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

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We use this protocol and it's working

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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.
Dilutesamples, controls, conjugates, and TMB as recommended in the steps.

Materials

MATERIALS

- 3,3′,5,5′-Tetramethylbenzidine **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**54827-17-7
- Anti-Chicken IgY, HRP Conjugate, 300ul **Promega Catalog #**G1351
- Greiner Clear-bottom polystyrene 96-well plates **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**M2936
- Peptide 579-601 of HIV-gp41
- Peptide 308-331 HIV-gp120
- Peptide 421-438 HIV-gp120

Troubleshooting

1 Coating buffer is prepared as follows: 3.7 g Sodium Bicarbonate ($NaHCO_3$) and 0.64 g Sodium Carbonate (Na_2CO_3) in 1L of distilled water.

2 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 Na_2HPO_4, 0.25g of KH_2PO_4, 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.

3 Blocking solution is prepared as follows: add 0.1 g KCl, 0.1 g K_3PO_4, 1.16 g Na_2HPO_4, 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.

4 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.

5 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.

6 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.

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

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

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

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

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

12 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.

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

14 The cut-off value is assessed from the mean optical density (OD) of the negative control times 2.

15 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.