Bioscientist: Jurnal Ilmiah Biologi
https://e-journal.undikma.ac.id/index.php/bioscientist

March 2026 Vol. 14, No. 1
e-ISSN: 2654-4571
pp. 165-174

Effect of Variations in Blood Component Supplementation on the Suitability of Blood Agar Plate Media for the Growth of *Salmonella typhi*

[1*]Fransisca Probo Setyoningrum
[1]Department of Applied Medical Laboratory Technology, Faculty of Health Sciences, Universitas Pelita Harapan, Tangerang, Indonesia.
*Corresponding Author e-mail: fransisca.setyoningrum@uph.edu
Received: January 2026; Revised: February 2026; Accepted: March 2026; Published: March 2026

Abstract: This study aimed to evaluate the effect of different human blood components added to Blood Agar Plate (BAP) media on the growth of *Salmonella typhi* O. An experimental design was employed with three treatment groups and a total of 27 replicates. *Salmonella typhi* O was inoculated onto BAP media containing different blood components, followed by incubation at 37°C for 24 hours. Bacterial colonies were then counted using the total plate count method. Statistical analysis was performed using the Shapiro–Wilk test and one-way ANOVA, followed by Bonferroni post hoc analysis. The results showed that variation in blood components significantly affected colony counts ($p = 0.0001$). Whole blood containing antibodies produced the lowest bacterial growth (mean 2.6×10^5 CFU/mL), whereas erythrocyte suspension (1.8×10^6 CFU/mL) and whole blood without antibodies (1.9×10^6 CFU/mL) showed substantially higher growth and did not differ significantly from each other ($p = 0.618$). These findings indicate that antibodies in human blood can inhibit the growth of *Salmonella typhi* through complement activation, while washed erythrocytes are effective as a substitute for sheep blood. Therefore, whole blood without antibodies or erythrocyte suspension may serve as viable and effective alternatives for the preparation of BAP media in laboratories with limited access to sheep blood.
Keywords: *Salmonella typhi* antibodies; blood agar plate; total plate count; complement system

How to Cite: Setyoningrum, F. P. (2026). Effect of Variations in Blood Component Supplementation on the Suitability of Blood Agar Plate Media for the Growth of Salmonella typhi. *Bioscientist: Jurnal Ilmiah Biologi*, *14*(1), 165–174. https://doi.org/10.33394/bioscientist.v14i1.19748

https://doi.org/10.33394/bioscientist.v14i1.19748

Copyright© 2026, Setyoningrum et al
This is an open-access article under the CC-BY-SA License.

INTRODUCTION

Blood Agar Plate (BAP), or blood agar medium, is a universal as well as differential bacterial growth medium that plays a crucial role in the identification of various pathogenic bacteria. Globally, BAP serves as a standard medium for cultivating bacteria such as *Salmonella typhi*, which require specific nutrients derived from blood proteins (Verma & Shahab, 2022; Imara, 2020).

In North America and Europe, sheep blood or horse blood has become the gold standard because of its ability to produce maximal and consistent hemolytic reactions. However, in tropical countries such as Indonesia, maintaining standard blood-producing wool sheep presents major environmental adaptation challenges, resulting in limited availability and relatively high costs for both clinical and research laboratories.

Consequently, many laboratories in Indonesia have shifted to the use of human blood as a substitute because of its easier accessibility. Nevertheless, although human blood is rich in nutrients such as hemoglobin and plasma proteins, its use often produces differences in colony morphology, color, and hemolysis patterns compared with standard sheep blood media. Therefore, a thorough analysis of the optimization of human blood components is needed to ensure the accuracy of laboratory results (Turista and Puspitasari 2019; Rahma Velina et al, 2016; Sanjaya et al, 2022; Rigby et al, 2022).

The main problem associated with the use of human blood as a bacterial growth medium lies in the presence of specific antibodies formed as a result of previous infections, such as antibodies against *Salmonella typhi*, which can inhibit the growth of compatible antigens through their bacteriolytic activity via the complement pathway. In addition, differences in erythrocyte morphology, size, and membrane permeability between human and sheep blood may interfere with clinical interpretation.

