JMU
Jurnal Medika Udayana
ISSN: 2597-8012 JURNAL MEDIKA UDAYANA, VOL. 14 NO.01, JANUARI, 2025
Received: 2024-01-04 Revision: 2024-10-30 Accepted:01-12-2024

EVALUATION OF EFFECTIVENESS OF RPMI AND PBS CULTURE MEDIUM ON *PLASMODIUM BERGHEI* PARASITEMIA IN MICE

Firsta Angela Trikira[1], Dewa Ayu Agus Sri Laksemi[2], Ni Luh Putu Eka Diarthini[2], I Made Sudarmaja[2], Putu Indah Budi Apsari[3]

[1] Undergraduate Medical Study Program, Faculty of Medicine, Udayana University
[2] Department of Parasitology, Faculty of Medicine, Udayana University
[3] Department of Microbiology and Parasitology, Faculty of Medicine and Health Sciences, Warmadewa University
e-mail: srilaksemi@unud.ac.id

ABSTRACT

Malaria is an infection caused by *Plasmodium* and transmitted to humans through the bite of female *Anopheles* mosquitoes. Resistance of *Plasmodium* to antimalarial drugs complicates treatment, necessitating research for new malaria drugs. In vivo research requires culture medium. Two commonly used culture medium are Roswell Park Memorial Institute Medium (RPMI) and Phosphate Buffered Saline (PBS). The objective of this study is to determine the difference in parasitemia levels in mice infected with *Plasmodium* using RPMI and PBS, as well as the difference in propagation time between these media. This experimental in vivo study used 12 mice, divided into RPMI and PBS groups. Mice were infected with *P. berghei* at 1 x 10^7 intraperitoneally. Blood samples were taken from the tail on the third, fifth, and seventh days, prepared as smears, and observed under a microscope. The average parasitemia was analyzed using the Independent T-Test. The average degree of parasitemia for RPMI was 17.00% on the third day, 21.66% on the fifth day, and 28.68% on the seventh day. For PBS, it was 15.95% on the third day, 18.45% on the fifth day, and 22.97% on the seventh day. Independent T-Test results showed *p-value* (0,000) < 0,05 on seventh day, indicating significant difference in the effectiveness and growth rate of RPMI and PBS as culture media. The study concluded that RPMI is more effective as a culture medium for *P. berghei* than PBS.

Keywords : Roswell Park Memorial Institute Medium (RPMI)., Phosphate Buffered Saline (PBS)., *Plasmodium berghei*

INTRODUCTION

Malaria is an infectious disease caused by *Plasmodium* species, transmitted to humans through the bite of a female *Anopheles* mosquito. Currently, there are 5 types of *Plasmodium* that can infect humans: *Plasmodium ovale*, *P. falciparum*, *P. vivax*, *P. malariae*, and *P. knowlesi*[1]. Globally, 86 countries in subtropical and tropical regions remain at risk of malaria transmission, including the Eastern Mediterranean, Southeast Asia, the Western Pacific, sub-Saharan Africa, and the Americas[2]. In 2020, malaria infected 241 million people worldwide[3]. In Indonesia, reported malaria cases reached 418,546 people in 2023[4].

The management of malaria is complicated by the resistance of *Plasmodium* parasites to antimalarial drugs such as chloroquine and artemisinin. This growing resistance underscores the need for ongoing research to develop new antimalarial medications. For this type of research, it is essential to work with a suitable Plasmodium species, with *Plasmodium berghei* being a common choice. Additionally, conducting in vivo studies requires an appropriate culture medium to support the growth of *Plasmodium*.

Two widely utilized culture media for this purpose are Roswell Park Memorial Institute Medium (RPMI) and Phosphate Buffered Saline (PBS).Roswell Park Memorial Institute Medium (RPMI) is a culture medium containing adequate nutrients, including glutathione, vitamins, amino acids, glucose, and inorganic salts. Developed by George E. Moore, Robert E. Gerner, and H. Addison Frank, RPMI contains albumin, which provides lipids, transport proteins, and minerals[5]. The cost for 1 liter of RPMI-1640 medium is Rp1,143,460.00[6]. Phosphate Buffered Saline (PBS) is a buffer solution that maintains cell osmolarity and prevents cell lysis by maintaining a neutral pH. PBS is composed of sodium chloride, sodium phosphate, and potassium phosphate which maintain the balance of salt concentration around the cells which prevents osmosis[7]. The price offered per liter of PBS tablet medium is Rp200,000.00[8]. Given this context, further research is necessary to compare culture media in terms of cost-effectiveness and propagation ability. This study aims to assess two objectives: to determine the differences in parasitemia degree in mice infected with *Plasmodium* and propagated using RPMI versus PBS, as well to identify any variations in propagation time between RPMI and PBS

