IVD_04 - Design of a new optimized VLP as a positive control of molecular diagnostic kits

Alexandre Rodrigues Calazans[1]; Pedro Henrique Cardoso[1]; Mônica Barcellos Arruda[1]; Beatriz Vasconcello de Souza Barreto[1]; Alexandre Vicente Frederico[1]; Marisa de Oliveira Ribeiro[1]; Antônio Gomes Pinto Ferreira[1]; Patrícia Alvarez Baptista[1].
[1]Fiocruz/Bio-Manguinhos

Introduction: All molecular diagnostic kits of Bio-Manguinhos – PCR based, carry a VLP (virus like particle), non-infectious, non-replicative as internal, negative and positive control. We use this VLP as control, to mimic all steps of extraction and amplification. Up to date all VLP are based on the molecular clone HIV-1 D Z2Z6, that has several attenuations as: deletion of Nef gene, truncated Envelope and insertion in the the Integrase gene. We have 6 VLP in Bio-Manghinhos, 3 of them dedicated to the NAT Kit, 1 negative and 2 positives. The 2 positives are CP-multi and CP2, which have minigen1 and 2 as inserts, respectively. Minigen 1 has the follow targerts: Dengue 1,2,3 and 4; HIV-1; RP; HCV; YFV; INF A/B; HBV; Malaria, Flavivirus general, SC2 E/N/N1; Zika; Chikungunya. While minigen2, has: Falciparum; Malarie; Ovale; Vivax; Malaria general 2, MPXV Africa/Congo/1/2; OPV; VZV; MOCV; VARV; PPV; RSV; HMPV; Adenovirus; HRV; Measles; Rubella; Mumps; SC2 E/N; RP; INFA A/B; Mayaro; H1 pandemic; INFA pandemic, H3, INF B Yamagata e Victoria.

Objectives: To design a new optimized VLP to be used as internal and positive control of molecular kits, carrying the minigen1 and minigen 2 inserts.

Methodology: The DNA of the VLP was synthetized as a plasmid. The optimized construction, instead of a full- length molecular clone, as Z2Z6, has only 5`LTR, Gag and Protease, from HIV-1 HXB2, plus minigen 1/2 sequences. As we used a bi-cistronic vector, we add HIV-1 tat and rev, on the other MCS to allow better expression of the VLP. This construction was named CP3. The plasmid, once built, was transfected into 293-T cells and the supernatant harvested 48 hours later.

Results: After transfection, we measure the VLP yield by ELISA p24 HIV-1 and qPCR (SARS-Cov2 N). In the ELISA test, we have an OD of 3,36 for CP3 and 2,82 for CP2. We obtained 2 CT higher for CP3, comparing to CP2, in the PCR.

Conclusion: An optimized VLP for control of the PCR kits were built. It has a smaller plasmid, contain all PCR targets (minigen 1/2), doesn't require the tat/rev plasmids transfection and a better yield than former CP2.

Keywords: VLP; Molecular diagnosis