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Evaluation of Alternative Culture Media for Cost-effective and Reliable *in vitro* Cultivation of *Leishmania*

Leishmania'nın Maliyeti Düşük ve Güvenilir in vitro Üretimi için Alternatif Kültür Besiyerlerinin Değerlendirilmesi

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ABSTRACT

Objective: This study aimed to evaluate the efficiency of commonly available culture media in routine microbiology laboratories for the cultivation of *Leishmania tropica* (*L. tropica*) promastigotes.

Methods: Various media including yeast extract agar, tryptic soy broth, sabouraud dextrose agar, brucella agar, and Columbia agar were tested. A total of sixteen media were prepared: eight blood-free (BY1-BY8) and eight supplemented with erythrocyte suspension blood-containing medium (KBY1-KBY8). Each medium was inoculated with *L. tropica* promastigotes at a concentration of 10^5 promastigotes/mL and incubated for 12 days. Daily promastigote counts were performed to monitor growth.

Results: Among the tested media, BY7, BY8, KBY7, and KBY8 showed the most favorable growth patterns. In BY7 and BY8, the promastigote count increased from 10^3/mL on day 1 to 10^4/mL by day 5. BY7 supported continuous growth, reaching 10^7 promastigotes/mL by day 8 and maintaining this level until day 12. BY8 peaked at 10^5/mL on day 8 but declined to 10^3/mL by day 12. KBY7 and KBY8 both demonstrated rapid growth, reaching 10^7/mL by day 8 and sustaining this level through the end of incubation.

Conclusion: The presence of Columbia agar in BY7, BY8, KBY7, and KBY8 media significantly enhanced *L. tropica* promastigote proliferation. Due to its low cost, ease of preparation, and availability in routine laboratories, Columbia agar is proposed as a practical and effective alternative to the conventional Novy-McNeal-Nicolle medium for promastigote culture.

Keywords: *Leishmania tropica*, Columbia agar, Novy-McNeal-Nicolle, promastigote

ÖZ

Amaç: Bu çalışmada rutin mikrobiyoloji laboratuvarlarında bulunabilecek besiyerlerinin *Leishmania tropica* (*L. tropica* promastigotlarının üretilmesindeki etkinliğinin test edilmesi amaçlanmıştır.

Yöntemler: Bu çalışmada rutin mikrobiyoloji laboratuvarında kullanılan yeast extract agar, triptik soy broth, saboroud dextroz agar, brucella agar ve Columbia agar denenmiştir. Sekiz adet kansız (BY1-BY8), sekiz adet kanlı (KBY1-KBY8) besiyeri oluşturulmuştur. *L. tropica* promastigotları 105 promastigot/mL konsantrasyonda tüm besiyerlerine inoküle edilmiş ve 12 gün boyunca inkübe edilmiştir. Besiyerlerindeki promastigot sayısı günlük olarak sayılmıştır.

Bulgular: Novy-McNeal-Nicolle (NNN) besiyeri ile karşılaştırıldığında, *L. tropica* promastigotlarının üremesinde en iyi performansı BY7, BY8 ve KBY7 ve KBY8 besiyerleri göstermiştir. BY7 ve BY8 besiyerlerinde 1. gün promastigot sayısı 10^3 promastigot/mL iken 5. günde 10^4 promastigot/mL'ye ulaşmıştır. BY7 besiyerinde 8. günde 10^7 promastigot sayılmış ve 12. güne kadar bu şekilde devam etmiştir. BY8 besiyerinde ise 8. günde 10^5 promastigot sayılırken sonraki günlerde azalarak 12. günde 10^3 promastigot/mL'ye düşmüştür. KBY7 ve KBY8 besiyerlerinin her ikisinde de 1. günde promastigot sayısı 10^3 promastigot/mL iken 8. günde 10^7 promastigot/mL'ye çıkmış ve 12. güne kadar bu şekilde devam etmiştir.