MATERIALS AND METHODS

This experimental in vivo study employed a randomized post-test-only comparison group design and was conducted from January to March 2024. The sample required for this study was 12 mice. The inclusion criteria for this sample were healthy male Balb/c mice aged 8-12 weeks weighing 25-30 grams. The exclusion criteria were mice that were lost or died. After meeting the inclusion criteria, the mice were divided into 2 groups: the RPMI and PBS group. This research was conducted at the Integrated Biomedical Laboratory, Faculty of Medicine, Udayana University. This study has obtained ethical clearance with the number 2762/UN14.2.2.VII.14/LT/2023 issued by the Research Ethics Commission Unit (KEP) of the Faculty of Medicine, Udayana University.

Preparation of Experimental Animals

Male mice were selected as experimental animals for research because they have similar biological characteristics and physiological traits to other mammals, easy to handle, considerable genetic variability, high reproductive rates, and short life cycles. Two donor mice were housed in a single cage, while 12 test mice were divided between two cages, with six mice per cage. The acclimatization period was carried out for 7-14 days under consistent environment and controlled conditions. Mice were kept in a room with a temperature of 22-25^{0}C with a 12-hour light-dark cycle. In addition, mice were given with standard feed and water *ad libitum*. Acclimatization aims to standardize feeding and allow mice to adapt and ensuring their suitability as research subjects[9,10].

Infection of Donor Mice with *Plasmodium berghei*

Plasmodium berghei stored in frozen stock at -20^{0}C was thawed by warming in hands, and used to infect 2 donor mice. When the parasitemia level of *Plasmodium berghei* in donor mice was >10%, 0.2 mL of blood was collected and stored in RPMI and PBS culture media. To determine the parasitemia of mice, blood samples were taken, a blood smear was made and observed under a microscope. Before blood was taken, mice were anesthetized with ketamine at a dose of 75 mg per kg body weight by intramuscular injection. After blood was taken, mice were euthanized by cervical dislocation. After blood collection, *Plasmodium berghei* samples were stored in RPMI and PBS in a refrigerator at -20^{0}C for 2 weeks.

Infection of test mice with *Plasmodium berghei*

Before being injected into test mice, the blood was resuspended with PBS and RPMI to achieve a *Plasmodium berghei* inoculum concentration of 1 x 10^7 for each mouse. Furthermore, 0.2 mL of inoculum is injected intraperitoneally into each test mouse. *Plasmodium berghei* parasitemia levels were observed from the third, fifth, and seventh day. Prior to blood collection, the mice are anesthetized with ketamine at a dose of 75 mg per kg body weight via intramuscular injection. After blood is taken, the mice are euthanized by cervical dislocation[9].

Blood Smear Preparation and Observation of Parasitemia Degree

Blood smears are prepared by obtaining a blood sample from the mouse's tail, and placing 1 drop of blood on the tip of the object glass. Another glass slide used to spread the blood into a thin smear. The second slide is then placed at a 45-60° angle and pushed to create a smear approximately 3-4 cm in length. After this stage, the dried blood smear is fixed with methanol for 2-3 minutes and stained with 10% Giemsa stain for 15 minutes. The next step is to wash the slide gently with running water and dry it. After drying, the blood smear can be observed under a microscope[9,11].

Parasite identification performed by first focusing the microscope lens at 100x magnification. After the lens is successfully focused, 1 drop of immersion oil is added, and the objective lens is switched to 100 x 10 magnification. The preparation is observed across 5 fields of view. After the observation is complete, the percentage of parasitemia is calculated using the following calculation formula:

$$\%\ \text{Parasitemia} = \frac{number\ of\ infected\ erythrocyte\ \times\ 100\%}{erythrocyte\ count}$$

Data Analysis

Data analysis was performed using the Statistical Package for the Social Science (SPSS) version 26. In this study, descriptive analysis, normality test, homogeneity test, and parametric Independent T-Test were carried out.

