IVD_12 - Detection of HIV-1 dilution panel by a new platform of Real Time digital PCR

Pedro Henrique Cardoso[1]; Daniele Ramos Rocha[1]; Monica Barcellos Arruda[1]; Elisabete Ferreira de Andrade[1]; Elaine Motta Costa[1]; Marcela Fontana do Carmo Machado Maurell[1]; Alexandre Calazans[2]; Patrícia Alvarez Baptista[1].
[1]Fiocruz/Bio-Manguinhos
[2]Fiocruz/CDTS

Introduction: It is known that digital PCR (dPCR) can increase the sensitivity and specificity of PCR assay. By partitioning the PCR mixture in thousands of nanoscales chambers, it is possible to count, in copies per chamber, the concentration of the sample tested. Using the Poisson distribution, the probability of 1 or more copies can be figured out. Furthermore, all the PCR inhibitors at the reaction will be diluted when partitioned increasing the efficiency of the enzymes. For detection of HIV-1, the gold standard method is the RT-PCR. However, to get the concentration of target per microliter and avoid PCR inhibitor, the dPCR can be easily performed.

Objectives: The main objective of this study was to evaluate and compare both RT-PCR and digital RT-PCR (RT- dPCR) for HIV-1 targets.

Methodology: It was performed a HIV-1 dilution panel with a well-known HIV-1 sample concentration of $5{,}41x10^5$ copies/mL quantified using COBAS® TaqMan® HIV-1 Kit, v2.0. (Roche® Diagnostics). A 2-fold serial dilution panel was done for limit of detection (LoD) analysis. For RT-PCR, the gold standard molecular detection was performed using the NATPLUS HIV/HCV/HBV/MAL Bio-Manguinhos kit, according to the instructions of use. For dRT-PCR, it was optimized an assay based on the NATPLUS assay target sequences, but in a singleplex format. The RT-dPCR reactions were performed using LOAA platform and 20K cartridges (OPTOLANE technologies) with 2X dRT-PCR master mix, according to manufactures instructions of use. To get the RT-dPCR optimized, a range of 200nM to 800nM was evaluated for primers and 200nM to 400nM for HIV-1 FAM probes.

Results: The LoD resulted was about 8,45 copies/mL for both methodologies analyzed. For RT-PCR, it was observed 38,6 of Ct value and it was detected 3 of 5 replicates tested. For RT-dPCR, only cartridges resulted in more than 18.000 valid partitions were considered. Thus, only 1 positive partition in a duplicate cartridge was detected. Furthermore, both methods were evaluated using a HIV-1 NIBSC panel diluted and the LoD resulted was at 30 copies/mL. For RT-PCR, 7 to 8 replicates were detected with 38,27 of Ct value. For RT-dPCR, 6 positive partitions were detected and the quantification was resulted in 72 copies/mL in a mean of the two cartridges tested.

Conclusion: These tests suggested that both methodologies showed to have the same LoD, since both could detect efficiently the target at the same dilution point. Accuracy tests must be carried out to determine what methodology could detect HIV-1 targets more efficiently.

Keywords: Digital PCR, HIV-1 detection, Molecular biology