The urgency of this study is supported by findings showing that antibodies in whole blood are significant inhibitors of media fertility. From a broader perspective, these findings indicate that the direct use of human blood is not recommended because of the risk of transmissible disease transmission and the potential failure of certain pathogenic bacteria to grow. International studies have also emphasized the need to modify human blood so that it more closely resembles sheep erythrocytes, thereby improving laboratory effectiveness in developing countries (Cardigan et al, 2020; Yougbare et al, 2020; Xie et al, 2020).

Therefore, comparing the effectiveness of different human blood components is urgently needed to identify practical solutions to the financial and logistical constraints associated with sheep blood procurement in local laboratories. This gap indicates that, although human blood is commonly used, the presence of heat-stable compounds such as antibodies and heat-labile compounds such as complement in plasma remains a major challenge that has not yet been fully resolved at the practical laboratory level (Pont et al. 2020; Bayly-Jones et al, 2017; Serna et al. 2016).

This problem requires an immediate solution through systematic technical problem-solving strategies. The novelty of the proposed method lies in optimizing blood-based BAP through the use of erythrocyte suspensions prepared by washing with 0.85% saline solution three times. Theoretically, this approach is capable of removing more than 98% of plasma proteins, antibodies, and complement components without damaging essential nutritional substrates such as hemoglobin within red blood cells (Ho et al, 2025; Miguel & Pereira, 2024; Barnum et al, 2020).

This washing method is expected to increase the proportion of erythrocytes with characteristics more favorable for bacterial growth, considering that packed red cells contain higher concentrations of hemoglobin and hematocrit than whole blood. This intervention is therefore critically important to ensure that BAP prepared from human blood no longer exerts immunological inhibitory effects, thereby allowing optimal growth of *Salmonella typhi* comparable to that achieved on standard international media.

The aim of this study was to comprehensively evaluate the effect of variations in human blood components on the fertility of BAP media, particularly for the growth of *Salmonella typhi* O. The principal contribution of this article lies in providing a scientific basis for the development of medical laboratory science and technology, offering an alternative pathway for laboratories facing logistical difficulties in obtaining sheep blood.

The variables investigated were clearly defined: the independent variable was blood type—whole blood containing antibodies, erythrocyte suspension, and whole blood without antibodies—while the dependent variable was the number of bacterial colonies measured in CFU/mL using the Total Plate Count (TPC) method. Based on relevant references, this study offers both theoretical insight into the effect of human antibodies on media fertility and practical value as a potential operational standard for culture media modification in healthcare facilities. Therefore, it is expected to support national laboratory technological self-reliance through the scientific and measurable optimization of locally available resources.

METHOD

This study employed an experimental research method and design to analyze the effect of variations in blood component supplementation on the fertility of Blood Agar Plate (BAP) media. The experimental design consisted of three main treatment groups: whole blood containing *Salmonella typhi* O antibodies, erythrocyte suspension, and whole blood without antibodies.

The study procedures were organized systematically, beginning with confirmation of the whole blood to be used through Widal and TUBEX testing, followed by preparation of Nutrient Agar (NA) as the base medium, collection and processing of human blood samples, and inoculation of *Salmonella typhi* O. All procedures were conducted under tightly controlled conditions, including an incubation temperature of 37°C, an incubation period of 24 h, and an 8% blood supplementation volume to maintain nutritional consistency.

The experimental workflow included rejuvenation of the pure bacterial strain in a universal medium, preparation of a bacterial suspension at a standardized concentration, colony counting, and data analysis. This design ensured that any differences observed in the final results were attributable to the variations in the blood components tested. The samples and research subjects in this study consisted of human blood selected according to specific criteria to meet the experimental requirements. The study population comprised all human blood samples; however, subjects were selected using a non-probability sampling method, specifically purposive sampling, based on strict inclusion and exclusion criteria.

