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Applications of Digital PCR in Clinical Laboratory Medicine: Pathogen Detection and Early Cancer Screening

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Abstract. This review focuses on the recent development of digital polymerase chain reaction (dPCR) in pathogen detection and cancer early screening. dPCR partitions samples into multiple independent units,enabling absolute quantification of target molecules. It surpasses traditional qPCR in sensitivity and accuracy. Nevertheless, it has limitations, such as the inability to distinguish between live and dead bacteria and relatively high costs. In virus detection, dPCR notably enhances the early diagnosis rate of COVID -19 and shortens the HIV detection window period. In cancer early screening, it demonstrates high sensitivity in detecting early-stage thyroid cancers. Despite existing drawbacks, with technological advancements, dPCR is expected to become a core molecular diagnostic tool, contributing significantly to public health and cancer prevention and control. Although there was still scope for improvement regarding cost and automation, digital PCR would develop more efficiently and cost-effectively through technological changes. it was anticipated that digital PCR would become a central tool for molecular diagnosis and play a bigger part in public health and in the prevention and treatment of cancer.

Keywords: digital PCR, clinical application, pathogen detection, early cancer screening

1. Introduction

With the continuous rise in global cancer incidence, data from the World Health Organization indicates that millions of people lose their lives to cancer each year. The majority of patients are diagnosed at the middle or advanced stages,which means they not only have to cope with greater psychological pressure and more challenging treatment processes, but also that ordinary families will face substantial medical expenses. Therefore, early cancer screening is particularly crucial for reducing the mortality rate of patients. The emergence of infectious diseases has posed a significant threat to global public health, with frequent outbreaks of novel pathogens such as COVID-19 and SARS causing severe socioeconomic disruptions and undermining public security worldwide. Current diagnostic technologies,however,often fall short in sensitivity,precision,and rapid response capability, particularly in detecting low-abundance targets. Furthermore,the rising prevalence of drug resistance in many infectious diseases has exacerbated the urgency for developing more accurate detection methods.

dPCR, an innovative molecular diagnostic technology, offers transformative solutions for cancer early screening and viral detection. A hallmark advantage of dPCR lies in its ability to perform

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absolute quantification,enabling precise identification of low-concentration nucleic acids in complex biological samples. This is particularly critical for detecting circulating tumor DNA and pathogen-specific sequences, where traditional methods often fail due to background interference. The exceptional sensitivity and reproducibility of dPCR have established it as a powerful tool for early cancer detection and treatment monitoring, facilitating timely personalized therapeutic decisions. In this review, the author summarizes the advancements in dPCR applications for pathogen detection and cancer early screening over the past year. The author also delves into the technical advantages, current limitations and future technological prospects of dPCR.

2. The principal of dPCR

dPCR operates by partitioning a sample into numerous independent micro-reactions,each containing at least one target nucleic acid molecule. After PCR amplification, fluorescence detection is performed to count the proportion of positive partitions at the endpoint. The initial concentration of the target gene is then calculated using the Poisson distribution,enabling absolute quantification of target molecules. This approach overcomes the relative quantification limitations of conventional qPCR,offering superior sensitivity and accuracy,particularly in detecting low-abundance targets like ctDNA or rare mutations [1,2].

2.1. Advantages and limitations of dPCR

In contrast to conventional quantitative PCR (qPCR), digital PCR (dPCR)offers absolute quantification without depending on standard curves which enhances the precision and dependability of data, has more tolerance for PCR inhibitors thus increasing sensitivity and exactness in complicated biological samples like plasma or tissue homogenates, shows greater tolerance for single-point flaws in templates, primers, or probes making it more efficient for detecting uncommon mutations. Its high reproducibility and comparability between laboratories render it useful for microbial gene detection, species recognition, and analysis of antimicrobial resistance genes [3]. One major shortcoming of dPCR is that it cannot tell apart live from dead bacteria which is crucial for some microbial diagnostic methods. Also, different microbial species might have varying sensitivity making the interpretation of results more complicated and it demands specific amounts of samples as smaller quantities could reduce its sensitivity. Moreover, the number of partitions has a limited range which might affect its performance in situations where a wide range of concentrations need to be detected. When it comes to speed and cost, it is still falling behind the quickest qPCR systems. Its costs for equipment and maintenance are too high for many people in low-income and middle-income countries to afford. Finally, it isn't fully integrated into completely automatic diagnostic systems which limits its extensive use in clinical settings.

