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Droplet digital PCR and assay calibration at Micropathology Ltd - Version: 1.0. Index: S - 3176. Printed: 12-Oct-2026 16:54

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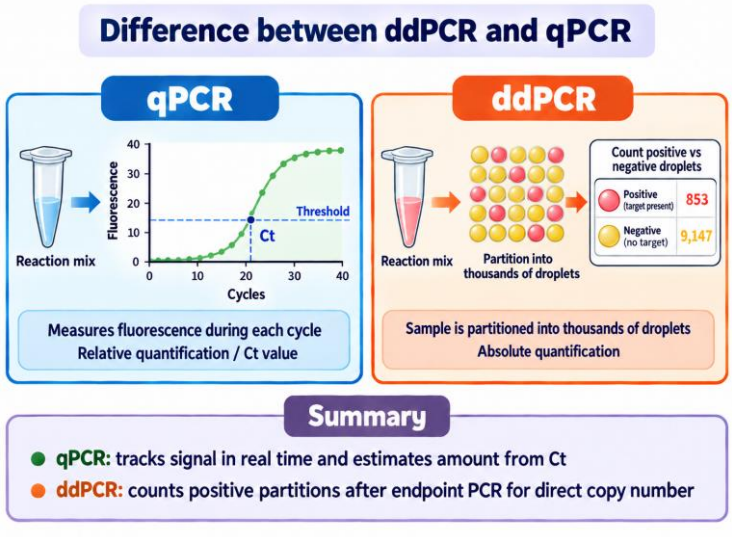
Principle of dPCR

Digital PCR (dPCR) is a PCR technique that provides absolute quantitation of a target molecule, unlike to traditional real-time quantitative PCR (qPCR), which only provides a relative quantitation of the target sequence using on a standard curve. In addition to providing higher sensitivity and greatly enhanced accuracy, dPCR also has the advantage of being less sensitive to PCR inhibitors than qPCR. The technique is relatively expensive and requires considerable scientific expertise, so it is rarely applied directly to patient samples, however it is an extremely valuable tool for assay development and calibration of standards.

The principle of dPCR relies on partitioning the PCR reaction into thousands of small individual reaction volumes (partitions), such that only some of the droplets contain the target sequence. At the end of the PCR, the fluorescence signal is measured in each partition and scored as a binary “digital” value: 1 (fluorescent signal detected, target present) or 0 (no signal, no target). The exact, absolute concentration of the target molecules is then calculated based on the total number of positive and negative partitions using Poisson distribution law, without the need for a calibration curve.

Two types of dPCR instruments are commercially available based on the partitioning technique used: partition into oil droplets (droplet digital PCR, ddPCR) and partition into physical microchambers or arrays (microchamber digital PCR, cdPCR).



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ddPCR at Micropathology

At Micropathology Ltd, we have been using the Bio-Rad droplet digital PCR technology (QX-200) since 2018 to calibrate our quantitative real-time PCR assays as well as to quantitate materials used for assay validation purposes (LLoD, precision…).

The performance of the method was initially verified using CE-IVD certified quantified molecular DNA and RNA controls[1,2] obtained from Vircell. ddPCR has since been routinely used to measure the concentration of our assay controls.

Most of the PCR controls (DNA and RNA) used at Micropathology Ltd are synthetic gene fragments[3,4], which are carefully designed in-house. These are quantitated by ddPCR and used as quantitation standards to generate standard curves in qPCR assays. We have continuous control stability monitoring (> 4years) in place to ensure the suitability of the storage conditions. In addition, assay calibration is verified periodically through EQA panels (INSTAND, QCMD, NEQAS) and daily through internal quality control assessment (Trend controls).

Most EQA providers are now using dPCR as a reference measurement procedure to establish the target values for External Quality Assessment (EQA) [5,6].

Conversion factors

WHO International Standards (IS) are universal reference materials which can be used to calibrate secondary reference materials and harmonise nucleic acid amplification techniques (NAAT). These are calibrated in IU and can be used to ensure accuracy and comparability of viral and bacterial load quantification across different laboratories and diagnostic platforms.

At Micropathology Ltd, since we use ddPCR to determine the absolute concentration of our controls, we preferentially report our quantitative results in copies/mL. However, for every quantitative assay in use at Micropathology Ltd, the latest WHO IS (if commercially available) has been run through our analytical process and its content measured by ddPCR in order to determine the conversion factor[4,5]. Conversion factors are either displayed on the test reports or available upon request. The current list of IS assessed for our quantitative assays include BKV, JCV, Parvovirus B19, Adenovirus, CMV, EBV, HHV-6B, HAV, HBV, HDV and HEV. We feel that with the advent of dPCR it makes more sense to report quantitative results in copies/mL, since this represents an absolute value, rather than a consensus value, which may vary from preparation to preparation. Nonetheless, we continue to report certain assays such as HBV in IU/mL, where this is standard practice.

Summary

In our experience, dPCR is a highly valuable tool for accurately quantifying controls and assay standards. We were early adopters of this technology and we now have many years of experience of using it for assay calibration. We take assay calibration extremely seriously and use multiple strategies to check the performance of our assays. Please contact us if you would like to discuss our calibration strategies or ddPCR generally, which we are sometimes able to offer as a service and we are often able to offer help or guidance.

References

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