The minimum sample size, or number of replicates, was calculated using the Federer formula. With three blood treatment groups, the calculation indicated a minimum of nine replicates per group, resulting in a total of 27 replicates. The inclusion criteria specified individuals with previous *Salmonella typhi* O infection, as demonstrated by a positive Widal test result. In contrast, the exclusion criteria included individuals who were actively suffering from typhoid fever at the time of blood collection, in order to avoid interference from an existing bacterial load in the bloodstream. Blood sampling was performed by trained personnel with phlebotomy expertise to minimize the risk of hematoma formation and pain in respondents.

The research instruments and procedures involved the use of standard laboratory equipment, including an autoclave for sterilization, an incubator for bacterial growth, and micropipettes and vacuum tubes to ensure volume accuracy. The validity of bacterial measurement was based on the Total Plate Count (TPC) method, an international standard in microbiology, as well as the use of the 0.5 McFarland standard to standardize the concentration of the bacterial suspension and obtain a uniform inoculum density.

The main procedure began with qualitative Widal testing to confirm the presence of antibodies in the respondents. The next crucial step was the preparation of an erythrocyte suspension through a red blood cell washing method using 0.85% NaCl solution three times. This washing procedure was intended to remove more than 98% of plasma proteins and antibodies that could inhibit bacterial growth without damaging hemoglobin, which serves as the principal nutrient source.

After the BAP media had been prepared with the respective blood variations, the bacteria were inoculated using a serial dilution technique and evenly spread with a hockey stick spreader. The procedure concluded with counting the colonies that grew on Petri dishes containing 30–300 colonies to ensure the reliability of the colony count data.

Data were analyzed quantitatively using SPSS version 21 to interpret the results in accordance with the predefined study objectives. The first analytical step was the Shapiro–Wilk normality test to verify whether the bacterial colony count data were normally distributed, given that the sample size was fewer than 50. Data were considered normally distributed when the significance value met the established criterion.

Once normality had been confirmed, the analysis proceeded with the parametric one-way ANOVA test to compare the mean bacterial counts among the three blood component variation groups. If a significant difference was identified, further post hoc analysis was performed using the Bonferroni test (pairwise comparisons) to determine which groups differed significantly in supporting bacterial fertility. This analytical approach enabled the researchers to scientifically test the hypothesis regarding the effect of human antibodies on the effectiveness of BAP media. The results of the data analysis were then interpreted in relation to the theoretical role of the complement system and IgG in bacterial lysis, thereby providing a meaningful contribution to the standardization of modified culture media in clinical laboratories.

RESULTS AND DISCUSSION

Sheep blood is the gold standard used for the preparation of Blood Agar Plate (BAP) culture media in Europe and America. The sheep blood used is derived from wool sheep. This type of sheep is not readily available in Indonesia because wool sheep are difficult to breed and are unable to adapt well to tropical climates such as that of Indonesia (Krihariyani et al. 2016). For developing countries such as Indonesia, importing wool sheep blood is also costly and associated with numerous logistical constraints (Sumilih, 2012). As a result, various studies have emerged to explore substitutes for sheep blood, one of which is the use of human blood because it can be obtained more easily.

Human blood contains antibodies. These antibodies are formed when an individual has previously been exposed to an antigen. Antibodies against *Salmonella typhi* are present in the blood of individuals who have been infected with *Salmonella typhi* (Setyoningrum et al. 2020; Dyson et al. 2024; Buzilă et al. 2025). In this study, the effect of antibodies present in human blood on antigens derived from *Salmonella typhi* was tested *in vitro*. This was performed by comparing variations in blood component supplementation during the preparation of Blood Agar Plate media, after which media fertility was assessed based on the number of bacteria growing on the Blood Agar Plate.