2.2. Differences between ddPCR and cdPCR

While chip-based digital PCR (cdPCR) is slightly less sensitive than droplet digital PCR (ddPCR) for ultra-low-abundance targets, it relies on pre-fabricated microchambers on solid-state chips,eliminating the need for emulsion formation. This simplifies operate and enables high-throughput parallel screening (e.g., 96 samples simultaneously), with lower long-term costs [1]. ddPCR is excellent at detecting low-abundance pathogens (for instance, HIV latent infection, hepatitis B cccDNA quantification) and analyzing rare mutations (such as influenza drug - resistant variants), while cdPCR is more appropriate for large - scale routine testing (like COVID - 19 mass

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screening) and standardized uses like gene expression quantification. These technologies complement each other instead of competing, so cdPCR could be used for initial screening first and then ddPCR was utilized for accurately quantifying positive samples, which balanced efficiency and precision.

3. Virus detection

Digital PCR technology, which has the features of great sensitivity and absolute quantification, shows remarkable benefits in the area of virus infection detection and had an indispensable part to play, especially in the early-stage diagnosis and disease surveillance of viruses like COVID and HIV.

3.1. Applications of digital PCR in COVID-19 detection

In the detection of COVID-19, digital PCR technology makes up for the drawback of traditional qPCR as in the initial phase of infection when the viral quantity is extremely minute, traditional qPCR frequently gives false-negative outcomes, but digital PCR splits the reaction system into ten thousand to a million separate micro-droplets so that each micro-droplet holds just a tiny amount or even a solitary viral nucleic acid particle [4]. After PCR amplification, positive micro -droplets are tallied via fluorescence signals and then absolute quantification is attained by integrating with the Poisson distribution principle [5]. In the early days of the COVID-19 outbreak, for certain asymptomatic infected persons or patients in the incubation period, the viral load in nasopharyngeal swab samples was extraordinarily low and qPCR often missed detection. Nevertheless, digital PCR could precisely seize these minuscule quantities of viral nucleic acids, considerably enhancing the early diagnosis rate. For instance, in the first study, clinicians in Wuhan, China noticed that a group of infected individuals who were negative for COVID-19 when tested with qPCR turned out positive when tested with ddPCR [6]. The second study found that droplet digital PCR significantly increased the diagnostic detection precision of SARS-CoV-2 from 28.2% to 87.4%, thus decreasing false negatives [7]. Additionally, when it came to disease surveillance, digital PCR was able to precisely measure the dynamic alterations of the viral burden in patients, which furnished a dependable foundation for assessing the efficacy of treatment and determining the prognosis [8]. For those patients undergoing antiviral therapy, digital PCR could still detect the minute traces of the virus even once the viral quantity had fallen beneath the detection threshold of qPCR, enabling clinicians to adjust treatment regimens promptly and diminishing the likelihood of viral relapse [9].

3.2. Applications of digital PCR in HIV detection

In the detection of HIV, digital PCR technology functions exceptionally well [10]. Since there was a rather long window period following HIV infection when traditional antibody detection found it hard to detect, nucleic acid detection became the crucial method to reduce the window period, so digital PCR, which has an ultrahigh level of sensitivity, could detect HIV nucleic acid in the peripheral blood more quickly after infection, further cutting down the window period. When it comes to disease surveillance and assessing the effectiveness of treatment, the benefits of digital PCR become even more evident. HIV patients on antiretroviral therapy (ART) had to regularly keep an eye on their viral load so as to figure out if the treatment was working [11]. When the viral load fell below the detection threshold of qPCR (generally 50 copies/mL), traditional approaches couldn't tell the difference between total virus suppression and low-level replication situations, but digital

