Comparison of Droplet Digital PCR and qPCR for the Quantification of the Autolysin Gene (*lytA*) of *Streptococcus Pneumoniae*

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Introduction

- Quantitative droplet digital polymerase chain reaction (ddPCR) is a novel technique providing absolute quantification of target nucleic acids without the need for reference material.
- *Streptococcus pneumoniae (S. pneumoniae)* is both an aggressive pathogen and a normal part of the human respiratory microbiome.
- There are few reports of the use of ddPCR for detection of clinical pathogens such as *S. pneumoniae* for which precise measurement is important.
- Quantification of target pathogens using ddPCR may be more precise than with quantitative real time polymerase chain reaction (qPCR) .
- This study compared the diagnostic potential of ddPCR and qPCR for precision and absolute quantitation of the autolysin gene *(lytA*).

Materials and Methods

- Replicates of ten-fold serial dilution of ATCC 496196 *S. pneumoniae* strain were extracted and amplified to establish a qPCR assay for *lytA* diagnosis on a Bio-Rad CFX96 platform (Fig 1 and Fig 2).
- The assay was transferred and optimized on ddPCR and performance compared (Fig 4A).
- Three replicate runs of samples with pneumococcal genomes were analyzed on both platforms. Controls were included in all runs (Fig 3 and Fig 4B).
- Measurements were compared (Fig 5).

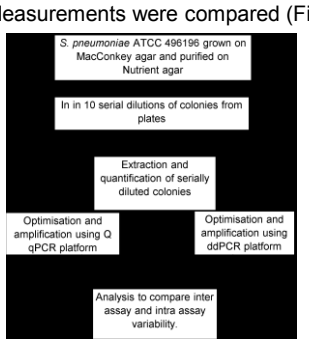


Fig 1: Sample processing workflow for comparison of droplet digital PCR and qPCR

Results

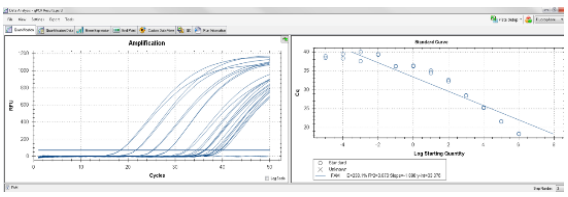


Fig 2: Amplification curves derived towards determination of limit of detection of *lytA* gene target on Bio-Rad CFX 96 platform 20

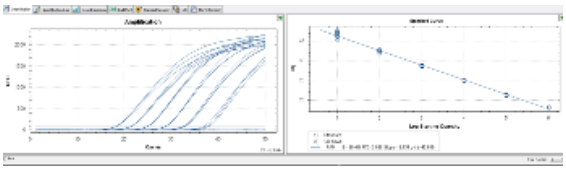
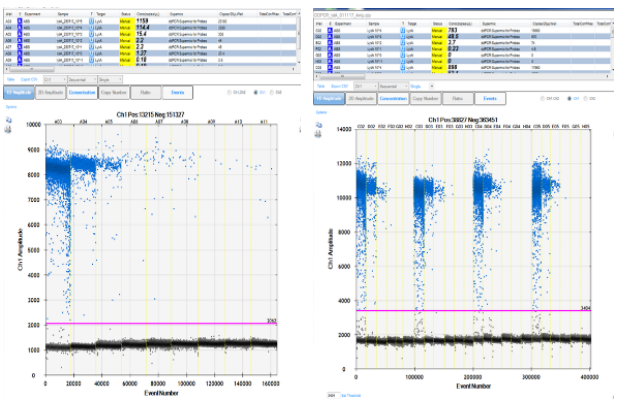


Fig 3: Amplification curves derived towards determination of coefficient of variation of *lytA* gene target on Bio-Rad CFX96 platform



A B

Fig 4: Determination of limit of detection of *lytA* gene target on ddPCR platform (A), and (B) Determination of coefficient of variation of *lytA* gene target on ddPCR platform.

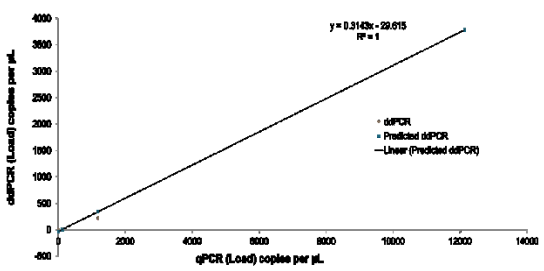


Fig 5: Correlation of quantitative real-time PCR and ddPCR methods following comparison of the measurements

Summary

•qPCR had lower sensitivity compared to ddPCR with a limit of detection at a Ct value of 36 while ddPCR had no limit of detection.

•The qPCR assay had a broader dynamic range compared to the ddPCR assay with saturation experienced for ddPCR at concentrations greater than 10^4 copies/µL.

•The coefficient of variations were lower for qPCR compared to ddPCR. However, there was no statistical difference of the CV between the methods (R^2 = 0.9999; P = 0.77396).

Conclusions

Accurate absolute quantification of the *lytA* target gene is possible with ddPCR

ddPCR shows promise to be in agreement with qPCR in *lytA* target quantification and is more sensitive compared to qPCR

Acknowledgements

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References

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