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CULTURE CONDITIONS AND THE PROMASTIGOTE PHENOTYPE OF *LEISHMANIA (VIANNIA) GUYANENSIS*: GROWTH VIABILITY MORPHOLOGY AND METACYCLOGENESIS

By

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Abstract

Axenic cultivation of promastigotes supports studies of Leishmania biology, diagnosis, drug screening, antigen production, and experimental infection. Culture medium selection influences both growth and population phenotype; cultures with similar densities may differ in viability, morphology, differentiation capacity, and infectivity. This structured narrative review examined media and culture conditions for promastigotes, with emphasis on Leishmania (Viannia) guyanensis and monophasic LIT or biphasic NNN/LIT systems. Twenty-two scientific publications were coded by year and primary contribution. Four studies (18.2%) provided evidence specific to L. guyanensis, whereas 18 (81.8%) supplied comparative, mechanistic, or methodological context. Direct evidence shows that strain MHOM/BR/75/M4147 can be maintained in distinct formulations and that morphological and functional changes associated with metacyclogenesis may be largely established by day four under defined conditions. Across other species, LIT and Schneider frequently supported cellular expansion, but viability and infectivity depended on supplementation and experimental purpose. Defined and serum-free media reduced compositional variability without producing uniform performance across species. The evidence does not identify a universally superior medium. Reproducible work with L. guyanensis requires full strain identification, an explicit distinction between monophasic LIT and biphasic NNN/LIT, control of inoculum and passage history, and combined assessment of density, viability, morphometry, metacyclogenesis, and infectivity.

Keywords: *axenic culture; Leishmania guyanensis; Liver Infusion Tryptose; metacyclogenesis; promastigotes.*

1. INTRODUCTION

Leishmaniases are diseases caused by protozoa of the genus *Leishmania* and transmitted mainly by phlebotomine sand flies. Cutaneous leishmaniasis is the most frequent clinical presentation and may produce persistent lesions and scars. In the Americas, species of the subgenus *Viannia*, including *L. (V.) guyanensis*, participate in heterogeneous transmission cycles and differ in geographic distribution, host interaction, and therapeutic response (Pereira *et al*., 2021; World Health Organization, 2023).

The *Leishmania* life cycle alternates intracellular amastigotes in vertebrate hosts with promastigotes in the digestive tract of the vector. An axenic promastigote culture is not biologically uniform. Growth, cell elongation, the relationship between body and flagellum length, metabolic state, and infective capacity vary across the growth curve and with environmental conditions. Parasite density per milliliter therefore cannot replace independent assessments of viability, morphology, or infective competence (Mendes *et al*., 2019; Serafim *et al*., 2012; Zakai *et al*., 1998).

NNN, LIT, Schneider, Grace, M199, and RPMI media have been incorporated into laboratory routines, but their effects are not always treated as part of the experimental design. Comparative studies show that formulation can modify doubling time, stationary-phase timing, differentiation, and macrophage infection. The same inoculum maintained in different media may yield populations that are not functionally equivalent (Costa *et al*., 2020; Lemesre *et al*., 1988; Santarém *et al*., 2014; Siripattanapipong *et al*., 2019).

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This issue is particularly important for *L. guyanensis*. Reference strain MHOM/BR/75/M4147 is used in studies of metacyclogenesis, intercellular communication, virulence, and genetics, yet published protocols use different systems, including biphasic NNN/LIT and supplemented Schneider, M199, or RPMI. Direct comparisons among these systems within the species remain scarce, which prevents attribution of superiority to one formulation (Hernández *et al*., 2014; Mendes *et al*., 2019; Pereira *et al*., 2021; Zakharova *et al*., 2022).

This review examined how culture media and related conditions influence the growth, viability, morphology, metacyclogenesis, and infectivity of promastigotes. It distinguishes findings demonstrated in *L. (V.) guyanensis* from evidence obtained in other species and defines minimum requirements for reproducible culture protocols.