The blood variations used were as follows:

1. Whole blood containing antibodies against *Salmonella typhi*.
2. Blood containing antibodies against *Salmonella typhi* that was washed three times with sterile 0.85% NaCl solution to remove antibodies, thereby producing an erythrocyte suspension.
3. Whole blood that did not contain antibodies against *Salmonella typhi*.

The *Salmonella typhi* antigen was obtained from a pure strain of *Salmonella typhi* ATCC® 27870™. The strain was subcultured one day before inoculation to ensure that the bacteria were in optimal condition for transfer to another medium. If bacteria enter the stationary phase, waste products accumulate, nutrients become depleted, pH changes occur, and other factors may suppress and disrupt the culture, thereby reducing the bacterial growth rate (Sumilih, 2012).

Colonies obtained after subculture were then suspended in sterile 0.85% NaCl and adjusted to the 0.5 McFarland standard, followed by dilution to 10^{-4} and 10^{-5}. A volume of 100 µL was inoculated onto Blood Agar Plate media containing the three blood-component variations, and the plates were incubated for 24 h at 37°C. The numbers of bacteria growing on BAP with each blood variation are presented in Table 1. Meanwhile, the results of the descriptive analysis are presented in Table 2.

Table 1. Number of bacteria growing on BAP with each blood variation (CFU/mL)

Replication	BAP Containing Antibodies	BAP with Erythrocyte Suspension	BAP Without Antibodies
1	$2{,}4 \cdot 10^5$	$1{,}2 \cdot 10^6$	$1{,}9 \cdot 10^6$
2	$2{,}8 \cdot 10^5$	$1{,}5 \cdot 10^6$	$2{,}0 \cdot 10^6$
3	$2{,}9 \cdot 10^5$	$1{,}7 \cdot 10^6$	$2{,}2 \cdot 10^6$
4	$2{,}8 \cdot 10^5$	$1{,}8 \cdot 10^6$	$1{,}9 \cdot 10^6$
5	$2{,}3 \cdot 10^5$	$1{,}8 \cdot 10^6$	$1{,}9 \cdot 10^6$
6	$2{,}9 \cdot 10^5$	$2{,}0 \cdot 10^6$	$1{,}9 \cdot 10^6$
7	$2{,}6 \cdot 10^5$	$2{,}0 \cdot 10^6$	$1{,}7 \cdot 10^6$
8	$2{,}6 \cdot 10^5$	$2{,}2 \cdot 10^6$	$1{,}7 \cdot 10^6$
9	$2{,}9 \cdot 10^5$	$2{,}0 \cdot 10^6$	$1{,}9 \cdot 10^6$
10	$2{,}1 \cdot 10^5$	$1{,}9 \cdot 10^6$	$2{,}1 \cdot 10^6$
Rerata	$\mathbf{2{,}6 \cdot 10^5}$	$\mathbf{1{,}8 \cdot 10^6}$	$\mathbf{1{,}9 \cdot 10^6}$

Table 2. Results of descriptive analysis

Variation	Mean	Median	Standard Deviation	Maximum	Minimum
A	$2{,}6 \cdot 10^5$	$2{,}7 \cdot 10^5$	0,28	$2{,}9 \cdot 10^5$	$2{,}1 \cdot 10^5$
B	$1{,}8 \cdot 10^6$	$1{,}9 \cdot 10^6$	2,88	$2{,}2 \cdot 10^6$	$1{,}2 \cdot 10^6$
C	$1{,}9 \cdot 10^6$	$1{,}9 \cdot 10^6$	1,54	$2{,}2 \cdot 10^6$	$1{,}7 \cdot 10^6$

Note:
A: BAP with whole blood containing antibodies against *Salmonella typhi*
B: BAP with erythrocyte suspension
C: BAP with whole blood without antibodies against *Salmonella typhi*

