IVD_09 - Evaluation of loop-mediated isothermal amplification (lamp) for htlv-1a detection in whole blood and dried blood spot samples

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Introduction: HTLV-1 is a virus of growing concern given its difficult diagnosis. The cosmopolitan subtype strain (also known as 1a) is a great concern in non-endemic countries, such as Brazil, with two main subgroups: transcontinental (HTLV-1aA) and Japanese subgroup (HTLV-1aB). HTLV-1 infection screening is done mostly by serological methods, with molecular techniques (PCR) being done as confirmatory diagnosis given its high complexity and cost. This system, however, yields a high rate of false negative and unspecific results. Loop- mediated Isothermal Amplification (LAMP) is a molecular methodology considered faster, simple and easy to perform. In addition, alternative specimens, such as, dried blood spot (DBS) could be used to enable HTLV-1 screening in low resource areas as it facilitates acid nucleic storage.

Objectives: To evaluate the effectiveness of LAMP to detect HTLV-1a RNA/proviral DNA in whole blood and DBS samples.

Methodology: Ninety-seven patients infected with HTLV-1a and 50 healthy individuals donated blood samples. Out of the HTLV patients, 37 were HTLV-1aA, 47 HTLV-1aB and 13 did not have genotype determined in a subgroup level, being characterized only as HTLV-1a. A subgroup of 60 individuals had DBS evaluated [40 patients with HTLV infection (HTLV-1aA: 14; HTLV-1aB: 19; HTLV-1a: 7) and 20 healthy]. RNA/proviral DNA was extracted using a commercial kit. Besides the extracted samples, *in natura* and inactive forms of samples were also evaluated in a smaller group (n=12). Posteriorly the sample preparation, a preheating stage was done followed by LAMP reaction at 63ºC for 60 minutes and enzyme inactivation at 80ºC for 10 minutes. Gel electrophoresis, fluorescence and colorimetric were tested for visualization.

Results: HTLV RNA/proviral DNA was detected in 92.7% (90/97) of whole blood samples and had a specificity of 100% (0/50). The test was also able to detect samples with DNA concentration as low as 1.7 ng/µL. Inactivated and *in natura* samples both had a sensitivity of 75% (9/12). Referring to HTLV-1a subgroups, LAMP showed a sensitivity of 94.6% (35/37) for HTLV-1aA and 93.6% (44/47) in HTLV-1aB. Using DBS, LAMP had a sensitivity of 90% (36/40) and specificity of 100%. Referring to HTLV-1a subgroups, DBS showed a sensitivity of 85.7% (12/14) for HTLV-1aA and 94.7% (18/19) for HTLV-1aB. All methods of detection (gel electrophoresis, fluorescence and colorimetric) showed equal results.

Conclusion: It was possible to employ LAMP to detect HTLV in whole blood and DBS with high sensitivity which makes it a promising method to approach the diagnosis in low-resources settings and as a point-of-care.

Keywords: LAMP, dried blood spot, HTLV