2. METHODOLOGY

2.1 Review design and question

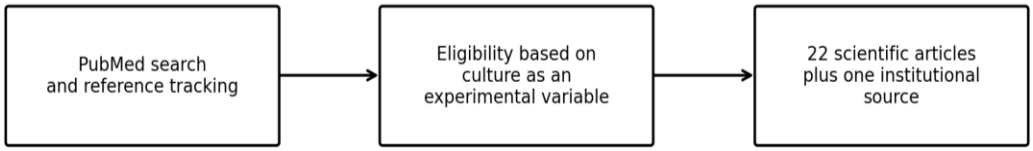
A structured narrative review was conducted to answer the following question: how do axenic media and culture conditions alter the growth, viability, morphology, metacyclogenesis, and infectivity of *Leishmania* promastigotes, particularly *L. (V.) guyanensis*? A narrative design was selected because studies differed substantially in species, strain, medium, supplementation, inoculum, temperature, follow-up time, and outcome, preventing a valid pooled estimate.

2.2 Search and eligibility

The primary search was conducted in PubMed/MEDLINE on September 13, 2026, without an initial language restriction and covering 1980-2026. Search concepts included *Leishmania*, promastigote, culture medium, cultivation, LIT, Schneider, Grace, growth, viability, morphology, and metacyclogenesis. References of key articles and institutional documents were also examined. Original studies were eligible when the medium or another culture condition was evaluated in relation to growth, viability, differentiation, morphology, or infectivity. Antileishmanial activity assays were excluded when culture served only as an operational step and was not an object of analysis.

Selection and extraction were performed by one reviewer. Because the review was narrative and no deduplicated search library was retained, raw retrieval and exclusion counts are not reported. The final evidence base comprised 22 scientific publications and one institutional source used only for disease context. Figure 1 summarizes the review process.

Figure 1 - Review process and composition of the evidence base. The diagram represents a structured narrative review rather than a registered systematic review.



Source: Prepared by the author for this narrative review.

2.3 Data extraction and evidence appraisal

Extracted variables included species and strain, basal medium, supplementation, temperature, initial density, culture duration, counting method, viability, morphology, metacyclogenesis, and infectivity. Information absent from the indexed abstract or consulted full text was not inferred. Evidence specific to *L. guyanensis* was distinguished from observations in other species. Greater inferential weight was assigned to direct comparisons that evaluated more than one outcome under controlled culture conditions.

2.4 Quantitative descriptive analysis

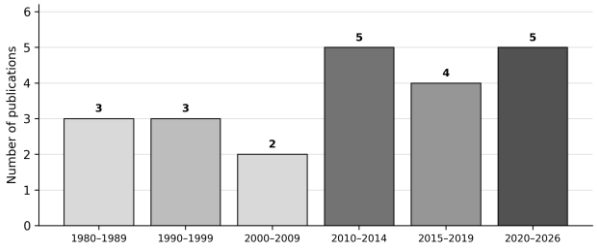
The 22 scientific publications were coded by publication year and one mutually exclusive primary contribution: evidence specific to *L. guyanensis*; comparative evaluation of culture media; defined or serum-free systems; or metacyclogenesis and phenotypic drift. The WHO source was excluded from these counts. Absolute and relative frequencies characterize the bibliography used in this review and do not represent effect estimates or a meta-analysis.

3. RESULTS AND DISCUSSION

3.1 Quantitative profile of the evidence base

Fourteen of the 22 publications (63.6%) were published from 2010 onward. Five studies appeared between 2010 and 2014, four between 2015 and 2019, and five between 2020 and 2026. The recent literature expanded comparisons of media and phenotypic outcomes, but the temporal distribution also shows that foundational evidence on defined media and long-term passage remains relevant (Figure 2).

Figure 2 - Temporal distribution of the 22 scientific publications included in the descriptive analysis. The institutional source was excluded.



