

Popular Article

Digital PCR: A Revolutionary Assay for Viral Disease Diagnosis

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Abstract

Digital PCR is a new-age PCR technology with increased sensitivity and reduced inter-lab variations. Its partitioning principle helps to reduce PCR inhibitors and the detection of rare gene sequences. Absolute quantification without the requirement of a standard curve is a feature that makes it advantageous over other conventional PCR amplification methods. This assay has better reproducibility and efficacy making this technology suitable for application in viral disease diagnosis.

Introduction

Digital PCR (dPCR) is a revolutionary method introduced to ease the absolute quantification of DNA. Initially, the partitions of templates were done with the help of a microtitre plate. Further automation of this partitioning principle was done by an invention in 2011 by Quantalife Co, Ltd which was based on water-oil emulsion droplet technology. In this droplet digital PCR (ddPCR) assay a reaction mix is fractionated into droplets in which template DNA is randomly distributed. Each of these fractionated droplets undergoes PCR amplification (Chen et al., 2021). Conventionally real-time PCR (qPCR) is used for the quantification of nucleic acid. However, qPCR requires a standard curve for quantification purposes. The absence of standard references and inter-laboratory quantification biases can make this process complex. A standard curve is not needed in ddPCR. ddPCR can also tolerate PCR inhibitors like SDS, heparin, and other nucleic acid extraction-based inhibitors. Variation in reaction efficiencies can also alter the quantification process of qPCR while this doesn't happen in ddPCR.

In an early viral infection, the concentration of DNA/RNA copies is low which is difficult to detect with conventional PCR methods. ddPCR has a detection limit of 1 copy/ μ L which is 10 times higher than qPCR. Hence rare viral gene quantification which was difficult to do with qPCR is now possible with ddPCR. ddPCR can be used for virus identification, quantification of viral load, detection of single nucleotide polymorphisms in the viral genome, viral drug resistance, and malignancies caused by viruses (Kojabad et al., 2021).

Quantification



In dPCR, the fundamental process is the distribution of nucleic acid containing samples into thousands of droplets. Ideally, single droplet should contain only a single amplification target. This partitioning of sample into droplets of the same volume reduces target competition and enables the detection of rare sequences. There are commercial equipment available that can aid in the partitioning of PCR reaction mix - Biomark® dPCR from Fluidigm is micro-fluid chamber based dPCR while QuantStudio12k flex dPCR and 3D dPCR from Life Technologies are micro-chip based dPCR. These can generate several hundreds of droplets. Droplet-based ddPCR QX100 and QX200 from Bio-Rad® and RainDrop from RainDance® can form up to 20,000 droplets and 10,000,000 respectively (Dong et al., 2015). This compartmentalization also reduces the concentration of probes and primers thereby further reducing the chances of mispairing of target sequences.

After the amplification process, end-point quantification is done, unlike qPCR where fluorescent signals are collected in the exponential phase. The absorbance from each compartment is recorded. These signals are counted as binary events either positive 1 or negative 0. Based on Poisson's distribution the proportion of droplets having a target sequence is predicted. Multiplex ddPCR assays can also be conducted by using probes with different fluorescent signals. Hence in a single reaction multiplexing can be done (Quan et al., 2018).

dPCR In Animal Viral Disease Detection and Quantification

ddPCR can detect 10 copies/reaction as recorded in most viral diseases which is way more sensitive than qPCR method. An early diagnosis of contagious and viral diseases of transboundary importance is crucial for control strategies. Swine viral diseases with a potential for outbreaks like African swine fever virus, Porcine circovirus type 2, and Porcine reproductive and respiratory syndrome can be detected with ddPCR (Vashi & Kumar, 2022). RT-ddPCR has also been developed for rapid detection of Japanese Encephalitis virus (Wu et al., 2017). It was found more sensitive when compared to qRT-PCR. Bovine leukemia virus causes milk production losses across farms and infected animals serve as a continuous source of infection as proviral genome integrates into the host genome. Quantification of this proviral load is important to determine the infectious status of animals. Using ddPCR even 3 copies per reaction can be detected and also the proviral load status of the animal can be determined (De Brun et al., 2022). Bovine Herpesvirus-1 (BoHV-1) causing infectious bovine rhinotracheitis is intermittently excreted in semen. Virus isolation is difficult due to cytotoxic activity of semen in cell culture. OIE recommends qPCR for screening



of bulls. However, Low viral loads in mixed semen samples from herds is a constraint for qPCR due to its lower sensitivity. ddPCR has been used to detect low copy numbers with detection limit of 4.45 copies of BoHV-1. A novel technique RT-ddPCR has also been developed for foot-and-mouth viral detection in clinical samples (Yu et al., 2022).

ddPCR In Human Viral Disease Detection and Quantification

ddPCR has been used in human patients to detect viruses affecting nervous system which include Varicella zoster virus, Herpes simplex virus, Human cytomegalovirus, and Epstein-Barr virus. These ddPCR results were found to correlate with clinical manifestations and treatment response. E gene-based Dengue virus detection for all serotypes can be done along with absolute quantification. For Zika virus limit of detection was found at 1 copy/ μ L from blood samples. The high sensitivity of ddPCR enables detection of only 11.1 copies/test for SARS-COV-2 from clinical samples (Chen et al., 2021). Multiplex ddPCR assay has also been developed for detection of enterovirus, parechovirus, herpes simplex virus-1, and 2 in CNS infections. The range of detection varied from 2000 to 2 copies per reaction (Zhu et al., 2022). Raindance droplet digital PCR has been used for ultrasensitive detection of simian immunodeficiency virus which serves as a model of Human immunodeficiency virus-1. The persistent latent infection in resting CD4⁺ Tcell even after anti-retroviral therapy requires ultrasensitive detection. SIV ddPCR can detect an average of 3 target copies per reaction and also give quantification for further anti-retroviral therapy (Long & Berkemeier, 2022).

Conclusion

In diagnostic procedures involving nucleic acid-based detection, the sensitivity of assay is a crucial factor. dPCR is a third-generation PCR technology that can provide ultrasensitive detection of viral antigen in early stages of the disease. Moreover, for diseases where cell culture-based and serological detection procedures are complex and time consuming this assay can be a solution. Absolute quantification to determine the level of infection and also a response to antiviral therapy without the need for standard curve generation could increase the efficaciousness of disease management.

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