The research data showed that, in BAP containing antibodies, the highest bacterial count was $2{,}9 \cdot 10^{-5}$, the lowest was $2{,}1 \cdot 10^{-5}$, and the mean bacterial count was $2{,}6 \cdot 10^{-5}$ CFU/mL. In BAP with erythrocyte suspension, the highest bacterial count was $2{,}2 \cdot 10^{-6}$, the lowest was $1{,}2 \cdot 10^{-6}$, and the mean bacterial count was $1{,}8 \cdot 10^{-6}$ CFU/mL. In BAP without antibodies, the highest bacterial count was 2.2 × 10^6, the lowest was $1{,}7 \cdot 10^{-6}$, and the mean bacterial count was $1{,}9 \cdot 10^{-6}$ CFU/mL. Based on these data, the highest bacterial count was observed in BAP without antibodies when compared with BAP containing erythrocyte suspension and BAP containing antibodies, as reflected by the mean values of the three blood-component variations.

The analysis of the effect of blood-component variation on the fertility of Blood Agar Plate for the growth of *Salmonella typhi* was initiated with a normality test using the Shapiro–Wilk test, which is appropriate for sample sizes below 50. The results are presented in Table 3.

Table 3. Evaluation of data normality distribution

Blood Variation	N	Sig.	Interpretation
A	10	0,127	Normally Distributed
B	10	0,375	Normally Distributed
C	10	0,197	Normally Distributed

Based on the normality test results in Table 3 using the Shapiro–Wilk test, the data are considered normally distributed when the significance value is p > 0,05. The significance values obtained were 0,127 for BAP with whole blood containing antibodies against *Salmonella typhi* O, 0,375 for BAP with erythrocyte suspension, and 0,197 for BAP with whole blood without antibodies against *Salmonella typhi* O. These findings indicate that the numbers of bacteria growing on BAP with the different blood-component variations were normally distributed. Therefore, the analysis was continued using the parametric One-Way ANOVA test. One-Way ANOVA was selected because the study involved more than two independent groups.

The analysis of statistical significance for the differences observed among the variables is presented in Table 4, which provides a detailed overview of the extent to which these variables differ from one another.

Table 4. Significance analysis of differences among variables

Variable	p	Interpretation
A vs B vs C	0.0001	There was a significant difference in total plate count values of bacteria inoculated on media A, B, and C

Note:
A: BAP with whole blood containing antibodies against *Salmonella typhi*
B: BAP with erythrocyte suspension
C: BAP with whole blood without antibodies against *Salmonella typhi*

In the One-Way ANOVA test, if the significance value is p < 0.05, then Ha is accepted and the data are considered significantly different. Based on the One-Way ANOVA results, the significance value obtained was less than 0.05, namely p = 0.0001. This indicates that Ha was accepted and demonstrates a difference in the mean number of *Salmonella typhi* O bacteria growing on BAP prepared with whole blood containing antibodies against *Salmonella typhi* O, BAP prepared with erythrocyte suspension, and BAP prepared with whole blood without antibodies against *Salmonella typhi* O.

Because a significant difference was observed among the three blood-component variation groups, a Post Hoc test was performed to identify which groups differed. The results of the Bonferroni pairwise comparisons are shown in Table 5.

Table 5. Significance test among variables

Blood Variation	p	Interpretation
A vs B	0.0001	There was a significant difference in bacterial total plate count between media A and B
A vs C	0.0001	There was a significant difference in bacterial total plate count between media A and C
B vs C	0.618	There was no significant difference in bacterial total plate count between media B and C

Note:
A: BAP with whole blood containing antibodies against *Salmonella typhi*
B: BAP with erythrocyte suspension
C: BAP with whole blood without antibodies against *Salmonella typhi*

In the pairwise significance test, a significance value below 0.05 indicates a difference between variations, meaning that Ha is accepted and H0 is rejected. Conversely, if the significance value is greater than 0.05, no difference exists between variations, meaning that Ha is rejected and H0 is accepted. In Table 5, both A vs B and A vs C yielded p = 0.0001, indicating that the presence of antibodies affected the fertility of the medium for *Salmonella typhi* O. In contrast, B vs C showed no significant difference because neither medium contained antibodies against *Salmonella typhi* O.