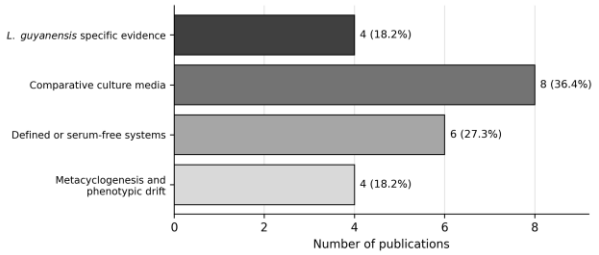
Source: Prepared by the author for this narrative review.

Only four publications (18.2%) formed the category specific to *L. guyanensis*. Comparative evaluations of culture media represented eight studies (36.4%), defined or serum-free systems represented six (27.3%), and metacyclogenesis or

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phenotypic drift represented four (18.2%). Thus, 18 of 22 publications (81.8%) served primarily as comparative or methodological support rather than direct evidence for the focal species (Figure 3).

Figure 3 - Distribution of publications according to their primary contribution. Categories are mutually exclusive; n = 22.



Source: Prepared by the author for this narrative review.

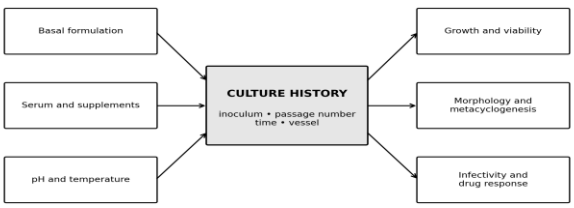
3.2 Culture medium as a biological variable

The first consequence of culture medium is nutritional, but its effects extend beyond the supply of carbon, nitrogen, and cofactors. Osmolarity, pH, heme and purine availability, lipid composition, and serum macromolecules influence division and differentiation. Initial density also has a consistent effect: cultures started with few promastigotes may show insufficient expansion, whereas larger inocula shorten lag and exponential phases (Lemesre *et al*., 1988; Nayak *et al*., 2018; Serafim *et al*., 2012).

Maximum density alone is an incomplete outcome. Media that sustain high biomass may select parasites with different morphology, metabolism, or infectivity. In *L. infantum*, distinct formulations altered cell cycle, metacyclic profile, differentiation into amastigotes, and infection. In *L. martiniquensis*, the preferred formulation depended on whether the purpose was long-term maintenance, biomass production, isolation, or metacyclogenesis (Santarém *et al*., 2014; Siripattanapipong *et al*., 2019).

Basal formulation interacts with supplementation, pH, temperature, inoculum, time, and passage history. The resulting population should therefore be characterized by several outcomes selected according to its intended downstream use (Figure 4).

Figure 4 - Conceptual model of culture variables that determine promastigote population phenotype. Arrows indicate experimental relationships that require joint control.



Source: Prepared by the author for this narrative review.

3.3 LIT and NNN LIT are not equivalent

LIT, or Liver Infusion Tryptose, is a liquid medium containing liver infusion, tryptose, glucose, salts, and hemin. NNN/LIT combines a Novy–MacNeal–Nicolle-type solid phase with a liquid phase based on LIT. The solid–liquid interface, blood-derived components, and nutrient availability distinguish the two systems. Methods, tables, and figure legends should therefore identify whether LIT was used alone or as the liquid phase of a biphasic system.

Direct evidence documents maintenance of strain MHOM/BR/75/M4147 in NNN/LIT supplemented with 20% fetal bovine serum at 25 °C, including protocols developed for genotyping Neotropical species. Other work with the same strain used Schneider at 26 °C, supplemented M199 at 23–25 °C, or RPMI at 24 °C. These protocols demonstrate adaptability but do not establish phenotypic equivalence among media (Hernández *et al*., 2014; Zakharova *et al*., 2022).

The performance of LIT was compared directly in *L. infantum*. Costa *et al*. observed greater growth in LIT and Schneider than in Grace, whereas albumin improved viability regardless of basal medium. Serum-supplemented LIT and Grace produced greater infection of J774A.1 macrophages than Schneider. These findings support LIT for that species and purpose, but they cannot be transferred directly to *L. guyanensis* (Costa *et al*., 2020).