RESULT

Parasitemia Degree Observation Results

The graphic showed that on the third day after *Plasmodium berghei* infection, the RPMI group had a parasitemia degree of 17.00% while the PBS group showed 15.95%. On the fifth day after infection, the RPMI group had a parasitemia degree of 21.66% compared to the 18.45% in the PBS group. By the seventh day, the RPMI group displayed a parasitemia degree of 28.68%, while the PBS group had 22.97%.

Figure 1. Graphic of observations of parasitemia levels

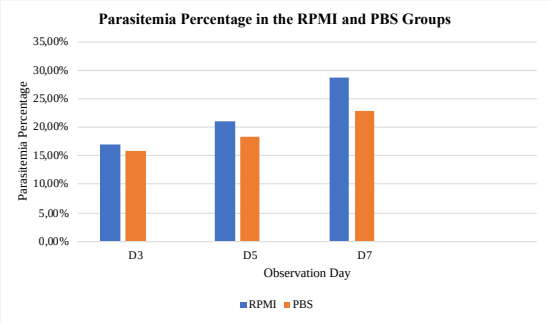

Table 1. Descriptive Analysis of Average Parasitemia Degree

Observation Day	% Parasitemia ± SD
The third day	16,38 ± 1,70
The fifth day	19,77 ± 2,13
The seventh day	25,82 ± 3,35

From the table above, it can be seen that the highest average parasitemia degree occurred on the seventh day of parasitemia observation, which was 25.82%, while the lowest average parasitemia degree occurred on the third day of parasitemia observation, which was 16.38%.

The image below shows the results of parasitemia degree observations in the RPMI and PBS groups taken on the third, fifth, and seventh days. It can be seen that the infected red blood cells have Schuffner dot. The arrows in the image indicate red blood cells infected with *Plasmodium*.

Figure 2. Infected red blood cell

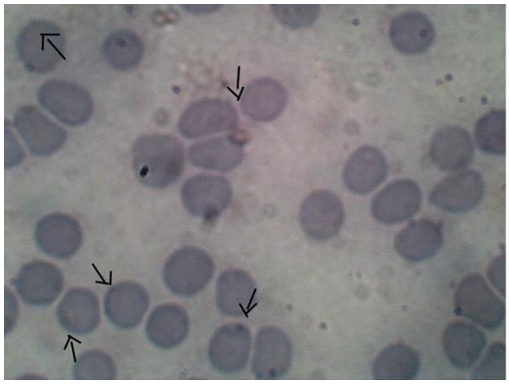

Figure 3. Erythrocytes infected with *Plasmodium berghei* (PBS) on day 3.

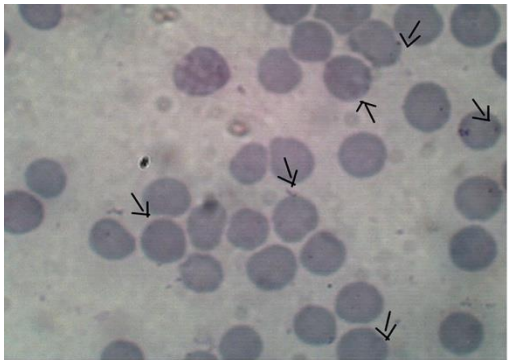

Figure 4. Erythrocytes infected with *Plasmodium berghei* (PBS) on day 5.

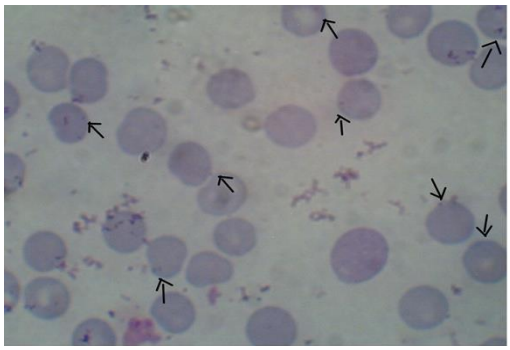

Figure 5. Erythrocytes infected with *Plasmodium berghei* (PBS) on day 7.

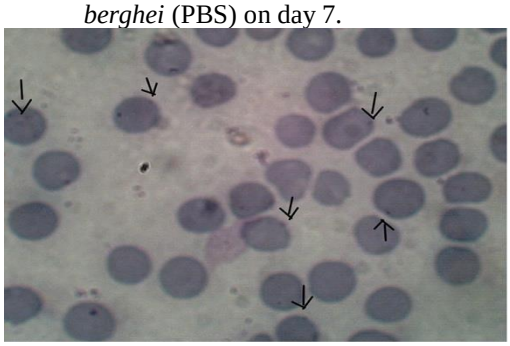

Figure 6. Erythrocytes infected with *Plasmodium berghei* (RPMI) on day 3.

