

Article

Cultivation of Protozoa Parasites In Vitro: Growth Potential in Conventional Culture Media versus RPMI-PY Medium

Germano Castelli , Eugenia Oliveri * , Viviana Valenza , Susanna Giardina, Flavia Facciponte ,
Francesco La Russa , Fabrizio Vitale and Federica Bruno 

Centro di Riferenza Nazionale per le Leishmaniosi (C.Re.Na.L.), WHOI Leishmania Reference Laboratory, Istituto Zooprofilattico Sperimentale della Sicilia, Via Gino Marinuzzi 3, 90129 Palermo, Italy

* Correspondence: oliverieugenia@gmail.com; Tel.: +39-0916565450

Simple Summary: Many different media have been used to grow protozoa parasites, which can be classified into two main categories: biphasic and liquid. All major biphasic media require blood as an essential component for parasite replication: Evans's modified Tobie and Novy–MacNeal–Nicolle media are the conventional media used for the cultivation of *Leishmania* and *Trypanosoma* parasites. These media are time-consuming and often complex, and they are based on relatively expensive reagents and blood components, which complicate immunological and biochemical studies. Our study shows that a new liquid medium, RPMI-PY, can be used for both diagnostic and research purposes since comparable and sometimes better results were found for parasite load compared with the reference media in *Leishmania* and *Trypanosoma*. The relative simplicity of RPMI-PY medium, the general availability of its components, and above all, the lack of fresh animal blood requirement renders this medium the best choice for many purposes in protozoa parasites studies. The elimination of fresh blood components is important for economic and ethical aspects, with no need for rabbits' housing and bleeding, as well as to avoid the complication of the blood components of media in immunological and biochemical studies. Our approach allowed us to clearly evaluate the significant changes in the biological parameters of promastigotes in each medium.

Abstract: The in vitro cultivation of *Leishmania* and *Trypanosoma* parasites plays an important role in the diagnosis and treatment of parasitic diseases. Although Evans's modified Tobie and Novy–MacNeal–Nicolle media, for *Leishmania* spp. and *Trypanosoma cruzi*, respectively, are the two commonly used media for both isolation and maintenance of strains in vitro, their preparation is expensive and laborious and requires fresh rabbit blood from housed animals. The purpose of this study was to evaluate the in vitro growth of both parasites with an alternative monophasic, blood-free, easy, and affordable medium called RPMI-PY, which was previously demonstrated suitable for the in vitro growth of *Leishmania infantum*. The potential growth of different *Leishmania* species and *Trypanosoma cruzi* was evaluated in traditional culture media versus RPMI-PY medium, and we recorded the protozoa parasites' morphology via orange acridine–ethidium bromide staining. The results of our study show that RPMI-PY medium can be used for *Trypanosoma cruzi*, *Leishmania amazonensis*, *Leishmania major*, and *Leishmania tropica* species since in all the species except *Leishmania braziliensis*, the exponential growth of the parasite was observed, in many cases higher than conventional media. The staining confirmed not only their growth during the 72 h investigation but also the optimal morphology and viability of the protozoa in the RPMI-PY medium.

Keywords: protozoa parasites; culture media; RPMI-PY medium; *Leishmania* spp.; *Trypanosoma cruzi*



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1. Introduction

Hemoflagellates include parasitic protozoa of medical relevance to humans and other vertebrates. *Leishmania* and *Trypanosoma* are the two genera in the group that have a

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