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PCR could precisely detect a viral load as low as 1 copy/mL, effectively pinpointing the virus reservoirs in "clinically cured patients" and offering a fresh technical method for evaluating the outcome of functional cure. Meanwhile, by dynamically monitoring the minute alterations in the viral load, digital PCR could give early warning about the appearance of virus-resistant mutations, directing clinicians to adjust treatment plans promptly to prevent treatment from failing. In conclusion, when it came to the nucleic acid detection of viruses like COVID-19 and HIV, digital PCR not only enhanced the sensitivity of early-stage diagnosis considerably, which laid the groundwork for the early detection and treatment of viral infections, but also furnished precise quantitative data support during disease surveillance, the assessment of treatment effectiveness, and the early warning of drug resistance, showing that it had a wide-ranging application prospect [12].

4. Early cancer screening

Digital PCR technology has distinct technical features and holds substantial application promise in the area of early cancer screening, presenting a fresh approach to tackle the drawbacks of conventional early-screening means. Starting from the technical concept all the way to clinical application, it was gradually reforming the pattern of early cancer screening, paving a new way for the early and precise diagnosis of cancer. The early detection of thyroid cancer frequently depends on ultrasound examination and fine - needle aspiration biopsy, but for papillary micro - carcinomas (with a diameter less than 1 centimeter) [13]. The misdiagnosis rate in ultrasound examination was rather high and fine - needle aspiration biopsy was intrusive, so the use of digital PCR in the early screening of thyroid cancer offered a new solution to this problem [14]. This study chose 277 patients who had thyroid nodules and all of them received fine - needle aspiration cytology (FNA) and PCR based on the amplification - refractory mutation system (ARMS-PCR) for the detection of the BRAF V600E mutation. Digital PCR found 101 cases with the BRAF V600E mutation while ARMS-PCR identified 96 cases and among the extra 5 cases that digital PCR detected, surgical pathology confirmed that 4 cases were papillary thyroid carcinoma [14].

Using surgical pathology as a benchmark, the sensitivity of digital PCR in detecting the BRAF V600E mutation during the diagnosis of PTC reached 91.3% and the specificity was 100%, while the sensitivity of ARMS-PCR was 83.1% and its specificity was also 100%. So, the sensitivity of digital PCR was considerably higher than that of ARMS-PCR [14]. However, when both methods were utilized together with cytology for screening surgical patients, digital PCR only detected one more potential patient compared to ARMS-PCR [14]. This study emphasized that digital PCR technology outperformed ARMS-PCR when it came to detecting the BRAF V600E mutation in FNA samples of thyroid nodules, particularly regarding detection sensitivity, as it was able to spot low-abundance mutations which traditional methods often overlooked and this held great significance for the precise diagnosis of thyroid nodules. Multiple clinical studies and practical cases have shown that digital PCR has brought about a revolutionary technological advance in early-stage cancer screening, although it has certain limitations. Its role in enhancing the early-diagnosis rate and improving patients' prognosis has been fully demonstrated, as the technology matured and its application deepened. It was anticipated that digital PCR would become one of the key technology for early-stage cancer screening, making significant contributions to the fight against cancer.

5. Conclusion

In brief, digital PCR having great sensitivity and the ability for absolute quantification showed substantial benefits in detecting pathogens and in early-stage cancer screening, whether it was

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precisely monitoring COVID-19 and HIV or giving an early warning about thyroid cancer, and it offered crucial support for clinical diagnosis and treatment. Although there was still scope for improvement regarding cost and automation, digital PCR would develop more efficiently and cost-effectively through technological changes. So, in the days to come, it was anticipated that digital PCR would become a central tool for molecular diagnosis and play a bigger part in public health and in the prevention and treatment of cancer.

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