Optimization of Cell Proliferation Assay for Goats using Primary Peripheral Blood Mononuclear Cells

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Abstract—*Cell mediated immune response can be measured by lymphocyte proliferation assay under the influence of mitogens and specific antigens. Although there are well established protocols available for lymphocyte proliferation assay for various species, goats are kind of exceptional one and need specialized modified protocol. The method described here is isolation and in vitro proliferation of goat lymphocytes under the influence of mitogens and goatpox virus (GTPV) antigen. Alamar blue and Resazurin (7-hydroxy-3H-phenoxazin-3-one 10-oxide) are redox dyes, which exhibits both colorimetric and fluorometric changes that relate to metabolic activity of cultured cell as well as proliferation indicator and they implicate safe, convenient and low cost technique with high applicability. Two colorimetric assays viz., alamar blue and resazurin cell proliferation assays were performed using various concentrations of mitogens such as Con A, LPS, PHA, pokeweed mitogen and purified GTPV antigen. The cells were maintained in three different modified culture mediumviz., AIM-V, RPMI-1640 and TCM-199 and incubated for 24 hours with 5% CO_2 atmosphere. The resazurin dye concentration and dye reduction time were optimized in order to detect highest proliferative activity. Out of three different media, modified TCM-199 provides better results. Both dyes take nearly 24 h for optimum dye reduction and yielded similar results. TCM-199 provides better survival environment and dye reduction as per goat lymphocytes are concerned. The purified GTPV antigen and PHAP induced maximum stimulation in the goat PBMCs. This finding would be beneficial in assessing CMI response in goats especially for vaccination studies.*