Indirect Enzyme Linked Immunosorbent Assay (ELISA) for Detection of Anti-HIV Antibodies in Human Serum

Angel A Justiz-Vaillant¹, Norma McFarlane-Anderson²

¹University of the West Indies St. Augustine; ²University of the West Indies, Mona

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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
This protocol was already used successfully to detect anti-HIV antibody in the serum of women with cervical dysplasia or cervical cancer in Jamaica, West Indies [1].

Reference

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1. The 96 well polystyrene microplate (U-shaped bottom; Sigma-Aldrich) is coated with 50 ng of a mixture of synthetic peptides (including the fragment 579-601 of the HIV gp41 and fragments 254-274, 308-331 and 421-438 of the HIV gp120) for 4 h at 37°C.

2. The microplate is blocked with 3% non-fat milk in PBS, 25 µl/well, 1h at room temperature (RT).

3. The microplate is washed 4X with PBS-Tween 20.

4. Duplicates of 25 µl of 1:16 diluted human sera are added.

5. After incubation for 90 min at RT the microplate is washed 4X with PBS-Tween 20.

6. Then, 25 µl of a chimeric commercially-prepared recombinant protein LA-HRP conjugate (Sigma-Aldrich) diluted 1:5000 is added.

7. After incubation for 90 min at RT and rewashing steps 25 µl TMB is added to each well for 15 min in the dark.

8. The reaction is stopped with 3M H2SO4.

9. The microplate is read in a microplate reader at 450 nm.

10. In the ELISA is included a pooled human sera with high titre of anti-HIV antibodies as positive control, a pooled sera from healthy individuals as negative control and 0.9% normal saline solution was used as the blank.

11. The cut-off point is calculated as mean optical density (XOD) of negative control plus two standard deviation (SD).