Based on the statistical analysis, the results clearly demonstrate that human antibodies influence the fertility of Blood Agar Plate media. The immunological interaction between anti-*Salmonella typhi* O antibodies dissolved in human blood and *Salmonella typhi* O antigens produced significant effects because antibodies are capable of inducing lysis through the complement activation pathway. IgG present in blood activates C1q, which subsequently activates C1r and then C1s. Activated C1s exhibits proteolytic and esterolytic properties and then activates C4, a glycoprotein that binds to the cell membrane associated with the antigen epitope recognized by C1q, and interacts with C1s to activate C2. Activated C2 binds to C4 to form the C4,2 enzyme (C3 convertase), which activates C3. C3 is then cleaved into two fragments, namely C3a, which is smaller, and C3b, which is larger. Both C3a and C3b are capable of associating with C4,2,3 (C5 convertase). C5 is subsequently cleaved and binds to C6 and C7 to form C567, which then activates C8 and C9. When C5b is deposited on the cell membrane and binds to C6, C7, C8, and C9, C5b678 and polymeric C9 are formed, generating the membrane attack complex (MAC), which subsequently lyses the bacteria.

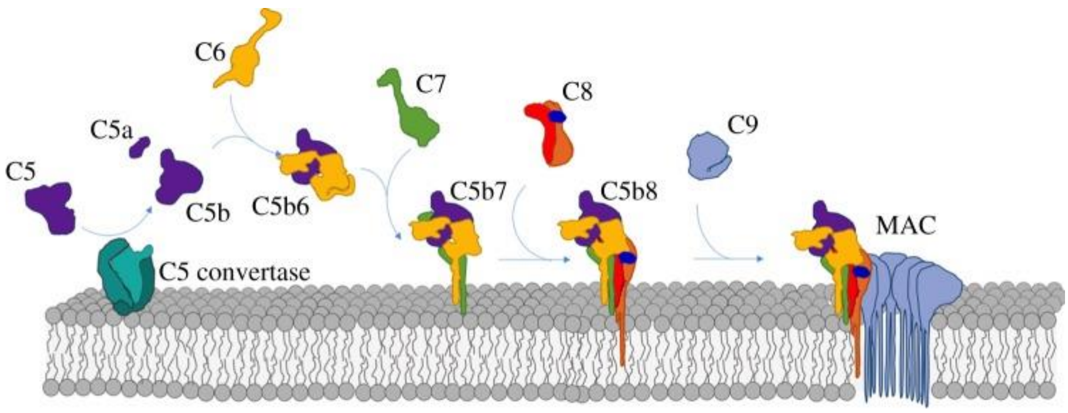

Figure 1. Process of complement system activation (Balyl et al, 2017)

The MAC process is recognized as a cytolytic effector that forms pores in the plasma membrane of pathogens or target cells, thereby causing osmotic lysis (Xie et al, 2020). Bacterial lysis prevents bacterial growth; consequently, the fertility and quality of the BAP medium also differ. Based on the results of this study, BAP with erythrocyte suspension and BAP with whole blood lacking anti-*Salmonella typhi* O antibodies showed comparable quality. This is because erythrocyte suspension provides sufficient nutrition due to its high protein content derived from hemoglobin and lacks growth-inhibiting components, namely antibodies. Therefore, the quality of BAP with erythrocyte suspension is comparable to that of BAP with whole blood not containing antibodies against *Salmonella typhi* O.

If a laboratory encounters difficulty in obtaining sheep blood for culture media preparation, Blood Agar Plate may be prepared using human blood as a substitute. Whole blood that does not contain antibodies against *Salmonella typhi* O may serve as an alternative blood source for media preparation. If only antibody-positive blood is available, it should first be washed with sterile 0.85% NaCl to obtain an erythrocyte suspension. Thus, both BAP prepared with whole blood lacking antibodies and BAP prepared with erythrocyte suspension may be used as alternative blood sources for enrichment media functioning as strain media.