Sonuç: *L. tropica*'nın üretilmesinde en iyi performansı içeriğinde Columbia agar bulunan BY7, BY8 ve KBY7, KBY8 besiyerleri göstermiştir. Columbia agar, ucuz, hazırlanması kolay ve rutin mikrobiyoloji laboratuvarlarında kolayca temin edilebilir olması nedeniyle bu tür uygulamalar için güçlü bir alternatif olarak öne çıkmaktadır. Bu nedenle, promastigotların kültüründe konvansiyonel NNN besiyerine pratik ve etkili bir alternatif olarak önerilebilir.

Anahtar Kelimeler: *Leishmania tropica*, Columbia agar, Novy-McNeal-Nicolle, promastigot

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Türkiye PARAZİTOLOJİ Dergisi

INTRODUCTION

Leishmaniasis is a vector-borne disease caused by protozoan parasites of the genus *Leishmania*, transmitted to humans through the bite of infected female phlebotomine sandflies. The World Health Organization estimates 700,000 to 1 million new cases annually, with 350 million people at risk (1). Classified as a neglected tropical disease (NTD), it disproportionately affects impoverished populations in developing countries and ranked second among all NTDs in 2017 in terms of disability-adjusted life years (DALYs), contributing to a global burden of 774,000 DALYs (2). The disease presents in three clinical forms: cutaneous leishmaniasis (CL), visceral leishmaniasis (VL), and mucocutaneous leishmaniasis, with CL being the most frequently encountered form (3).

A wide range of diagnostic methods is employed for leishmaniasis. Microscopy remains the gold standard, owing to its high specificity. However, its sensitivity can be limited by factors such as insufficient sample volume, low parasite burden, smear preparation errors, and lack of technical expertise among laboratory personnel. In the case of VL, bone marrow biopsies are commonly used, since splenic biopsies—though highly sensitive—pose a significant risk of severe hemorrhage (4,5). For CL, microscopic examination of parasites in skin lesions is a routine method, with reported sensitivity ranging between 42% and 70% (6).

Immunodiagnostic methods that detect *Leishmania* antigens or antibodies still play a fundamental role in diagnosis. Among antibody-based tests, immunochromatographic test (ICT) and enzyme-linked immunosorbent assay are widely used, employing antigens such as crude soluble antigen, recombinant K39 (rK39), and synthetic peptides in both commercial kits and in-house assays. The performance of these tests varies depending on the type and purity of the antigen, with rK39 ICT being a rapid and reliable method. However, the high cost of these tests and their limited applicability in all laboratories constitute a significant limitation in the diagnostic process (7,8).

Molecular methods, such as direct DNA extraction from clinical samples, are also widely employed to detect *Leishmania* infection and determine strain types. However, PCR-based sequencing techniques can struggle to differentiate between closely related species, particularly when parasite DNA levels in the sample are low (9,10). Recently, emerging technologies have started to complement traditional diagnostic methods. Promising innovations include nanoparticles, nanobiosensors, and novel point-of-care platforms such as breath analysis, portable PCR, nanotechnology-assisted rapid tests, next-generation sequencing, immunoinformatics, and artificial intelligence-based diagnostic systems (7).

Although clinical findings contribute to diagnosis, definitive identification of leishmaniasis relies on the direct detection of the parasite in clinical specimens and/or the cultivation of promastigotes using appropriate culture techniques. These methods are considered diagnostic gold standards, making culture media critical to the process. In 1904, Novy, McNeal, and Nicolle successfully cultured the promastigote form in a biphasic medium, which they named Novy-McNeal-Nicolle (NNN). This medium remains in widespread use today (11).

Culturing the parasite not only enhances diagnostic precision but also yields clinical isolates that facilitate advanced genetic studies-such as analyses of parasite evolution, lineage tracing, hybridization events, and identification of drug resistance genes. Expanding the collection of clinical isolates thus represents a valuable resource for *Leishmania* research.

This study aimed to achieve rapid and efficient cultivation of *Leishmania tropica (L. tropica)* promastigotes using culture media that are readily available in routine microbiology laboratories.

