A comprehensive analysis of viability assessment methods for *Giardia lamblia* and *Trichomonas vaginalis*: a systematic review

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Background: *Giardia lamblia* (*G. lamblia*) and *Trichomonas vaginalis* (*T. vaginalis*) are flagellated protozoan parasites responsible for giardiasis and trichomoniasis, respectively. These infections, often asymptomatic, can manifest with gastrointestinal and genitourinary symptoms [1,2]. Metronidazole serves as the primary pharmacological treatment for both diseases, but it has limitations, including adverse effects and the emergence of drug-resistant strains [1,3]. *In vitro* models are essential for the development of novel treatment options [4]. However, the diversity of viability assessment methods has led to inconsistent results and challenges in comparing studies. **Aim:** This study provides a comprehensive overview of commonly employed methods for assessing the viability of trophozoites of these parasites in the presence of compounds with therapeutic potential. **Methods:** In September 2023, a literature search was conducted on PubMed, ScienceDirect, and Web of Science using predefined search terms, including "*in vitro*", "method", "susceptibility", "viability", "sensitivity", "giardia" and "trichomonas". Research articles published in English or Portuguese were included, specifically focusing on *in vitro* methodologies for evaluating the viability of trophozoites in *G. lamblia* and *T. vaginalis*. Studies addressing cyst viability or lacking explicit method descriptions were excluded, as were reviews, systematic reviews, and meta-analyses. No temporal restrictions were imposed, and the Preferred Reporting Items for a Systematic Review and Meta-Analyses (PRISMA) statement was followed. **Results:** The search identified 29 experimental studies with multiple viability assays. Only five methods were common to both parasites: a colorimetric approach (dye exclusion test with trypan blue), two fluorometric methods (resazurin and SYBR green I), and two morphophysiological methods (cell count and cell motility). **Conclusion: T**he fluorometric resazurin method emerges as the most suitable choice for assessing trophozoite viability in both parasites, offering cost-effectiveness, flexibility, reliability, and ease of use. This method shows promise for standardized assessments of potential therapeutic compounds for *G. lamblia* and *T. vaginalis* infections.

Keywords: cell viability; *Giardia lamblia*; parasitic diseases; *Trichomonas vaginalis*; trophozoites.

Acknoldgements
This research did not receive external funding.

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