3.4 Classical defined and serum free media

Schneider, Grace, M199, and RPMI differ in amino acids, vitamins, salts, and energy substrates. Schneider was formulated for insect cells and is widely used for promastigotes; M199 and RPMI provide known basal compositions but often require supplementation. Direct comparisons produced species-dependent results. Alternative low-cost media also supported growth, but their performance remained composition dependent. No formulation was consistently optimal across species and intended uses (Castelli *et al*., 2014; Costa *et al*., 2020; Lemesre *et al*., 1988; Özel *et al*., 2025; Santarém *et al*., 2014; Siripattanapipong *et al*., 2019).

Chemically defined media provide greater control of composition and are useful for metabolomic and proteomic studies. Nayak *et al*. obtained sustained growth and infectivity comparable to a semidefined medium and identified amino acids required for viability. Merlen *et al*. maintained several species in a medium without serum or macromolecules while preserving morphology and infectivity under the evaluated conditions (Merlen *et al*., 1999; Nayak *et al*., 2018).

Serum removal does not yield a single transferable solution. Semidefined mixtures, urine-containing media, albumin, and other substitutes may support growth, but results depend on species and outcome. Studies using serum from different animals also show that source and concentration modify proliferation. Replacement of fetal bovine serum therefore requires functional validation rather than visual comparison of growth curves alone (Ali *et al*., 1998; Evans, 1986; Habibzadeh *et al*., 2021; Sharief *et al*., 2008).

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Table 1 compares the practical characteristics of recurrent culture systems. It is not a collection of preparation recipes; it identifies the variables that must be reported when a culture platform is selected.

Table 1 - Comparative characteristics of systems used to cultivate Leishmania promastigotes (Castelli *et al*., 2014; Costa *et al*., 2020; Hernández *et al*., 2014; Merlen *et al*., 1999; Nayak *et al*., 2018; Santarém *et al*., 2014; Sharief *et al*., 2008; Siripattanapipong *et al*., 2019).

System	Basis	Frequent application	Critical reporting point
Biphasic NNN/LIT	Blood-containing solid phase and liquid LIT phase	Isolation, recovery, and maintenance	Describe the solid phase, blood source, and supplementation
Monophasic LIT	Liver infusion, tryptose, glucose, salts, and hemin	Expansion and biomass production	Complex composition and preparation-dependent variability
Schneider	Nutrient-rich insect-cell medium	Maintenance, expansion, and differentiation	Phenotype depends on pH, serum, and species
Grace	Insect-cell medium	Cultivation and infectivity assays	May yield lower growth in some comparisons
M199	Medium of known basal composition, often supplemented	Maintenance and experimental manipulation	Heme, purines, serum, and antibiotics vary among protocols
RPMI 1640	Widely used defined basal medium	Drug assays and maintenance	Often requires serum or additional supplementation
Defined serum-free media	Specified amino acids, salts, vitamins, and cofactors	Omics, metabolism, and standardization	Validate species, infectivity, and phenotypic stability

Source: Prepared by the author based on the studies analyzed in this review.

3.5 Serum albumin heme and purines

Fetal bovine serum supplies lipids, carrier proteins, growth factors, and molecules that bind potentially toxic compounds. It also introduces lot variability, cost, and animal-derived material. Concentration, heat inactivation, manufacturer, and lot should be reported whenever reproducibility across experiments is relevant (Ali *et al*., 1998; Costa *et al*., 2020; Evans, 1986; Habibzadeh *et al*., 2021).

Albumin and protein substitutes may improve viability without reproducing every effect of serum. In the comparative *L. infantum* study, albumin increased viability in all three media, whereas infectivity depended on the interaction between basal formulation and supplement. Growth, membrane integrity, and infection should therefore be measured as distinct outcomes (Costa *et al*., 2020).

Heme and purines are particularly relevant to metabolism and differentiation. Purine depletion may promote metacyclogenesis without being equivalent to simple growth restriction. Serafim *et al*. showed that adenosine and other purines regulate this process, supporting a conceptual distinction between proliferation and differentiation (Serafim *et al*., 2012).

