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Immunoblot analysis for immunodetection of HIV proteins.

Angel A Justiz-Vaillant[1]

[1]University of the West Indies St. Augustine

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

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

ABSTRACT

Enzyme-Linked Immunosorbent Assay (ELISA) was used for screening while the Western Blot was used for confirmation.

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1 Aliquots of 3-5 µl of the serum are applied to the gel and run on a protein electrophoresis (SDS-PAGE).

2 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).

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

4 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.

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

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

7 A positive test displays two or more HIV proteins.

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

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