Comparison of dPCR and qPCR for detection of Epstein-Barr Virus (EBV) in blood plasma using Clarity™ Digital PCR System

I. Overview

Untreated EBV infections can cause post-transplantation lymphoproliferative disease (PTLD) and Nasopharyngeal Carcinoma[1]. This is notable as PTLD has mortality rates ranging from 56-73%[2]. Complications arising from EBV can be treated if detected early and prior to the development of symptoms[3].

Assays have been developed to monitor the viral population by measuring their nucleic acids (DNA/RNA). The detection of viruses allows physicians to target the opportunistic pathogen endangering the patient in turn increase the success of organ transplant[4]. This study seeks to validate the Clarity™ EBV quantification kit using the Clarity™ Digital PCR (dPCR) system.

II. Clarity™ digital PCR system detected EBV virus in blood plasma to a level of 0.003%

EBV DNA from 32 EBV-positive and 17 EBV-negative achieved samples were analyzed using the Clarity™ EBV quantification kit. These samples were previously evaluated using the ELITe MGB® EBV Kit. Each reaction mixture was sub-divided into 10,000 partitions within Clarity™ tube-strip. This was followed by PCR and data was analyzed using the Clarity™ reader. As shown in Table 1, the Clarity™ EBV quantification kit has a 96.88% positive percentage agreement (PPA) and 52.94% negative percentage agreement (NPA) when compared to the ELITe qPCR assay. Intraclass correlation coefficient (ICC) analysis also showed that both assays exhibited excellent reliability in absolute agreement (ICC=0.936).

Table 1. Qualitative performance of EBV dPCR test compared to the EBV ELITe qPCR assay. 32 EBV-positive and 17 EBV-negative samples tested using CE-marked quantitative polymerase chain reaction (qPCR) ELITE®EBV Test.

		ELITe assay		
		Pos	Neg	Total
Clarity EBV Kit	Pos	31	8	39
	Neg	1	9	10
	Total	32	17	49
		PPA = 96.88% (95% CI 82.89-100%)	NPA = 52.94% (95% CI 30.96-73.84%)	

PPA, positive percentage agreement;
NPA, negative percentage agreement;

III. Conclusion

Our results show Clarity™ EBV dPCR and ELITe qPCR assays having good quantitative agreement, with ICC>0.9. High PPA (96.88%) also indicates good sensitivity between the 2 assays. The lower NPA could be attributed to the differences in LoD between the assays, as Clarity dPCR could detect positive samples among those detected as negative by the ELITe assay. This study showed that Clarity EBV dPCR assay could potentially be employed in clinical setting for viral load monitoring. Beyond this, digital PCR has many advantages over qPCR, and shows promise for various diagnostic uses in the clinical settings.

References

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