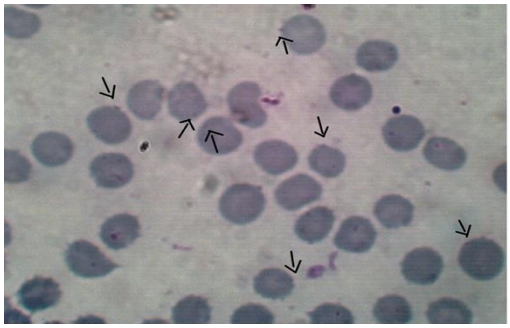

Figure 7. Erythrocytes infected with *Plasmodium berghei* (RPMI) on day 5.

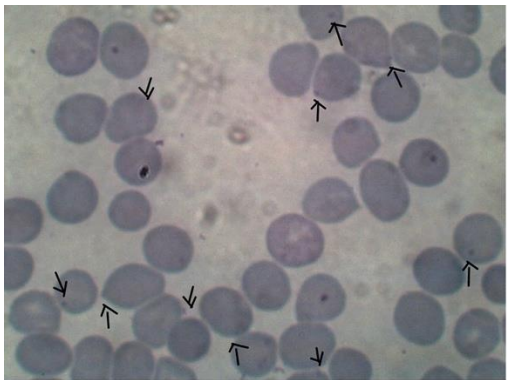

Figure 8. Erythrocytes infected with *Plasmodium berghei* (RPMI) on day 7.

Normality Test

The Shapiro-Wilk test was employed due to the sample size being less than 50, specifically 12 mice. Normality of the data was concluded if the p-value was greater than 0.05[12]. The results of the Shapiro-Wilk test showed a p-value > 0.05, indicating that the degree of parasitemia in both groups follows a normal distribution.

Table 3. Normality test

Day	Culture Medium	Statistics	P value
Day 3	RPMI	0,915	0,472
	PBS	0,971	0,900
Day 5	RPMI	0,919	0,119
	PBS	0,934	0,427
Day 7	RPMI	0,875	0,245
	PBS	0,916	0,475

Homogenity Test

The method used in this study is the Levene test, the variance can be concluded to be homogeneous if the p value>0.05[12]. Based on the results of the Levene test, the p-value > 0.05, therefore it can be concluded that the degree of parasitemia in both groups is distributed homogeneously.

Table 4. Results of homogeneity test with Levene's test

Observation day	Sample (n)	Levene (Sig.)
The third day	6	0,760
The fifth day	6	0,092
The seventh day	6	0,871

Independent T-test

Based on the results of the Independent T-test, on the third day of observation p-value > 0.05 [12]. It can be concluded that there is no significant difference. On the fifth and seventh day of observation p-value < 0.05, so it can be concluded that there is a significant difference in the effectiveness and growth rate of RPMI and PBS as culture media.

Tabel 5. Results of *independent t test*

Observation day	Sig.
Day 3	0,224
Day 5	0,022
Day 7	0,000

DISCUSSIONS

The results of this study indicate that *Plasmodium berghei* can grow in both culture media, spesifically RPMI and PBS. In addition, there is also a difference in effectiveness between RPMI and PBS as a culture medium for *Plasmodium berghei* infected in male mice of the Balb/c strain. Observations on the fifth and seventh days proved that there was a significant difference.

In the process of cell growth in culture media, sufficient nutrition is needed to meet the needs of cell life. Both culture media, RPMI and PBS, both have KCl and NaCl as good buffer components to maintain physiological pH in a 5–10% CO_2 environment[13].

Roswell Park Memorial Institute Medium (RPMI) as a frequently used culture medium has a composition such as glucose, amino acids, vitamins, and salts[13]. Glucose is a source of energy in culture media for cell metabolism[14]. Meanwhile, amino acids are needed as basic materials for the protein synthesis process in cells[15]. Vitamins are useful as cofactors for various enzymes that are essential for their function. The absence of vitamins in culture can cause reduced cell growth, cell death, and loss of cell productivity[16]. Inorganic salts found in culture media function as nutrition, help maintain the balance of cell culture media by providing structural integrity to cells, and as ion transporters in the plasma membrane[17].

