INNOVAPATH™
The Premier Journal Bridging Discovery & Innovation

Review Article
https://doi.org/10.63501/hrm56e02

Digital PCR versus Quantitative PCR in Diagnostic Pathology: Principles, Clinical Applications, Validation, and Practical Workflow Recommendations - A Comparative Review

Daman Tariq[1,3], Khadija Mubashir[1], Fariha Noor[1], Rahimeen Rashid[1], Sana Tariq[1], Amna Khalid[2]

[1]Quaid-e-Azam Medical College, Bahawalpur, Pakistan

[2]Federal Medical College, Islamabad, Pakistan

[3]Corresponding Author Email: Damanshahzadi101@gmail.com

ABSTRACT:

Polymerase chain reaction (PCR)-based technologies are central to molecular diagnostic pathology. Quantitative PCR (qPCR) has historically served as the standard method for nucleic acid quantification, offering robust performance, scalability, and established clinical workflows. However, digital PCR (dPCR) has emerged as a powerful alternative that enables absolute quantification through sample partitioning and Poisson statistical modeling, improving analytical precision, sensitivity, and reproducibility, particularly in low-abundance targets.

This comparative review critically evaluates qPCR and dPCR across analytical performance, clinical applications, and validation frameworks. Unlike traditional narrative descriptions, a structured comparative synthesis demonstrates that qPCR remains dominant in routine high-throughput diagnostics, whereas dPCR provides superior performance in applications requiring rare variant detection, minimal residual disease monitoring, copy number variation analysis, and inhibitor-resistant quantification.

The review further discusses validation requirements and workflow integration strategies, emphasizing that both technologies should be viewed as complementary rather than competing platforms within modern diagnostic pathology.

KEYWORDS: *digital PCR; quantitative PCR; diagnostic pathology; liquid biopsy; minimal residual disease; copy number variation; assay validation; molecular diagnostics*

INTRODUCTION:

Quantitative PCR (qPCR), introduced in the 1990s, revolutionized molecular diagnostics by enabling real-time detection of nucleic acid amplification through fluorescence-based measurement during exponential amplification cycles. Its reliance on standard curves allows relative or semi-quantitative analysis, making it highly adaptable for clinical diagnostics, including infectious disease monitoring, gene expression analysis, and mutation screening. Despite its widespread adoption, qPCR performance

Review Article
https://doi.org/10.63501/hrm56e02

is inherently influenced by amplification efficiency, reference standard quality, and assay variability, which can limit accuracy in low-copy or borderline detection scenarios.

Digital PCR (dPCR), developed as an evolution of endpoint PCR quantification, overcomes these limitations by partitioning samples into thousands of micro-reactions, enabling absolute quantification without reliance on standard curves. Using Poisson-distribution-based statistical modeling, dPCR minimizes dependence on amplification efficiency and significantly improves precision in detecting low-abundance targets.

Although dPCR offers clear analytical advantages, its clinical adoption remains limited by cost, lower throughput, and reduced multiplexing capacity. Therefore, its role in diagnostic pathology is best understood as complementary to qPCR rather than as a substitute.

This review aims to provide a structured comparative analysis of both technologies, focusing on their analytical characteristics, clinical utility, validation requirements, and workflow integration strategies.

2. METHODS

2.1 Literature Search

A structured literature search was performed using PubMed, Scopus, and NCBI databases covering publications from 2000 to 2025. Search terms included “digital PCR,” “quantitative PCR,” “liquid biopsy,” “minimal residual disease,” and “copy number variation.”

2.2 Inclusion Criteria

Studies comparing qPCR and dPCR in terms of analytical performance, clinical applications, or diagnostic validation were included. Studies lacking direct comparative or quantitative data were excluded.

2.3 Data Synthesis

Data were synthesized qualitatively, with an emphasis on direct comparisons between qPCR and dPCR across key performance metrics, including sensitivity, precision, reproducibility, inhibitor tolerance, and clinical applicability.

2.4 Quality Assessment

Validation parameters such as limit of detection (LOD), limit of quantification (LOQ), amplification efficiency, and partition accuracy were extracted and evaluated where available.

3. RESULTS

3.1 Analytical Performance Comparison

Both qPCR and dPCR demonstrate strong analytical utility; however, their performance differs fundamentally due to methodological principles.

qPCR provides reliable relative quantification based on fluorescence thresholds and standard curves, but its accuracy is dependent on amplification efficiency and calibration quality. In contrast, dPCR provides absolute quantification by partitioning samples into thousands of independent reactions and applying Poisson statistics, eliminating the need for external standards.