METHODS

Ethical Approval

No human or animal materials were used in this study. The experiments were conducted using parasite strains preserved in liquid nitrogen. Therefore, ethical approval is not required.

Parasite Isolate

For the evaluation of the modified media, the *L. tropica* strain MHOM/TR/2012/CBCL-LT, obtained from the Parasitology Bank of the Manisa Celal Bayar University Faculty of Medicine was used.

Preparation of NNN Medium

1.4 g of agar and 0.6 g of sodium chloride were dissolved in 90 mL of distilled water and sterilized in an autoclave (Hirayama HG-80, Japan) at 121 °C for 15 minutes. After cooling to 50-55 °C, 10 mL of defibrinated rabbit blood and 0.2 mL of penicillin/streptomycin solution were added to the medium. Then, 4 mL of the prepared medium was poured into sterile screw-cap tubes, which were placed at a 10° angle to allow solidification of the medium. The tubes were stored at +4 °C until use.

Preparation of Roswell Park Memorial Institute-1640 (RPMI-1640) Stock Liquid Medium

Commercially obtained RPMI-1640 was supplemented with 10% fetal calf serum (FCS), 1% penicillin/streptomycin, and 1% gentamicin to prepare the stock liquid medium. The medium was stored at +4 °C until use.

Preparation of Modified Culture Medium

To formulate the solid phase of the modified media, culture media commonly available in routine microbiology laboratories were used, including yeast extract agar (113116, Merck), tryptic soy broth (TSB) (100525, Merck), sabouraud dextrose agar (SDA) with 2% glucose (110413, Merck), brucella agar (110490, Merck), and columbia agar (100214, Merck). Each medium was prepared in slant tubes following the manufacturer's instructions.

A total of sixteen media formulations were created:

Eight blood-free modified media (BY1-BY8)

Eight blood-containing modified media (KBY1-KBY8)

Human erythrocyte suspension was added to the blood-containing media. For the liquid phase, either normal saline or RPMI-1640 was used. None of the media contained FCS. To prevent bacterial contamination, 1% penicillin/streptomycin and 1% gentamicin were included in all media formulations.

Inoculation into Modefied and NNN Medium

The strain retrieved from liquid nitrogen was thawed in a water bath at 37 °C (Memmert WBN-10, Germany) and inoculated into NNN medium for growth. The tubes were then incubated

at 26 °C (Miprolab MSI-120, Türkiye). Upon observation of promastigote proliferation, the culture was transferred to RPMI-1640 liquid medium for further analysis. To obtain a sufficient number of promastigotes, fresh RPMI-1640 medium was added to the culture flasks every 2-3 days. *L. tropica* promastigotes were cultured until the logarithmic growth phase was reached with invert misroscope (Olympus CKX-41, Japan). Subsequently, the parasites were adjusted to a concentration of 10^5 promastigotes/mL for inoculation into the test media. The modified culture media were incubated at 26 °C for 12 days.

Promastigotes in all tested media were counted daily using a Thoma hemocytometer under 40× magnification with a light microscope (Zeiss Primo Star, Germany). The results were compared with those obtained from the reference NNN medium. All experiments were repeated three times on independent days. All aseptic procedures included in the experimental design of the study were carried out in biosafety level II cabinets (Miprolab, Türkiye) while wearing personal protective equipment. The detailed compositions of the prepared media are provided in Table 1.

Statistical Analysis

All statistical analyses were performed using Python 3.10 with the pandas, numpy, scipy, and matplotlib libraries. The growth data of *L. tropica* promastigotes were collected over 12 consecutive days for each culture medium. The Shapiro-Wilk test was used to assess the normality of data distribution. As the data did not follow a normal distribution, non-parametric tests were employed. The Kruskal-Wallis test was initially used to assess overall differences among groups. For selected media (BY7, BY8, KBY7, and KBY8), pairwise comparisons with the standard NNN medium were conducted using the Wilcoxon signed-rank test. P-values less than 0.05 were considered statistically significant. Graphical representations of promastigote growth were plotted on a logarithmic scale, and the most successful media were visually emphasized using distinct colors and symbols.