The use of human blood as a substitute for sheep blood in the preparation of Blood Agar Plate media has increasingly become the subject of research. Previous studies have focused on eliminating compounds that inhibit bacterial growth and optimizing the characteristics of human erythrocytes so that they resemble those of sheep erythrocytes (Niederstebruch et a,l 2017). Modifying human blood is one way to optimize Blood Agar Plate when used as an enrichment medium. The best modification that can be performed is erythrocyte washing, because it can reduce or even eliminate antibodies without damaging other essential substrates, thereby producing Blood Agar Plate that is more optimal for the growth of *Salmonella typhi*.

Salmonella typhi was selected because this bacterium survives in human blood; therefore, it is reasonable that a suitable medium for its growth should contain blood, one example being Blood Agar Plate. In addition, when *Salmonella typhi* is present in human blood, stable antibodies are produced because the affinity between antigen and antibody becomes increasingly complex and stronger. Accordingly, this study provides a scientific basis showing that antibodies in human blood influence the fertility of Blood Agar Plate media.

CONCLUSION

This study demonstrated that the presence of *Salmonella Typhi* antibodies in whole blood significantly increased the growth of *Salmonella Typhi* on BAP medium, with the highest mean bacterial growth reaching 2.6 CFU/mL, compared with erythrocyte suspension (1.8 CFU/mL) and whole blood without antibodies (1.9 CFU/mL). Statistical analysis confirmed a significant difference ($p < 0.05$) between bacterial growth in the whole blood medium containing antibodies and that in the other two groups. In contrast, no significant difference was observed between erythrocyte suspension and whole blood without antibodies ($p = 0.618$). These findings indicate that antibody components in whole blood play an important role in supporting or facilitating the growth of *Salmonella Typhi* in culture media, whereas erythrocyte components alone do not produce a similar effect.

RECOMMENDATION

Further studies are recommended using different bacterial species, such as Gram-positive bacteria, to broaden the scope and improve the generalizability of these findings.

ACKNOWLEDGMENT

The authors would like to express their sincere gratitude to Universitas Pelita Harapan for providing research facilities for lecturers and to the Medical Laboratory Technology Study Program for its continuous encouragement and support throughout the research process.

REFERENCES

Barnum, S. R., Bubeck, D., & Schein, T. N. (2020). Soluble membrane attack complex: Biochemistry and immunobiology. *Frontiers in Immunology, 11*, 1–14. https://doi.org/10.3389/fimmu.2020.585108

Bayly-Jones, C., Bubeck, D., & Dunstone, M. A. (2017). The mystery behind membrane insertion: A review of the complement membrane attack complex. *Philosophical Transactions of the Royal Society B: Biological Sciences, 372*(1726), 20160221. https://doi.org/10.1098/rstb.2016.0221

Buzilă, E. R., Dorneanu, O. S., Trofin, F., Sima, C. M., & Iancu, L. S. (2025). Assessing *Salmonella Typhi* pathogenicity and prevention: The crucial role of vaccination in combating typhoid fever. *International Journal of Molecular Sciences, 26*(9), 1–27. https://doi.org/10.3390/ijms26093981

Cardigan, R., New, H. V., Tinegate, H., & Thomas, S. (2020). Washed red cells: Theory and practice. *Vox Sanguinis, 115*(8), 606–616. https://doi.org/10.1111/vox.12971

Dyson, Z. A., Ashton, P. M., Khanam, F., Chirambo, A. C., Shakya, M., Meiring, J. E., Tonks, S., et al. (2024). Pathogen diversity and antimicrobial resistance transmission of *Salmonella enterica* serovars Typhi and Paratyphi A in Bangladesh, Nepal, and Malawi: A genomic epidemiological study. *The Lancet Microbe, 5*(8), 1–12. https://doi.org/10.1016/S2666-5247(24)00047-8

Ho, B. H. T., Spicer, B. A., & Dunstone, M. A. (2025). Action of the terminal complement pathway on cell membranes. *Journal of Membrane Biology, 258*. https://doi.org/10.1007/s00232-025-00343-6