In terms of sensitivity, dPCR consistently outperforms other methods for detecting low-frequency alleles, particularly in liquid biopsy applications where mutant allele fractions may fall below 0.1%. qPCR typically performs well above 1% allele frequency thresholds but loses accuracy near detection limits.

Precision is also improved in dPCR, with significantly lower coefficient of variation at low template concentrations, whereas qPCR variability increases due to exponential amplification dependence.

Review Article
https://doi.org/10.63501/hrm56e02

For copy number variation analysis, qPCR requires normalization against reference genes, introducing potential bias, while dPCR provides absolute quantification and improved discrimination of subtle genomic gains or losses.

Regarding inhibitor resistance, dPCR is more robust in the presence of PCR inhibitors due to sample partitioning, which reduces competition effects within individual reactions. qPCR, in contrast, is more susceptible to inhibition, particularly in complex clinical matrices such as FFPE tissues and plasma.

3.2 Clinical Applications

In infectious disease diagnostics, qPCR remains the established standard due to its high throughput, compatibility with automation, and validated clinical thresholds for viral load monitoring in infections such as HIV, HBV, and HCV. However, dPCR has demonstrated superior sensitivity in detecting low-level viremia and residual viral reservoirs, making it particularly valuable in treatment monitoring and cure research.

In oncology, particularly liquid biopsy, qPCR is widely used for mutation detection but is limited in detecting low-frequency circulating tumor DNA variants. dPCR significantly improves detection sensitivity for clinically relevant mutations such as EGFR, KRAS, and BRAF, especially in early-stage disease or post-treatment monitoring.

For minimal residual disease, dPCR provides an enhanced ability to detect rare leukemic clones and fusion transcripts at extremely low abundance, enabling earlier relapse detection compared to qPCR.

In gene expression studies, qPCR remains widely used due to established normalization strategies; however, dPCR allows absolute transcript quantification without reliance on housekeeping genes, reducing normalization bias and improving reproducibility in translational research settings.

3.3 Workflow and Validation Considerations

qPCR validation focuses on amplification efficiency, linear dynamic range, standard curve reliability, and assay reproducibility. It benefits from well-established regulatory frameworks and standardized protocols.

In contrast, dPCR validation emphasizes consistency in partition volume, accuracy in Poisson distribution, determination of the limit of blank, and droplet stability. Proper control of partition occupancy is essential to avoid saturation effects that can compromise the accuracy of quantification.

Both platforms require strict quality control measures, including positive and negative controls, strategies to prevent contamination, and internal amplification controls. However, dPCR additionally requires optimization of partition uniformity and droplet generation consistency.

From a workflow perspective, qPCR is preferred for high-throughput screening and routine diagnostics, while dPCR is better suited for confirmatory testing, low-abundance detection, and precision molecular applications.

4. DISCUSSION

This comparative review demonstrates that qPCR and dPCR represent complementary rather than competing molecular diagnostic technologies. qPCR continues to dominate routine clinical workflows due to its cost-effectiveness, scalability, and established diagnostic thresholds. Its utility remains particularly strong in infectious disease monitoring and standardized gene expression analysis.

In contrast, dPCR offers distinct advantages in analytical precision, sensitivity, and resistance to technical variability. These strengths make it especially valuable in applications requiring the detection of rare variants, including liquid biopsy, minimal residual disease monitoring, and copy number variation assessment.

Despite these advantages, dPCR is limited by higher operational costs, reduced multiplexing capacity, and evolving regulatory standardization. Therefore, its integration into clinical laboratories should be guided by specific diagnostic needs rather than by replacing qPCR platforms.

A key implication of this comparison is the need for standardized reporting frameworks. While qPCR relies on cycle threshold (Ct) values, dPCR requires reporting of absolute copy numbers with associated confidence intervals. Harmonization of these reporting standards is essential for inter-laboratory comparability and clinical translation.

Future developments in microfluidics, automation, and multiplexing capacity are expected to further enhance the clinical utility of dPCR and may expand its role in routine diagnostics.