3.6 Growth viability and morphology

A typical curve includes adaptation, exponential growth, stationary phase, and decline, but the duration of each phase depends on inoculum, medium, and species. Hemocytometer counts estimate total density; without an integrity marker they do not distinguish viable parasites from immobile or damaged structures. Dye exclusion, metabolic activity, standardized motility, or flow cytometry can complement enumeration (Costa *et al*., 2020; Lemesre *et al*., 1988; Limoncu *et al*., 2004).

Morphology provides information on population composition. Promastigote stages vary in body length and width, flagellum length, position of the nucleus and kinetoplast, and motility. Rosette formation may accompany high density and cell–cell interaction, but it should not be interpreted alone as evidence of viability or metacyclogenesis (Mendes *et al*., 2019; Pereira *et al*., 2021; Zakai *et al*., 1998).

Mendes *et al*. described reduced body size, a longer flagellum relative to the cell body, greater complement resistance, and increased infectivity during *L. guyanensis* metacyclogenesis. Under their conditions, the process was essentially complete by day four, and parasites not agglutinated by *Bauhinia purpurea* lectin concentrated metacyclic traits. Day four is therefore a useful reference point for that protocol, not a universal collection time for every medium, strain, or inoculum (Mendes *et al*., 2019).

Strain M4147 has also been used in cell-cycle and morphometric analyses. Zakharova *et al*. measured the cell body, flagellum, nucleus, and kinetoplast in M199 cultures and showed that genetic alteration can change the proportion of biflagellated forms without producing identical effects on all growth measures. Enumeration and morphometry therefore provide nonredundant information (Zakharova *et al*., 2022).

3.7 Successive passages and phenotypic drift

Prolonged axenic maintenance selects parasites adapted to the flask environment. *L. donovani* promastigotes cultured for years lost infectivity and virulence and changed in size, growth pattern, and antigen composition. Successive passage

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of *L. amazonensis* produced metabolomic changes associated with attenuation. Passage number, thaw date, and frequency of return to cryopreserved stock should be part of the experimental record (Crepaldi *et al*., 2019; Nolan & Herman, 1985).

Phenotypic drift can affect drug assays and macrophage-interaction experiments. Differences attributed to a compound or parasite line may partly reflect culture age, formulation, or passage history. Comparative designs should use cultures synchronized by phase and passage number and preferably derived from the same cryostock lot (Crepaldi *et al*., 2019; Nolan & Herman, 1985; Santarém *et al*., 2014).

3.8 Evidence specific to Leishmania guyanensis

Available evidence confirms the plasticity of *L. guyanensis* but does not provide a paired comparison of LIT, Schneider, Grace, M199, and RPMI using the same strain and experiment. Lemesre *et al*. found that *L. guyanensis* followed a general growth pattern similar to other *Viannia* species and was strongly affected by initial density. Later studies documented successful use of NNN/LIT, Schneider, M199, and RPMI for different experimental purposes (Hernández *et al*., 2014; Lemesre *et al*., 1988; Pereira *et al*., 2021; Zakharova *et al*., 2022).

Pereira *et al*. showed that clinical *L. guyanensis* isolates can differ in growth, metacyclic proportion, and antimony response. Communication between populations in coculture also changed density and drug response. Strain identity is therefore an experimental variable: clinical origin, resistance profile, and population interactions may modify the observed phenotype (Pereira *et al*., 2021).

Strain MHOM/BR/75/M4147 carries Leishmania RNA virus 1 in many laboratory preparations and has become a model for functional genetics. Viral status and manipulation history should be reported because they can alter host interaction and the interpretation of virulence. Culture comparisons should document LRV1 status when known and should not treat M4147 as representative of every *L. guyanensis* isolate (Zakharova *et al*., 2022).

3.9 Synthesis of the central studies

Table 2 summarizes the studies that support the main arguments of this review. Heterogeneity of species, formulations, supplements, and outcomes prevents a single ranking of media. The most consistent finding is that performance depends on the interaction among formulation, supplement, parasite, and experimental purpose.

