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Resazurin Based Colorimetric Proliferation Assay for PBMCS of Goats and Sheep

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A B S T R A C T

Resazurin dye-based colorimetric assay is simple and cost-effective to evaluate viability and cytotoxicity. This dye also used for evaluating proliferative responses of PBMCs against mitogens and antigens especially to assess the CMI response against vaccines and pathogens. However, the assay needs to be optimized for different cell types and animal species. So far, resazurin based assay is not available for sheep and goats. In the present study, the assay was optimized for PBMCs of goat with two density gradients viz., Histopaque 1.077 and 1.083, and different culture conditions. The cell yield obtained through Histopaque 1.083 was 10.5 times more than cells obtained through 1.077. Out of three tested media, cells cultivated with TCM-199 showed the highest growth followed by RPMI-1640, and AIM-V. The resazurin dye @600 µM and 24 h dye reduction time yielded a better result. Among four mitogens tested PHA-P induced higher proliferation followed by ConA, PWM, and LPS. The result was correlated with the result obtained in the chemiluminescence based BrdU ELISA. Further, the resazurin assay also validated in sheep PBMCs.

Introduction

Cell-mediated immune response (CMI) is one of the major wings of immune responses that provide protection against various pathogens. Evaluation of CMI response is required for various research works including disease diagnosis and vaccines. Delayed type hypersensitivity test (DTH) is well known *in vivo* method to detect CMI responses whereas lymphocyte proliferation assay has been widely used in *ex vivo*.

The evaluation of CMI response broadly categorized into stimulation (leukocyte migration method), effector activity (cytokine kinetics), and cell proliferation activity (Nikbakht *et al.*, 2019).