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VIROLOGIA

HIV-1 DNA QUANTIFICATION: A COMPARISON STUDY BETWEEN "HIV-1 DNA TEST" AND A PROTOTYPE DEVELOPED FOR AN ACCURATE NORMALIZATION BY A SELECTED HOUSEKEEPING GENE

E. Carloni [1], E. Omiccioli [1], C. Orlandi [2], B. Canovari [3], Z. Pasquini [3], F. Barchiesi [3], A. Casabianca [2], M. Magnani [2]

[1]*Diatheva srl, Cartoceto, Fano (PU)*

[2]*Dipartimento di Scienze Biomolecolari, Università di Urbino, Urbino (PU)*

[3]*Malattie Infettive, Azienda Ospedaliera Ospedali Riuniti Marche Nord, Pesaro (PU)*

INTRODUZIONE

The most widely used marker of HIV persistence in infected cells is total HIV-DNA that varies among patients, according to the stages of HIV disease and the effectiveness of anti-HIV therapy. The "HIV-1 DNA Test" (Diatheva srl) is a real-time PCR (qPCR) that showed good amplification performances ensuring reliable quantification results of HIV-1 DNA confirmed also by comparative studies by digital-PCR. The kit contains an exogenous Internal Control to detect inhibitors in sample and require the measurement of DNA concentration for the correct HIV DNA copies quantification. The "HIV-1 DNA Test PRO" is a new prototype that allows to simultaneously amplify HIV DNA and the endogenous human Telomerase Reverse Transcriptase gene (hTERT). The endogenous gene allows to determine if there are inhibitors in the PCR assay, to verify the success of the extraction step and to obtain HIV copies number quantification without quantify the extract DNA.
The aims of this work are: a) validation of the amplification performances of the prototype; b) comparison between the prototype and "HIV-1 DNA Test"; c) determination of the prototype reproducibility in HIV DNA quantification, analysing different DNA quantities without previous quantification.

METODI

"HIV-1 DNA Test PRO" standard curves were amplified to study its calibration functions. DNA from 16 blood samples collected from HIV+ patients were analysed using the "HIV-1 DNA Test" and the prototype to check their correlation using the Pearson Coefficient with a 95% CI (GraphPad Prism 5).
Three, 5, 10 and 20 µl of 6 HIV+ samples were amplified using the prototype in 7500 and Rotor-Gene Q instruments.

RISULTATI

Testing the "HIV-1 DNA Test PRO", the efficiency and R^2 of the HIV standard curve were 99.1%±0.04 and 0.997±0.001 respectively, and for the hTERT were 108.5%±0.03 and 0.997±0.002, allowing to obtain accurate quantification. Positive samples ranged from 24.6 to 555.0 HIV cps/10^6 cells and the median [IQR] value was 121.2 [78.7-229.4] cps/10^6 cells. The analyses of same samples by "HIV-1 DNA Test" showed a range from 16.7 to 982.0 HIV cps/10^6 cells and a median [IQR] value of 169.7 [102.2-271.7] cps/10^6 cells. Pearson Coefficient value was 0.863 ($P<0.0001$). Quantification results obtained analysing different DNA quantities by prototype, ranging from 0.1 to 3.2 µg DNA/reaction, were similar to each other in terms of HIV cps/10^6 cells.

CONCLUSIONI

The "HIV-1 DNA Test PRO" showed good amplification performances ensuring reliable quantification in comparison with the previously validated "HIV-1 DNA Test". The HIV DNA and hTERT co-amplification, testing different DNA quantities, has no side effect in HIV DNA quantification.