Droplet Digital PCR – A Superior Complementary Technique for SARS-CoV-2 Detection

I. M. Hussaini1*, S. Gide2, B. Musa1, M. A. Sulaiman1 and A. Usman3

1Department of Microbiology, Ahmadu Bello University, Zaria, Nigeria.
2Desert Research Centre, Yobe State University, Damaturu, Nigeria.
3Department of Microbiology, Kaduna State University, Nigeria.

Authors’ contributions
This work was carried out in collaboration among all authors. Authors IMH and SG designed the study and wrote the first draft of the manuscript. Authors IMH and SG managed the literature searches. Authors BM, MAS and AU revised the first draft. All authors read and approved the final manuscript.

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ABSTRACT
Accurate and timely SARS-CoV-2 detection in suspected persons is crucial in the fight against its spread. Many techniques have been developed to meet up with the continuously growing demand, however some of these techniques lack the required accuracy, sensitivity and specificity. The current reference standard technique for SARS-CoV-2 detection is RT-PCR, but studies have shown that false-negative results are inevitable and data can be non-reproducible when samples and primers are not appropriately verified and validated. Droplet digital PCR (ddPCR) is a newly introduced technique that performs precise nucleic acid quantification. Researchers have evaluated the efficacy of ddPCR and the technique has shown promising results even in specimens with low viral load. ddPCR has shown increased accuracy, precision, sensitivity and specificity. Furthermore, it is less affected by annealing and amplification inhibitors. This suggests that ddPCR can be used as a complementary detection technique especially in convalescent cases.

Keywords: SARS-CoV-2; ddPCR; RT-PCR; nucleic acids; viral load.

*Corresponding author: E-mail: hussainibrahim269@gmail.com;
1. INTRODUCTION

Measures have been taken to control the transmission of the current SARS-CoV-2 pandemic. Such measures depend primarily on the early and precise SARS-CoV-2 detection in infected persons [1].

Recently, numerous techniques were developed for SARS-CoV-2 detection so as to meet up with the increasing demand for COVID-19 diagnosis. These techniques include computed tomographic chest examination, immunoglobulin detection [2] and nucleic acid detection techniques. RT-PCR, isothermal nucleic acid amplification assay and Microarray assay are the commonly used nucleic acid detection techniques for COVID-19 diagnosis [2,3]. Presently, real-time PCR (RT-PCR) serves as the current gold standard for diagnosis [2,3]. Presently, real-time PCR (RT-PCR) serves as the current gold standard for COVID-19 diagnosis [2,3].

The false-negative results associated with RT-PCR in clinical diagnosis are inevitable due to low viral load in some patient’s specimen, less precision in low-concentration samples, presence of sample inhibitors, poor amplification efficiency, subjective cut-off, calibration curve-dependent quantification [5,9,10], inadequate residual protein dilution and presence of inhibitors of annealing especially chemical contaminants [11].

In a study carried out recently by Suo et al. [2], 26 RT-PCR negative samples from COVID-19 patients were tested positive by ddPCR. Also six out of the fourteen discharged convalescents still had detectable SARS-CoV-2 nucleic acid 5-12 days post-discharge. Summary of the efficacy of RT-PCR and ddPCR as reported by Suo et al. [2] is presented in Table 1.

2. PRINCIPLE OF DDPCR

This PCR technique is based on water-in-oil partitioning and absolute quantification at PCR end-point [2]. In this technique, the specimen to be analyzed is partitioned into uniform droplets

Table 1. Comparative performance of ddPCR and RT-PCR in the detection of SARS-CoV-2

| Statistic                                | ddPCR performance | RT-PCR performance |
|------------------------------------------|-------------------|---------------------|
| Sensitivity (%)                          | 94 (95% CI: 83-99) | 40 (95% CI: 27-55)  |
| Specificity (%)                          | 100 (95% CI: 48-100) | 100 (95% CI: 54-100) |
| PPV (%)                                  | 100               | 100                 |
| NPV (%)                                  | 63 (95% CI: 36-83) | 16 (95% CI: 13-19)  |
| Negative likelihood ratio                | 0.06 (95% CI: 0.02-0.18) | 0.6 (95% CI: 0.48-0.75) |
| Accuracy (%)                             | 95 (95% CI: 84-99) | 47 (95% CI: 33-60)  |
| LoD (copies/reaction):                   |                   |                     |
| ORF1ab primes/probe sets                 | 2.1 (95% CI: 1.5-4.2) | 1039 (95% CI: 763.2-1862) |
| N primes/probe sets                      | 1.8 (95% CI: 1.4-3.3) | 873.2 (95% CI: 639.8-1633.2) |
| Reportable range (copies/reaction):      |                   |                     |
| ORF1ab primes/probe sets                 | 10 to 5×10⁴ (R² = 0.9935) | 1000 to 10⁷ (R² = 0.9921) |
| N primes/probe sets                      | 10 to 5×10⁴ (R² = 0.9908) | 1000 to 10⁷ (R² = 0.9898) |

Key: LoD = limit of detection, PPV= positive predictive value, NPV= negative predictive value
randomly with target templates contained in only a number of the droplet partitions. However, all the droplet partitions contain the reagents for template amplification. These partitions are then amplified to the end-point and then droplet reader is used to determine the amount of positive partitions [2].

After the amplification process, the amount of positive droplets (droplets with detectable amplified sequence) and the amount of negative Droplets (droplets with no detectable amplification) are determined by detecting the emitted fluorescence by individual droplet. The absolute quantification is estimated using Poisson statistics considering the positive droplets proportion in relation to the droplets total number and volume [16].

3. THE SUPERIORITY OF DDPCR AS A DIAGNOSTIC TOOL

DDPCR has proven to be a technique with high sensitivity and the analytical sensitivity of this technique is improved by the effective concentration of template molecules within the micro reaction. This technique has also shown increased precision [17] and sensitivity in the detection of low copies of target nucleic acid [18] compared to other types of PCR. This is a result of reduced competition between the different amplification targets within the reaction mixture [2,19].

The principle of ddPCR negates the challenges of calibrators and normalization hence it gives absolute quantification of target molecules [20]. Quantification by RT-PCR is dependent on the purity of the sample, initial copies of the target, primer dimers and efficiency of the reaction since the quantification is by amplification curve and a quantification cycle (Cq) value. However, PCR inhibitors have relatively little or no effect on ddPCR [20,21] and directly provide the result of detection in number of copies/µL of reaction with 95% CI [12].

End-point quantitation of nucleic acid by ddPCR allows direct nucleic acid quantification with no need for standard curves hence the data are more precise and reproducible [11]. The data from ddPCR analysis are highly reproducible [20] and negate the need for replication [12], as a result of specimen partitioning into tens of thousands micro-reactions [20].

DDPCR has a lower Limit of Detection (LoD) compared to RT-PCR using probit regression analysis [2]. This low LoD suggests that ddPCR can be used for quantification of the low copies of nucleic acid target.

All these superiorities of ddPCR over RT-PCR (and other PCR variants) has led to the proposed use of ddPCR in quantifying reference materials for calibration curves construction [22].

4. CONCLUSION

DDPCR has shown lower limit of detection improved precision, accuracy, sensitivity and specificity in SARS-CoV-2 detection. ddPCR should therefore be used as a complementary technique in SARS-CoV-2 detection and ddPCR negative results should be part of the criteria for discharge.

COMPETING INTERESTS

Authors have declared that no competing interest exits.

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