RESULTS

No promastigote growth was observed in BY1, BY2, KBY1, and KBY2 media, and no viable promastigotes were detected in these media even as early as day 2. While no growth occurred in BY4 medium, in BY3 medium, 10^2 promastigotes/mL were counted on day 4, and 10^3 promastigotes/mL on day 5. After day 5, the promastigote count gradually declined, and by day 12, no live promastigotes were observed. In KBY3 and KBY4 media, the initial promastigote concentration of 10^2/mL on day 1 peaked at 10^5/mL on day 7, then gradually declined to 10^3/mL and 10^1/mL, respectively, by day 12. In BY5 medium, the initial count of 10^2 promastigotes/mL on day 1 increased to 10^5/mL by day 6 and remained stable at this level until day 12. In BY6 medium, the promastigote count increased from 10^2/mL on day 1 to 10^3/mL on day 2, then progressively declined, with no promastigotes detected by day 9. In KBY5 and KBY6 media, the promastigote count started at 10^3/mL on day 1, peaked at 10^7/mL and 10^5/mL, respectively, by day 8, and then declined to 10^4/mL (KBY5) and 10^2/mL (KBY6) by day 12.

In BY7 and BY8 media, the initial count of 10^3 promastigotes/mL on day 1 increased to 10^4/mL by day 5. In BY7, the count reached 10^7/mL by day 8 and remained stable until day 12 (p=0.801). In BY8, 10^5 promastigotes/mL were observed on day 8, decreasing to 10^3/mL by day 12 (p=0.798). In both KBY7 and KBY8 media, the promastigote count started at 10^3/mL on day 1, rose to 10^7/mL by day 8, and remained stable through day 12 (p=0.987 for both KBY7 and KBY8). In the control NNN medium, the initial promastigote concentration of 10^3/mL on day 1 increased to 10^7/mL by day 7 and then declined to 10^5/mL by day 12. Promastigote counts in blood-containing and blood-free media over time are presented in Table 2 and Table 3, and their graphical representations are shown in Figure 1 and Figure 2.

Statistical comparison was performed using the Wilcoxon paired-samples test. No statistically significant difference was observed in promastigote counts between the modified media and the NNN medium (BY7: p=0.139, BY8: p=0.110, KBY7: p=0.110, KBY8: p=0.110, false discovery rate-adjusted). These results suggest that both blood-free (BY7, BY8) and blood-containing (KBY7, KBY8) formulations exhibited a comparable level of promastigote growth to the standard NNN medium. Furthermore, BY7 and KBY7 demonstrated the highest yield, reaching 10^7 promastigotes/mL by day 8 and maintaining this level through day 12. Based on these findings, the modified culture media BY7-BY8 and KBY7-KBY8 can be proposed as reliable alternatives to the traditional NNN medium for the *in vitro* cultivation of *L. tropica* promastigotes.

DISCUSSION

In the diagnosis of leishmaniasis, confirmation can be achieved either by demonstrating the parasite through various staining methods or by culturing promastigote forms. Culturing promastigotes not only supports clinical diagnosis but also enables the acquisition of a large number of parasites for scientific

Table 1. Ingredients of the prepared culture media

Media	Solid phase	Liquid phase
BY1/KBY1	Yeast extract agar + sabouraud dextrose agar with 2% glucose + tryptic soy broth	RPMI-1640
BY2/KBY2	Yeast extract agar + sabouraud dextrose agar with 2% glucose + tryptic soy broth	Normal saline
BY3/KBY3	Sabouraud dextrose agar with 2% glucose	RPMI-1640
BY4/KBY4	Sabouraud dextrose Agar with 2% glucose	Normal saline
BY5/KBY5	Brucella agar	RPMI-1640
BY6/KBY6	Brucella agar	Normal saline
BY7/KBY7	Columbia agar	RPMI-1640
BY8/KBY8	Columbia agar	Normal saline

BY1-BY8: Blood-free medium, KBY1-KBY8: Blood-containing medium, RPMI-1640: Roswell Park Memorial Institute-1640