The results showed that the growth rate of *Plasmodium berghei* in RPMI was higher than pbs. This happened because the media has a high glutamine content. L-glutamine is an essential amino acid that functions as an energy source in cell metabolism. The energy utilization process begins by converting glutamine to glutamate in the mitochondria. Then, glutamate together with alanine and pyruvic acid is converted into alpha-ketoglutarate, which plays a role in the tricarboxylic acid (TCA) cycle to produce ATP. ATP is the main source of energy in cell life, and contributes to the formation of amino acids and nucleotides[18].

In addition to L-glutamine, RPMI also contains glutathione in its media. Glutathione is a water-soluble tripeptide that has active thiols that are essential for cell growth and viability. Glutathione prevent cells from oxidative stress and maintain a good redox environment for the cell. It provides reduction for the regeneration of sulfhydryl compounds and antioxidants, which convert peroxides to alcohols or water. Glutathione would convert

organic hydroperoxides to water and organic alcohols and hydrogen peroxide to water, so it would detoxify the intra- and extracellular environment. Ascorbate is a valuable vitamin because its role as antioxidant, cell adhesion, copper mobilization, and amino acid catabolism. Glutathione role is to regenerates ascorbate[19].

PBS is less effective than RPMI for several reasons. First, some PBS formulations do not contain calcium, which is essential for a variety of cellular functions, such as promoting cell adhesion and signaling, and helping to maintain cell integrity and stability[20]. Second, some PBS formulas do not contain magnesium, which is essential for the activation of enzymes related to DNA replication, RNA transcription, and protein synthesis[21]. Third, PBS has phosphate which can bind calcium and can interfere with calcium-dependent processes in cells[22]. PBS also lacks the nutrients and growth factors that RPMI has, making it less than ideal for cell growth culture media.

CONCLUSIONS AND SUGGESTIONS

Based on the results of the research, the following conclusions can be drawn, namely that there is a significant difference on the degree of parasitemia in mice infected with *Plasmodium berghei* using RPMI and PBS, with RPMI being the more effective culture medium. RPMI and PBS have no significant difference in the degree of parasitemia on the third day after infection. However, on the fifth and seventh days there is a significant difference in the degree of parasitemia. Additionally, there is a difference in the duration of propagation between RPMI and PBS.

Based on the conclusions of this study, there are several suggestions that can be given. First, further research should use RPMI media because it can provide a more optimal development of the degree of *Plasmodium berghei* parasitemia. Second, long-term observations can be carried out to see whether this significant difference continues or changes over time. This can provide further insight into the dynamics of infection and the effectiveness of the culture media used. Third, it can be considered to conduct a comparative study with other culture media that may be more effective or have a different composition.

