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MEETING ABSTRACT



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Development of a molecular platform for HTLV confirmatory diagnosis: importance of the internal amplification control

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Background

Brazil may harbor the largest absolute number of HTLV-1-infected individuals worldwide. The current HTLV diagnosis is based mainly on antibodies detection. However, these tests exhibit high proportion of indeterminate results. Several real-time PCR techniques have been developed for the detection of HTLV-1/2. However, up to day the major drawbacks of HTLV-1/2 molecular diagnosis are the lack of standard molecular tests and the absence of suitable internal amplification control (IAC). The aim of this study was to develop a multiplex qualitative real-time PCR for the simultaneous detection and discrimination of HTLV-1/2 and to design an IAC for reaction monitoring.

Methods

After multiple sequence alignments of the full genomes of HTLV-1/2 subtypes, a conserved tax region was chosen for the design of specific primers and probes. The IAC was generated after the annealing of synthetic nucleotide sequences and cloned into TOPO TA[®] vector. MT-2 and Gu cell lines were used as positive controls for HTLV-1 and HTLV-2, respectively.

Results

The developed multiplex real-time reaction detected both HTLV-1 and HTLV-2 (105 to 101 copies/reaction) at the presence of IAC in the same reaction. Analytical sensitivity was 1.2 copies/reaction for HTLV-1 and 19.1 copies/reaction for HTLV-2. The analytical sensitivity analysis was performed in singleplex format.

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Conclusion

The detection of HTLV-1/2 and IAC by multiplex realtime PCR was efficient. The developed IAC is suitable for the molecular diagnosis and its presence ensures that the negative results are not due to failure in pre-PCR procedures.

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