Table 2. Number of promastigotes grown in blood-free media by day (promastigotes/mL)

	BY1	BY2	BY3	BY4	BY5	BY6	BY7	BY8	NNN
Day 1	10^2	0	10^2	0	10^2	10^2	10^3	10^3	10^3
Day 2	10^1	0	10^2	0	10^3	10^3	10^3	10^3	10^3
Day 3	0	0	10^2	0	10^3	10^2	10^3	10^3	10^3
Day 4	0	0	10^2	0	10^4	10^2	10^4	10^4	10^4
Day 5	0	0	10^3	0	10^4	10^1	10^4	10^4	10^4
Day 6	0	0	10^2	0	10^5	10^1	10^5	10^4	10^5
Day 7	0	0	10^1	0	10^5	10^1	10^6	10^4	10^6
Day 8	0	0	10^1	0	10^5	10^1	10^7	10^5	10^7
Day 9	0	0	10^1	0	10^5	0	10^7	10^5	10^7
Day 10	0	0	10^1	0	10^5	0	10^7	10^4	10^7
Day 11	0	0	0	0	10^5	0	10^7	10^4	10^6
Day 12	0	0	0	0	10^5	0	10^7	10^3	10^5

BY: Blood-free medium, NNN: Novy-McNeal-Nicolle medium

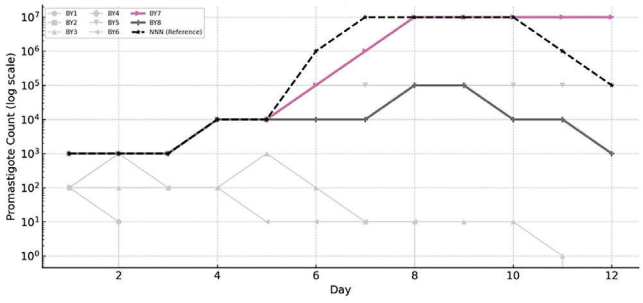

Figure 1. Growth curves of *Leishmania tropica* promastigotes in blood-free modified media over 12 days. Promastigote counts (promastigotes/mL) were monitored daily for each blood-free modified medium (BY1-BY8) and compared with the standard NNN medium. BY7 and BY8 demonstrated the highest proliferation, reaching 10^7 promastigotes/mL by day 8, comparable to NNN medium performance. No growth was observed in BY1, BY2, and BY4 throughout the incubation period. Data represent mean values of three independent experiments

BY1-BY8: Eight blood-free modified media, NNN: Novy-McNeal-Nicolle

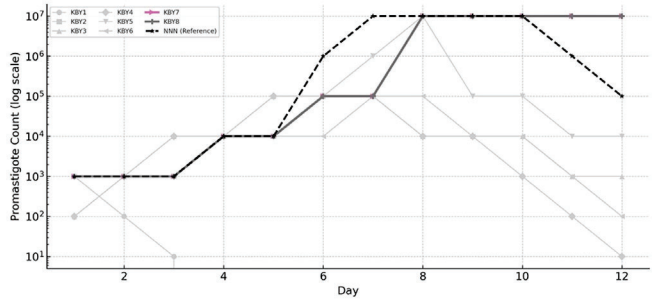

Figure 2. Growth curves of *Leishmania tropica* promastigotes in blood-containing modified media over 12 days. Promastigote counts (promastigotes/mL) were monitored daily for each blood-containing modified medium (KBY1-KBY8) and compared with the standard NNN medium. KBY7 and KBY8 media reached 10^7 promastigotes/mL on day 8 and maintained this level through day 12, demonstrating growth performance comparable to NNN. No growth was observed in KBY1 and KBY2 media throughout the incubation period. Data represent the mean values of three independent experiments

KBY1-KBY8: Eight blood-containing modified media, NNN: Novy-McNeal-Nicolle

studies. These cultured parasites are essential for applications such as animal model development, vaccine research, elucidation of disease pathogenesis, evaluation of immune responses, development of diagnostic kits, screening of drug candidates, and assessment of therapeutic efficacy.

