PARASITO2025
29º CONGRESSO BRASILEIRO DE PARASITOLOGIA

SIMPLIFIED FLUORESCENT LABELING OF *LEISHMANIA* SPP. PARASITES AS A TOOL FOR STUDYING EARLY EVENTS OF PARASITE-HOST CELL INTERACTIONS

MARIANA R. G. SILVEIRA[1]; FREDERICO MARTINEZ[3], ALIZA KHAITIN[3] JADE LIZ F. M. SOUZA[1]; IGOR R. G. SILVEIRA[1]; ANA C. D. ANDRADE[1]; CLÁUDIA I. BRODSKYN[1], DEBORAH B. M. FRAGA[1], SERENE MOUSSAOUI[3]; JULIANA P. B. DE MENEZES[1], MARIA C. GIMENEZ[3]; MAURÍCIO R. TEREBIZNIK[3], PATRÍCIA S. T. VERAS[1,2]

1 Laboratory of Parasite-Host Interaction and Epidemiology (LaIPHE) - Gonçalo Moniz Institute - Fiocruz, Salvador, Brazil;

2 National Institute of Science and Technology of Tropical Diseases (INCT-DT) - National Council for Scientific and Technological Development (CNPq);

3 Department of Biological Sciences, University of Toronto at Scarborough, Toronto, ON M1C 1A4, Canada.

ABSTRACT

INTRODUCTION The interaction between *Leishmania* and macrophages is crucial to understanding the mechanisms of cellular infection. Fluorescent labeling of parasites enables visualization through confocal microscopy or flow cytometry, allowing for detailed analysis. In this context, Oregon Green and Calcein Red are potentially cell tracers due to their high fluorescence intensity and low cytotoxicity. Given the limited literature, a detailed evaluation of their performance was conducted. **OBJECTIVE** Evaluate the effectiveness of Oregon Green and Calcein Red labelling on various *Leishmania* species as a tool for investigating early parasite-host cell interactions. **METHODOLOGY** *Leishmania braziliensis (Lb)* promastigotes were labeled with Oregon Green or Calcein Red, and their fluorescence intensity was assessed via flow cytometry over four days post-labeling. The growth of *Lb* cultures labeled with Oregon Green was monitored for six days. Promastigotes from *Lb, Leishmania amazonensis* and *Leishmania infantum* were labeled with concentrations of 1 mM, 5 mM or 10 mM of Oregon Green, and fluorescence intensity evaluated using fluorescence-activated cell sorting (FACS). **RESULTS** Oregon Green proved more effective for labeling *Leishmania* promastigotes than Calcein Red, showing decrease in fluorescence intensity from 24 h after labeling. Oregon Green does not hinder the development and growth of *Lb* promastigotes. A 10 mM concentration of Oregon Green exhibited the highest fluorescence intensity, making it the optimal concentration for evaluation via FACS and confocal microscopy, enhancing the visibility of the promastigotes and allowing more detailed and clear observation. **CONCLUSION** Oregon Green has proven to be an effective Cell-Trace for *Leishmania* promastigotes in studies involving confocal microscopy and FACS, with experiments extending up to 24 h post-infection. Further research is underway to evaluate the fluorescence of Oregon Green in the intracellular form of the parasite.

Supported by: Postgraduate program in Pathology (PgPAT); (UFBA/Fiocruz) Coordination for the Improvement of Higher Education Personnel (CAPES); National Council for Scientific Research and Development (CNPq); Foundation for the Support of Research in the State of Bahia (FAPESB); INOVA Program – Fiocruz (79700287000); National Institute of Science and Technology in Tropical Diseases (INCT-DT-465229/2014-0).

Keywords: Cell-Trace; Oregon Green; *Leishmania* spp.