REFERENCES

1. Buck E, Finnigan NA. Malaria. StatPearls [Internet]. 2021 Aug 11 [cited 2022 Feb 28]; Available from: https://www.ncbi.nlm.nih.gov/books/NBK551711/
2. Varo R, Chaccour C, Bassat Q. Update on malaria. Med Clin (Barc). 2020 Nov 13;155(9):395–402.
3. WHO. Malaria [Internet]. 2022 [cited 2022 Feb 22]. Available from: https://www.who.int/news-room/fact-sheets/detail/malaria
4. Kementerian Kesehatan Republik Indonesia. Cegah dan Kendalikan Malaria [Internet]. Jakarta; 2024 May [cited 2024 Aug 23]. Available from: Cegah dan Kendalikan Malaria
5. Rani U, Singh M, Saith A, Jain S, Aggarwal A, Aggarwal S. Evaluation of Use of RPMI Medium to Preserve Cell Morphology for Pleural/Peritoneal Fluid Cytology. J Cytol [Internet]. 2022 Jan 1 [cited 2024 Nov 11];39(1):26. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC8955699/
6. Sigmaaldrich. RPMI-1640 Medium [Internet]. 2022 [cited 2022 Apr 5]. Available from: https://www.sigmaaldrich.com/ID/en/product/sigma/r8758?gclid=CjwKCAjw0a-SBhBkEiwApljU0kN3AHQq6_P3w8GMjoXurjfkF4ml6jZelYax5-QfO-cRejbexofGDhoCKqsQAvD_BwE
7. Sigmaaldrich. Phosphate Buffered saline (PBS) [Internet]. 2024 [cited 2024 Nov 11]. Available from: https://www.sigmaaldrich.com/ID/en/products/chemistry-and-biochemicals/biochemicals/biological-buffers/phosphate-buffer-saline-pbs
8. Sigmaaldrich. Phosphate Buffered Saline [Internet]. 2022 [cited 2022 Apr 5]. Available from: https://www.sigmaaldrich.com/ID/en/product/sigma/p7059
9. Laksemi DAAS, Arijana IGK, Sudarmaja IM, Ariwati NL, Tunas K, Damayanti PAA, et al. Ethanol Extract of Spondias pinnata Leaves Reduce Parasite Number and Increase Macrophage Phagocytosis Capacity of Mice Infected by Plasmodium berghei. Indones Biomed J. 2021;13(1):40–7.
10. Mutiarahmi CN, Hartady T, Lesmana R. Use Of Mice As Experimental Animals In Laboratories That Refer To The Principles Of Animal Welfare: A Literature Review. Indones Med Veterinus. 2021 Jan 31;10(1):134–45.
11. Devi DNMKMS, Laksemi DAAS, Diarthini NLPE, Sudarmaja IM. View of The Effect of Pandan Wangi Roots Ethanol Extract (Pandanus amaryllifolius Roxb.) on the Degree of Plasmodium Berghei Parasitemia In Vivo. J Med Udayana [Internet]. 2024 [cited 2024 Nov 13];13. Available from: https://ojs.unud.ac.id/index.php/eum/article/view/110819/54201
12. Nuryadi, Astuti TD, Utami ES, Budiantara M. Dasar-Dasar Statistik Penelitian [Internet]. Vol. 1. Yogyakarta: Sibuku Media; 2017. Available from: www.sibuku.com
13. Thermo Fisher. Roswell Park Memorial Institute (RPMI) 1640 Medium [Internet]. 2024 [cited 2024 Jul 22]. Available from: https://www.thermofisher.com/id/en/home/life-science/cell-culture/mammalian-cell-culture/cell-culture-media/rpmi.html

14. Scientific Bioprocessing. Understanding the Role of Glucose in Cell Culture Media [Internet]. 2024 [cited 2024 Jul 22]. Available from: https://www.scientificbio.com/blog/understanding-the-role-of-glucose-in-cell-culture-media/
15. Salazar A, Keusgen M, Von Hagen J. Amino acids in the cultivation of mammalian cells. Amino Acids [Internet]. 2016 May 1 [cited 2024 Jul 22];48(5):1161. Available from: /pmc/articles/PMC4833841/
16. Schnellbaecher A, Binder D, Bellmaine S, Zimmer A. Vitamins in cell culture media: Stability and stabilization strategies. Biotechnol Bioeng [Internet]. 2019 Jun 1 [cited 2024 Nov 13];116(6):1537. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC6594077/
17. Yao T, Asayama Y. Animal- cell culture media: History, characteristics, and current issues. Reprod Med Biol [Internet]. 2017 Apr 1 [cited 2024 Jul 22];16(2):99. Available from: /pmc/articles/PMC5661806/
18. Mirveis Z, Howe O, Cahill P, Patil N, Byrne HJ. Monitoring and modelling the glutamine metabolic pathway: a review and future perspectives. Metabolomics 2023 198 [Internet]. 2023 Jul 23 [cited 2024 Nov 13];19(8):1–24. Available from: https://link.springer.com/article/10.1007/s11306-023-02031-9
19. Sigmaaldrich. Glutathione in Cell Culture [Internet]. 2024 [cited 2024 Jul 22]. Available from: https://www.sigmaaldrich.com/ID/en/technical-documents/technical-article/cell-culture-and-cell-culture-analysis/mammalian-cell-culture/glutathione
20. Conrad DR. Calcium in Cell Culture [Internet]. 2024 [cited 2024 Sep 20]. Available from: https://www.sigmaaldrich.com/ID/en/technical-documents/technical-article/cell-culture-and-cell-culture-analysis/mammalian-cell-culture/calcium-in-cell-culture
21. Waters L. Cell Culture Medium: 6 Critical Components to Include [Internet]. 2022 [cited 2024 Sep 20]. Available from: https://bitesizebio.com/37034/cell-culture-medium/
22. Guelph HPS from U of. Calcium and Phosphate Regulation. 2024.