Over the years, various culture media have been developed for cultivating *Leishmania* species, primarily categorized as biphasic (semi-solid) and monophasic (liquid) types. Commonly used media, including RPMI-1640, Medium 199, brain heart infusion, NNN, and Schneider's Drosophila medium, often require supplementation with fetal bovine serum (FBS) or blood to support long-term proliferation of *Leishmania* promastigotes (12,13). FBS contains crucial components such as hormones, vitamins, growth factors, and carrier proteins, all of which are vital for the maintenance and propagation of cultured parasites (14).

Among these, the NNN medium remains the most widely used and accepted for both diagnostic and research purposes. While it is relatively easy to prepare, its reliance on rabbit blood and costly FBS limits its accessibility in routine microbiology laboratories. Therefore, recent years have seen a growing interest in developing cost-effective, serum-free, and autoclavable alternative media (15,16).

The use of easily accessible and cost-efficient media in routine microbiology laboratories can significantly improve the detection of rare leishmaniasis cases, especially in non-endemic regions. In this context, various studies in Türkiye have investigated the potential of alternative biphasic and liquid culture media. In 1997, Limoncu et al. (17) compared a liquid P-Y medium supplemented with 10% FBS (containing peptone and yeast extract) with NNN and RPMI-1640+10% FBS media, reporting comparable results. Similarly, Ozbilgin et al. (18) observed promastigotes in nutrient

Table 3. Number of promastigotes grown in blood-containing media by day (promastigotes/mL)

	KBY1	KBY2	KBY3	KBY4	KBY5	KBY6	KBY7	KBY8	NNN
Day 1	10^3	0	10^2	10^2	10^3	10^3	10^3	10^3	10^3
Day 2	10^2	0	10^3	10^3	10^3	10^3	10^3	10^3	10^3
Day 3	10^1	0	10^4	10^4	10^3	10^3	10^4	10^4	10^3
Day 4	0	0	10^4	10^4	10^4	10^4	10^4	10^4	10^4
Day 5	0	0	10^5	10^5	10^4	10^4	10^5	10^5	10^4
Day 6	0	0	10^5	10^5	10^5	10^4	10^5	10^5	10^5
Day 7	0	0	10^5	10^5	10^6	10^5	10^6	10^6	10^6
Day 8	0	0	10^4	10^4	10^7	10^5	10^7	10^7	10^7
Day 9	0	0	10^4	10^4	10^5	10^4	10^7	10^7	10^7
Day 10	0	0	10^4	10^3	10^5	10^4	10^7	10^7	10^7
Day 11	0	0	10^3	10^2	10^4	10^3	10^7	10^7	10^6
Day 12	0	0	10^3	10^1	10^4	10^2	10^7	10^7	10^5

KBY: Blood-containing medium, NNN: Novy-McNeal-Nicolle medium

broth medium containing 20% FBS as early as day 1, whereas detection in NNN medium began on day 2. Yereli et al. (19) also successfully cultivated *L. infantum* and *L. tropica* promastigotes in nutrient broth enriched with 10% FBS, reaching 3×10^6 parasites/mL by day 5. However, the use of FBS in these media diminishes their cost advantage.

Internationally, several research groups have proposed alternative methods as well. Ali et al. (20) developed an egg-based biphasic medium free of FBS and blood, showing comparable proliferation to modified Tobie + FBS and Medium 199 + FBS. Kaddu and Nyamori (21) successfully cultured *L. donovani, L. major, L. adleri*, and a rodent *Leishmania* species in nutrient broth. Sadigursky and Brodskyn (22) reported similar promastigote proliferation in a serum-free liver infusion broth + tryptose (LIT) medium supplemented with 1% RPMI + Medium 199 (R9), compared to NNN, Warren medium, and LIT + 5% FBS. As also emphasized in a recent review, a major challenge in *Leishmania* culture remains the absence of a standardized, inexpensive, easy-to-prepare, and high-performance medium suitable for drug sensitivity testing (23).

