral RNA, and immunoassays for viral proteins, are unable to distinguish between intact and inactivated viruses. In the PMA assisted RT-qPCR assay, the photoreactive PMA dye selectively permeates into inactivated viruses and interacts with the RNA, preventing it from being detected by RT-qPCR. The authors found that using this method, they were able to obtain comparable results to the gold standard plaque assay for detecting infectious SARS-CoV-2 virus from serial dilutions and samples taken from plastic surfaces. Results show the assay successfully detected as few as 8 plaque-forming units of intact virus in the positive PCR samples.

The authors concluded that the PMA assisted assay provided a culture-free method with comparable results to the standard plaque assay for detecting intact SARS-CoV-2 virus.

How PMA was used

PMA was used in a RT-qPCR based assay for rapid detection of intact SARS-CoV-2 viruses. SDS (sodium dodecyl sulfate) was used as a modest membrane destabilizing agent that improves the permeability of PMA through the membranes of inactive SARS-CoV-2 virions, but not intact virions. The assay was tested on serial dilutions of cultured SARS-CoV-2 viruses, as well as SARS-CoV-2 viruses sampled from plastic surfaces.

Takeaways

- Unlike conventional RT-qPCR, the SDS and PMA-assisted assay was able to discriminate between intact and inactive SARS-CoV-2 virions.
- The PMA assisted assay was shown to give results comparable to the gold standard plaque assay, but required only 3 hours instead of 5 days.
- The PMA assisted RT-qPCR was able to detect as few as 8 PFU (plaque-forming unit) live viruses.
- This rapid and culture-free method could be used to support environmental monitoring for effective prevention and control of SARS-CoV-2.

Products Used

Product	Cat. No.	Unit Size
PMA Dye	40013	1 mg
PMA Dye, 20 mM in H_2O	40019	100 uL
PMA-Lite™ LED Photolysis Device	E90002	1 each

PMA-Combined RT-qPCR Correlates Well with the Plaque-Assay for Detection of Intact Murine Norovirus

Lee, M., Seo, D. J., Seo, J., Oh, H., Jeon, S. B., Ha, S. Do, Myoung, J., Choi, I. S., & Choi, C. (2015). Detection of viable murine norovirus using the plaque assay and propidium-monoazide-combined real-time reverse transcription-polymerase chain reaction. Journal of Virological Methods, 221, 57–61. https://doi.org/10.1016/J.JVIROMET.2015.04.018

Summary

Human noroviruses (HuNoVs) are the leading cause of acute viral gastroenteritis throughout the world. Murine norovirus (MNV) is often used as a model to evaluate sanitization methods for HuNoVs. Molecular techniques such as RT-qPCR are the primary method for detection of NoVs in foods and stools. However, unlike time-consuming culture-based methods, these molecular detection methods are unable to assess infectivity. Treatment with azo dyes followed by RT-qPCR has been used to discriminate between intact and thermally-inactivated capsid viruses. However, further study is needed to evaluate whether results from this method correlate with standard culture-based assays.

Lee et al. assessed PMA combined with RT-qPCR as a method for detection of intact MNVs, and compared the results with a standard plaque assay. Propagated
MNV-1 viruses were heat treated at varying temperatures from 65°C to 90°C. Samples were then treated with either PMA or EMA, followed by photoactivation and RT-qPCR analysis. The plaque assay was also performed on heat-treated MNV samples for comparison. Results showed that PMA differentiated between intact and heat-inactivated MNVs better than did EMA. Moreover, results from PMA-combined RT-qPCR correlated well with the plaque assay results, displaying decreased detection of intact or viable MNVs with increasing temperatures for both assays. The authors also produced an equation for calculating a PMA value that could be used as an interpretive tool for assessing the integrity of MNVs.

How PMA was used

PMA and EMA combined with RT-qPCR was evaluated as a method for the detection of intact MNV viruses after heat treatment. Propagated MNV-1 viruses were heat treated at varying temperatures. Samples were then treated with either PMA or EMA with photoactivation and subsequent RT-qPCR analysis. The plaque assay was also performed on MNV titers heat treated at the same temperatures for comparison.

Takeaways

- Detection of propagated MNVs using PMA-combined RT-qPCR was significantly reduced following heat treatment.
- Results from PMA-combined RT-qPCR and the standard plaque assay correlated well when assessing intact and heat-inactivated MNVs.
- Treatment with PMA before RT-qPCR differentiated between intact and heat-inactivated MNVs better than did treatment with EMA.
- The authors provide an equation to determine a PMA value that could be used as an interpretive tool to discriminate between inactive and intact viral capsids.

Products Used

Product	Cat. No.	Unit Size
PMAxx™, 20 mM in H_2O	40069	100 uL
PMA Dye	40013	1 mg
PMA Dye, 20 mM in H_2O	40019	100 uL

Have more questions?
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