Table 2 - Central studies on media and culture conditions for promastigotes. Findings apply to the species and protocols indicated.

Study	Model	Comparison	Principal contribution
Nolan & Herman (1985)	*L. donovani*	Modified Tobie medium and prolonged passage	Long-term culture reduced infectivity and altered growth and antigens
Evans (1986)	12 reference strains	Fetal-calf-serum substitute	Treated adult serum supported growth comparable to fetal serum
Lemesre *et al*. (1988)	Several species, including *L. guyanensis*	GLSH, RPMI, and LITR9	Initial density altered the duration of growth phases
Zakai *et al*. (1998)	Four species	Schneider with 20% serum	Acidic pH increased metacyclic traits, complement resistance, and infectivity
Ali *et al*. (1998)	*Leishmania* spp.	M199 with urine and no serum	Growth was similar to or greater than serum-supplemented M199
Merlen *et al*. (1999)	Cutaneous and visceral species	Defined CDM/LP	Continuous serum-free cultivation preserved morphology and infectivity
Limoncu *et al*. (2004)	*Leishmania* spp.	TBH, PYH, BKH, and RPMI	Growth peaks occurred on different days according to formulation
Sharief *et al*. (2008)	*Leishmania* spp.	Serum-free CML and RPMI	Growth and J774 infection were comparable under the evaluated conditions
Rodrigues *et al*. (2010)	*L. amazonensis*	PBHIL	High biomass, prolonged viability, and amastigote-like differentiation
Serafim *et al*. (2012)	*L. amazonensis* and *L. infantum*	Purine modulation	Adenosine availability regulated metacyclogenesis and proliferation
Santarém *et al*. (2014)	*L. infantum*	Multiple media and cRPMI	Media altered morphology, cell cycle, metacyclic profile, and infectivity
Castelli *et al*. (2014)	*L. infantum*	RPMI-PY and Tobie-PY	Modified media increased early growth and parasite load
Nayak *et al*. (2018)	*Leishmania* spp.	Defined medium	Sustained growth and identification of essential amino acids
Mendes *et al*.	*L. guyanensis*	Axenic culture	Metacyclogenesis was essentially

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(2019)			complete by day four under specific conditions
Siripattanapipong *et al*. (2019)	*L. martiniquensis*	M199, RPMI, Grace, and Schneider	The preferred medium depended on maintenance, biomass, isolation, or differentiation
Crepaldi *et al*. (2019)	*L. amazonensis*	Successive passages	Attenuation was accompanied by metabolic reprogramming
Costa *et al*. (2020)	*L. infantum*	LIT, Schneider, and Grace	LIT and Schneider supported growth; supplementation modified viability and infectivity
Habibzadeh *et al*. (2021)	*L. major* and *L. tarentolae*	Serum-free CSFM	Morphology, protein expression, and infectivity were maintained
Pereira *et al*. (2021)	*L. guyanensis*	Clinical isolates and coculture	Growth, metacyclic proportion, and drug response varied among populations
Zakharova *et al*. (2022)	*L. guyanensis* M4147	Supplemented M199	Morphometry and cell-cycle analysis revealed phenotypes missed by density alone

Source: Prepared by the author based on the studies analyzed in this review.

3.10 Minimum requirements for reproducible protocols

A protocol for *L. guyanensis* should report strain identity, origin, LRV1 status when available, passage number, complete medium, supplementation, temperature, vessel, culture volume, volume-to-vessel-capacity relationship, initial density, and counting frequency. The phrase cultured in LIT is insufficient when an NNN phase, blood, serum, or antibiotics are present (Hernández *et al*., 2014; Lemesre *et al*., 1988; Zakharova *et al*., 2022).

Minimum outcomes depend on the intended use. Expansion studies require density and viability; infection experiments should add metacyclogenesis and a functional assay; morphological work should report body and flagellum measurements from a defined number of cells, preferably using blinded criteria. Table 3 provides a minimum reporting framework.

Table 3 - Recommended minimum information for comparative studies of promastigote cultures.

