



Aug 17, 2020

# Immunoblot analysis for immunodetection of HIV proteins.

Angel A Justiz-Vaillant<sup>1</sup><sup>1</sup>University of the West Indies St. Augustine**1** Works for me [dx.doi.org/10.17504/protocols.io.bjtpknnm](https://dx.doi.org/10.17504/protocols.io.bjtpknnm)

University of the West Indies

[angel.vaillant@sta.uwi.edu](mailto: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.

## DOI

[dx.doi.org/10.17504/protocols.io.bjtpknnm](https://dx.doi.org/10.17504/protocols.io.bjtpknnm)

## PROTOCOL CITATION

Angel A Justiz-Vaillant 2020. Immunoblot analysis for immunodetection of HIV proteins.. **protocols.io**  
<https://dx.doi.org/10.17504/protocols.io.bjtpknnm>

## LICENSE

————— This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

## CREATED

Aug 17, 2020

## LAST MODIFIED

Aug 17, 2020

## PROTOCOL INTEGER ID

40527

## 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legal, medical, clinical, or safety advice, or otherwise; content added to [protocols.io](https://protocols.io) is not peer reviewed and may not have undergone a formal approval of any kind.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](https://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.

- 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 4°C 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.