

IVD_02 - Breaking barriers in HTLV-1 diagnosis: VHH anti-P24 antibodies for the construction of efficient optical and electrochemical biosensors

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Introduction: Human T-lymphotropic virus type 1 (HTLV-1) is one of the etiological agents of T-cell leukemia/lymphoma in adults and HTLV-1-associated myelopathy, called tropical spastic paraparesis (HAM/TSP). About 5 and 10 million individuals may be infected with the virus worldwide. The scarcity of epidemiological data on HTLV-1 infection is a limiting factor due to the lack of screening and diagnosis of the infection. In different locations around the world, no gold standard exists for diagnosis of HTLV infection. In Brazil, HTLV was added to the national list of mandatory notification of diseases, conditions, and public health events in 2024. The Brazilian Unified National Health System (SUS) necessitates resources that streamline the diagnosis of HTLV-1 infection.

Objectives: With the perspective of developing inputs applicable to the detection of infections caused by HTLV- I, we developed VHH-monoclonal antibodies of camelid immunoreactive with the virus's p24 protein.

Methodology: VHHs anti-p24 antibodies were applied in the construction of optical and electrochemical biosensors, using gold nanoparticles AuNPs and gold electrodes, respectively, and evaluated for their application for diagnosing active infection caused by the HTLV-1 virus.

Results: From the description of the AuNPs and characterization by transmission electron microscopy (TEM), it was possible to establish the conditions for bioconjugation of VHH52-anti/p24 at a concentration of 8 µg and pH 9, in the presence of 75 mM of the acid stabilizing agent N-hydroxysuccinimide-acrylic ester (AANHS). The immunoreactivity of the biosensors by dot blot assay demonstrated the ability of the bioconjugates to recognize different concentrations of HTLV-1 recombinant p24. Regarding the application of VHH52-anti/p24 as an electrochemical biosensor, it was possible to obtain a sensor capable of detecting up to 1 ng/µL of recombinant HTLV-I p24.

Conclusion: The results show that anti-p24 VHHs can be used as inputs to create optical and electrochemical immunosensors that detect the HTLV-1 p24 protein. These sensors may construct a variety of detecting platforms.

Keywords: Construction of Biosensors; HTLV Diagnosis; Single-Domain Antibodies