5. CONCLUSION

qPCR and dPCR serve distinct but complementary roles in diagnostic pathology. While qPCR remains the backbone of routine molecular diagnostics, dPCR provides superior analytical precision for low-abundance and high-precision applications. Optimal diagnostic strategies should leverage both technologies based on clinical context, analytical requirements, and resource availability.

Ethical Approval

Not applicable (no new human or animal subjects were involved; this narrative is based on published data).

Conflict of Interest:

None

Declaration of AI Use:

This letter was drafted and revised with the assistance of an AI language model (ChatGPT, GPT-5, OpenAI) for grammar refinement, structural reorganization, and clarity enhancement. All intellectual content, interpretation, and final approval of the text are solely the responsibility of the authors.

Funding: None

REFERENCES

1. Mackay IM, Arden KE, Nitsche A. Real-time PCR in virology. *Nucleic Acids Res.* 2002;30:1292–1305.
2. Hindson BJ, Ness KD, Masquelier DA, Belgrader P, Heredia NJ, Makarewicz AJ, Bright IJ, Lucero MY, Hiddessen AL, Legler TC, Kitano TK. High-throughput droplet digital PCR system for absolute quantitation of DNA copy number. Analytical chemistry. 2011 Nov 15;83(22):8604-10.
3. Whale, A. S., Huggett, J. F., Cowen, S., Speirs, V., Shaw, J., Ellison, S., Foy, C. A., & Scott, D. J. (2012). Comparison of microfluidic digital PCR and conventional quantitative PCR for measuring copy number variation. *Nucleic acids research*, *40*(11), e82. https://doi.org/10.1093/nar/gks203.
4. Huggett JF, Foy CA, Benes V, Emslie K, Garson JA, Haynes R, Hellemans J, Kubista M, Mueller RD, Nolan T, Pfaffl MW. The Digital MIQE Guidelines: M inimum I nformation for Publication of Q uantitative Digital PCR E xperiments. Clinical chemistry. 2013 Jun 1;59(6):892-902.
5. Beaver JA, Jelovac D, Balukrishna S, Cochran RL, Croessmann S, Zabransky DJ, Wong HY, Valda Toro P, Cidado J, Blair BG, Chu D. Detection of cancer DNA in plasma of patients with early-stage breast cancer. Clinical cancer research. 2014 May 15;20(10):2643-50.
6. Heredia, N. J., Belgrader, P., Wang, S., Koehler, R., Regan, J., Cosman, A. M., Saxonov, S., Hindson, B., Tanner, S. C., Brown, A. S., & Karlin-Neumann, G. (2013). Droplet Digital™ PCR quantitation of HER2 expression in FFPE

breast cancer samples. *Methods (San Diego, Calif.)*, *59*(1), S20–S23. https://doi.org/10.1016/j.ymeth.2012.09.012.

7. Strain MC, Lada SM, Luong T, Rought SE, Gianella S, Terry VH, Spina CA, Woelk CH, Richman DD. Highly precise measurement of HIV DNA by droplet digital PCR. PloS one. 2013 Apr 3;8(4):e55943.
8. Oxnard GR, Paweletz CP, Kuang Y, Mach SL, O'Connell A, Messineo MM, Luke JJ, Butaney M, Kirschmeier P, Jackman DM, Jänne PA. Noninvasive detection of response and resistance in EGFR-mutant lung cancer using quantitative next-generation genotyping of cell-free plasma DNA. Clinical cancer research. 2014 Mar 15;20(6):1698-705.
9. Drandi, D., Kubiczkova-Besse, L., Ferrero, S., Dani, N., Passera, R., Mantoan, B., Gambella, M., Monitillo, L., Saraci, E., Ghione, P., Genuardi, E., Barbero, D., Omedè, P., Barberio, D., Hajek, R., Vitolo, U., Palumbo, A., Cortelazzo, S., Boccadoro, M., Inghirami, G., … Ladetto, M. (2015). Minimal Residual Disease Detection by Droplet Digital PCR in Multiple Myeloma, Mantle Cell Lymphoma, and Follicular Lymphoma: A Comparison with Real-Time PCR. *The Journal of Molecular Diagnostics*, *17*(6), 652–660. https://doi.org/10.1016/j.jmoldx.2015.05.007.
10. Dong L, Meng Y, Sui Z, Wang J, Wu L, Fu B. Comparison of four digital PCR platforms for accurate quantification of DNA copy number of a certified plasmid DNA reference material. Scientific reports. 2015 Aug 25;5(1):13174.