Immunoblot analysis for immunodetection of HIV proteins.

Angel A Justiz-Vaillant

1University of the West Indies St. Augustine

University of the West Indies  angel.vaillant@sta.uwi.edu

Angel A Justiz-Vaillant
University of the West Indies St. Augustine

DISCLAIMER

DISCLAIMER – FOR INFORMATIONAL PURPOSES ONLY; USE AT YOUR OWN RISK

The protocol content here is for informational purposes only and does not constitute legal, medical, clinical, or safety advice, or otherwise; content added to protocols.io is not peer reviewed and may not have undergone a formal approval of any kind. Information presented in this protocol should not substitute for independent professional judgment, advice, diagnosis, or treatment. Any action you take or refrain from taking using or relying upon the information presented here is strictly at your own risk. You agree that neither the Company nor any of the authors, contributors, administrators, or anyone else associated with protocols.io, can be held responsible for your use of the information contained in or linked to this protocol or any of our Sites/Apps and Services.

ABSTRACT

Enzyme-Linked Immunosorbent Assay (ELISA) was used for screening while the Western Blot was used for confirmation.
Aliquots of 3-5 µl of the serum are applied to the gel and run on a protein electrophoresis (SDS-PAGE).

Gels are transferred to nitrocellulose membranes (Immobilon-Nc, pore size 0.45 µm, during 75 min at 40 mAmps using a semi-dry electroblotter, HEP-1 Model, Owl Scientific Inc).

The running buffer contains 25mM Tris, 192mM glycine pH 8.3 and 20% methanol.

The nitrocellulose membranes are blocked overnight in 10% nonfat skim milk in PBS with 0.05% Tween-20 pH 7.4 and then washed 4 times for 10 minutes with PBS-Tween 20.

Peroxidase-labeled anti-HIV conjugate are added and incubated at 4ºC overnight.

Membranes are washed as above and then tetra-methyl-benzidine are added and the reaction is stopped with deionised water.

A positive test displays two or more HIV proteins.

Only patients with positive confirmatory tests are classified as HIV positive.