Lymphocyte proliferation in poultry species

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Abstract

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Protocol

Step 1.
The heparinized blood samples were added to separation medium Histopaque®-1077 (cat# 10771, Sigma, USA).

Step 2.
Samples were centrifuged at 1030 xg for 20 min at 4°C.

Step 3.
Peripheral blood mononuclear cells (PBMCs) were isolated and washed twice with RPMI-1640 (Invitrogen Corp., Grand Island, NY, USA) and then re-suspended in 2 ml of RPMI-1640 complete culture medium.

Step 4.

The viable lymphocytes were detected using Trypan Blue dye and plated in triplicate wells (96-well plate) at 1×10^{6} cells per well.

Step 5.
A 50 µl of either Concanavalin-A (Con-A, 45 µg/ml, cat# C5275, Sigma, USA) or Lipopolysaccharide (LPS, 10 µg/ml, cat# L4391, Sigma, USA) was added to selected wells to induce the proliferation of T lymphocyte and B lymphocyte, respectively.

Step 6.
Control wells received 50 µl of RPMI-1640 medium.

Step 7.

Cells were then incubated for 48 h at 42 °C with 5 % CO_2.

Step 8.
After incubation, 15 µl of 3-[4,5-dimethylthiazol]-2,5-diphenyltetrazolium bromide (MTT, 5 mg/ml, cat# M2128 , Sigma, USA) was added to each well and the cells were incubated for another 4 h.

Step 9.
Subsequently, 100 µl of 10% sodium dodecyl sulfate dissolved in 0.04 M HCl solution was added to each well to lyse the cells and solubilize the MTT crystals.

Step 10.
Finally, the absorbance at 570 nm was recorded using an automated ELISA microplate reader

(ChroMate® Microplate Reader-4300, Awareness Technology Inc., Palm City, FL, USA).

Step 11.

Stimulating index (SI) for either T or B cells was calculated as follows: SI = OD570 $_{\text{(stimulated cells)}}$ / OD570 $_{\text{(unstimulated cells)}}$.