Domain	**Minimum information**
Identity	Species, subgenus, international strain code, origin, and confirmation method
History	Thaw date, passage number, cryostock lot, and LRV1 status when known
Medium	Name, manufacturer or recipe, lot, pH, relevant osmolarity, and LIT or NNN/LIT distinction
Supplements	Serum or albumin, concentration, heat inactivation, hemin, antibiotics, urine, and other additives
Culture	Temperature, atmosphere, vessel, volume, agitation or static condition, and duration
Inoculum	Initial density, source-culture phase, counting method, and replicate number
Growth	Daily or predefined-interval counts, complete curve, and dispersion measure
Viability	Method independent of total count and standardized reading time
Morphology	Stain, magnification, number of cells, body and flagellum measurements, and classification criteria
Function	Complement resistance, macrophage infectivity, or another assay aligned with the study objective

Source: Prepared by the author based on the studies analyzed in this review.

3.11 Knowledge gaps and experimental agenda

The principal gap is the absence of a paired comparison of monophasic LIT, biphasic NNN/LIT, Schneider, Grace, M199, and RPMI using the same *L. guyanensis* strain. A suitable design should use one cryostock lot and passage range, inoculate equivalent densities, control temperature and volume, and follow cultures through decline. Density, viability, and morphometry should be measured in parallel.

Metacyclogenesis should be assessed by more than one indicator. Compatible morphology, complement resistance, lectin-based separation, and macrophage infectivity answer

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related but nonidentical questions. For M4147, LRV1 status and passage history should be incorporated into both design and interpretation.

Poor comparability among serum lots and commercial formulations is another unresolved source of variation. Factorial experiments combining basal medium, supplement concentration, and initial density could identify interactions missed by one-variable-at-a-time designs. The preferred condition should be the one that reproducibly produces the phenotype required for the next experiment, rather than the condition that yields the highest cell count alone.

3.12 Review limitations

This review used PubMed as its principal structured database and supplemented the search through reference tracking. It was not preregistered, and one reviewer performed selection, extraction, and coding without duplicate assessment or a formal risk-of-bias tool. The absence of a retained deduplicated library prevents reconstruction of a systematic study-flow count. The included studies also differed in parasite species, strain, medium composition, supplements, inoculum, passage history, follow-up, and outcome definitions, precluding quantitative effect comparison. Evidence from species other than *L. guyanensis* was used to identify mechanisms and reporting requirements, not to claim biological equivalence.

4. CONCLUSION

Culture medium is part of the experimental definition of the promastigote phenotype. LIT and Schneider support expansion in several models, but comparable cell density does not establish equivalent viability, morphology, differentiation, or infectivity. Serum, albumin, heme, purines, pH, temperature, inoculum, and passage history modify the population recovered at the selected time point. Culture conditions should therefore be interpreted as interacting variables rather than as a neutral background procedure.

Evidence specific to *Leishmania (Viannia) guyanensis* remains limited. Published studies show that MHOM/BR/75/M4147 and clinical isolates can be maintained in several formulations and that metacyclic traits may become prominent by day four under defined conditions. They also demonstrate variation among isolates, effects of population interaction, and phenotypes associated with cell-cycle or lipid-metabolism changes. These findings do not identify one medium as universally superior or establish that results obtained with M4147 apply to every *L. guyanensis* isolate.

A defensible comparison requires the same cryostock and passage range, equivalent inocula, explicit differentiation between monophasic LIT and biphasic NNN/LIT, and control of vessel, volume, temperature, and sampling time. Cell density should be accompanied by an independent viability measure, quantitative morphometry, an assessment of metacyclogenesis, and a functional outcome aligned with the intended use. Reporting serum lot, complete supplementation, and LRV1 status when known is necessary to distinguish culture effects from strain or reagent effects.

The next experimental priority is a paired, multifactorial comparison of the principal media within the same *L. guyanensis* strain and across representative isolates. Such work should determine which conditions reproducibly preserve the phenotype required for biomass production, macrophage infection, drug testing, or differentiation studies. Until those data are available, medium selection should be justified by the downstream question and validated with more than a growth curve.

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