In this study, the selected culture media are widely used in routine microbiology laboratories and serve different purposes in microbial cultivation. Yeast extract agar provides essential vitamins and nitrogen sources for the growth of various bacteria, yeasts, and molds. TSB supports the growth of both aerobic and anaerobic bacteria such as *Staphylococcus aureus*, *Escherichia coli* and *Bacteroides fragilis*. SDA, characterized by its high glucose content and slightly acidic pH, is ideal for the isolation of pathogenic fungi like *Candida albicans*. Brucella agar promotes the cultivation of fastidious and anaerobic microorganisms. Columbia agar, enriched with casein, heart, and meat peptones, provides a rich base for the growth of demanding bacteria such as *Streptococcus* and *Haemophilus* species (24).

In the our study, we evaluated the potential of routinely available microbiological media for the cultivation of *L. tropica* promastigotes. Compared to the NNN medium, the fastest and highest levels of proliferation were observed in Columbia agar-based BY7/BY8 and their blood-containing variants KBY7/KBY8. BY7 and BY8 media, which do not contain FBS or blood, achieved statistically comparable growth levels to NNN medium (p=0.139 for BY7, p=0.110 for BY8). This renders them advantageous in terms of cost and ease of preparation. Similarly, KBY7 and KBY8 media demonstrated comparable performance (p=0.110 for both). The use of human erythrocytes, which can be obtained from blood banks, instead of animal blood, adds practical utility. Additionally, promastigotes cultured in these media exhibited greater motility and typical fusiform morphology, suggesting preservation of their infective potential.

The enhanced growth observed in Columbia agar-based media (BY7, BY8, KBY7, KBY8) may be attributed to the balanced nutrient composition and favorable oxygen diffusion of the biphasic structure. Peptones, yeast extract, and maize starch provide essential amino acids, vitamins, and energy sources that support continuous promastigote metabolism, while the semi-solid matrix ensures optimal gas exchange similar to the sand fly midgut environment. In blood-containing variants, erythrocytes may further contribute to redox balance and iron availability, promoting sustained proliferation comparable to the NNN medium.

These culture media, which are easy and inexpensive to prepare and support high promastigote production, present a promising potential for anti-leishmanial drug screening when redesigned with viability-indicating colorimetric markers and supplemented with defined drug dilutions. Furthermore, the integration of such media into future automated analysis systems could facilitate the standardization of drug efficacy testing and enable the development of high-throughput screening platforms.

Study Limitations

The most notable limitation of this study is the lack of evaluation of parasite growth directly from clinical specimens in the high-performing media. Future studies are planned to assess the diagnostic potential of these media using both animal models and clinical samples.

CONCLUSION

Columbia agar (base) offers a highly supportive growth environment due to its rich composition of peptones derived

from casein, meat, and heart tissue, along with yeast extract and maize starch. Yeast extract serves as a vitamin source, while maize starch contributes as an energy source. These nutritional components likely contributed to the enhanced proliferation of *L. tropica* promastigotes observed in the BY7, BY8, KBY7, and KBY8 media. Columbia agar stands out as a strong alternative for such applications, as it is inexpensive, easy to prepare, and readily available in routine microbiology laboratories. Therefore, it can be recommended as a practical and effective substitute for conventional NNN medium in promastigote cultivation.

*Ethics

Ethics Committee Approval: No human or animal materials were used in this study. The experiments were conducted using parasite strains preserved in liquid nitrogen. Therefore, ethical approval is not required.

Informed Consent: No human or animal materials were used in this study.

Footnotes

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*Authorship Contributions

Concept: Y.Ö., Design: Y.Ö., İ.Ç., G.V.Ü., M.Ü., A.Ö., Data Collection or Processing: Y.Ö., İ.Ç., G.V.Ü., M.Ü., A.Ö., Analysis or Interpretation: Y.Ö., İ.Ç., G.V.Ü., M.Ü., A.Ö., Literature Search: Y.Ö., İ.Ç., G.V.Ü., M.Ü., A.Ö., Writing: Y.Ö., İ.Ç.

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