ELISA for anti-HIV peptide antibodies in the egg yolks of brown Leghorn layer hens.

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ABSTRACT

These are three ELISAs for detection of anti-HIV antibodies. These test were highly specific, sensitive and reproducible. They detect specific immunoglobulin Y against HIV peptides in the egg yolk of brown Leghorn layer hens. A modification of these methods were used to detect anti-HIV antibodies in Humans.

GUIDELINES

Stop reaction as scheduled.

Dilute samples, controls, conjugates, and TMB as recommended in the steps.

MATERIALS

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3,3',5,5'-Tetramethylbenzidine Sigma
Aldrich Catalog #54827-17-7

Anti-Chicken IgY, HRP Conjugate, 300ul Promega Catalog #G1351

Greiner Clear-bottom polystyrene 96-well plates Sigma – Aldrich Catalog #M2936

Peptide 579-601 of HIV-gp41 Contributed by users

Peptide 308-331 HIV-gp120 Contributed by users

Peptide 421-438 HIV-gp120 Contributed by users
Coating buffer is prepared as follows: 3.7 g Sodium Bicarbonate (NaHCO3) and 0.64 g Sodium Carbonate (Na2CO3) in 1L of distilled water.

Phosphate buffered-saline Tween-20 (10% PBS-Tween 20, pH 7.2) is prepared as follows: Dissolve the following: 0.2g of KCl, 8g of NaCl, 1.45g of Na2HPO4, 0.25g of KH2PO4, and 2ml of tween-20 in 800 ml of distilled water. After that, adjust pH to 7.2, add additional distilled water to adjust the volume to 1L, and then may sterilize by autoclaving.

Blocking solution is prepared as follows: add 0.1 g KCl, 0.1 g K3PO4, 1.16 g Na2HPO4, and 4.0 g NaCl to 500 ml distilled water, pH 7.4. Then, to complete the preparation of this solution, 15g of non-fat dry milk should be added.

Sample/Conjugate Diluent is prepared as follows: Add 15 g of non-fat dry milk and 2.5 ml of 10% Tween 20 to 500 ml of PBS.

The 96-well polystyrene microplates (U- shaped bottom, Sigma-Aldrich) is coated with 100 ng of 579-601 of the HIV gp41, fragments 308-331 or 421-438 from HIV gp120 in coating buffer for 4.1 h at 37°C.
Then, each microplate is washed four times with 10% PBS-Tween 20 and the blocking solution (3% non-fat milk in PBS) added in the amount of 51 µl into each well.

The microplate is incubated at 1.30 h at RT. After that, the microplate is washed as previously.

Fifty µl of water soluble fraction (WSF) diluted 1:50 with the sample diluent is added to the wells.

Then, each microplate is incubated for 1h at RT and washed four times as previously.

Then, 50 µl of horseradish peroxidase labeled anti-IgY conjugate (Sigma-Aldrich) diluted 1:30,000 is poured into each well.

Each microtiter plate is incubated again for 1h at RT and then, washed four times.

A volume of 50 µl of tetramethylbenzidine (TMB, Sigma-Aldrich) is added; and after a further incubation of 16 min in the dark, the reaction is stopped with a solution of 3M HCl.

After that, each microplate is read in a microplate reader at 450 nm.

The cut-off value is assessed from the mean optical density (OD) of the negative control times 2.
The cut-off points of ELISAs for the detection of anti-HIV peptide (579-601), anti-HIV peptide (308-331) and anti-HIV peptide (421-438) were 0.42, 0.40 and 0.44 respectively.