Imara, F. (2020). *Salmonella Typhi* bakteri penyebab demam tifoid. *Prosiding Seminar Nasional Biologi di Era Pandemi COVID-19*, 1–5. http://journal.uin-alauddin.ac.id/index.php/psb/

Krihariyani, D., Woelansari, E. D., & Kurniawan, E. (2016). Pola pertumbuhan *Staphylococcus aureus* pada media agar manusia, 191–200. https://ejurnal.poltekkesjakarta3.ac.id/index.php/jitek/article/download/99/78/

Miguel, C. F., & Pereira, C. C. (2024). Evaluation of the feasibility for replacing sheep blood with human blood in culture media used in microbiological diagnostics. *Anais da Academia Brasileira de Ciências, 96*(2), 1–11. https://doi.org/10.1590/0001-3765202420231168

Pont, S., Fraikin, N., Caspar, Y., van Melderen, L., Attrée, I., & Cretin, F. (2020). Bacterial behavior in human blood reveals complement evaders with some persister-like features. *PLoS Pathogens, 16*(12), 1–22. https://doi.org/10.1371/journal.ppat.1008893

Rahma Velina, V., Hanif, A. M., & Efrida. (2016). Gambaran hasil uji Widal berdasarkan lama demam pada pasien suspek demam tifoid. *Jurnal Kesehatan Andalas, 5*(3), 687–691. https://doi.org/10.25077/jka.v5i3.602

Rigby, J., Elmerhebi, E., Diness, Y., Mkwanda, C., Tonthola, K., Galloway, H., Miles, R., et al. (2022). Optimized methods for detecting *Salmonella Typhi* in the environment using validated field sampling, culture and confirmatory molecular approaches. *Journal of Applied Microbiology, 132*(2), 1503–1517. https://doi.org/10.1111/jam.15237

Sanjaya, D. A., Meriyani, H., Juanita, R. R. A., & Siada, N. B. (2022). Kajian literatur: Profil resistensi *Salmonella Typhi* dan pemilihan antibiotik pada demam tifoid. *JPSCR: Journal of Pharmaceutical Science and Clinical Research, 7*(2), 107. https://doi.org/10.20961/jpscr.v7i2.56656

Serna, M., Giles, J. L., Morgan, B. P., & Bubeck, D. (2016). Structural basis of complement membrane attack complex formation. *Nature Communications, 7*, 1–7. https://doi.org/10.1038/ncomms10587

Setyoningrum, F. P., Iriyanti, D. B., & Situmorang, D. (2020). Edukasi dan simulasi pemeriksaan typoid metode Widal kepada siswa SMA Tunas Bangsa Sunter, 296–301.

Sumilih, J. (2012). *Meningkatkan kemampuan menumbuhkan Streptococcus pneumoniae pada media agar darah*, 1–77.

Turista, D. D. R., & Puspitasari, E. (2019). The growth of *Staphylococcus aureus* in the blood agar plate media of sheep blood and human blood groups A, B, AB, and

O. *Jurnal Teknologi Laboratorium, 8*(1), 1–7. https://doi.org/10.29238/teknolabjournal.v8i1.155

Verma, S., & Shahab, A. S. (2022). Isolation and identification of *Salmonella Typhi* from different blood samples. *10*(3), 955–958.

Xie, C. B., Jane-Wit, D., & Pober, J. S. (2020). Complement membrane attack complex: New roles, mechanisms of action, and therapeutic targets. *American Journal of Pathology, 190*(6), 1138–1150. https://doi.org/10.1016/j.ajpath.2020.02.006

Yougbare, I., McTague, A., He, L., Choy, C. H., Su, J., Gajewska, B., & Azizi, A. (2020). Anti-FIM and anti-FHA antibodies inhibit *Bordetella pertussis* growth and reduce epithelial cell inflammation through bacterial aggregation. *Frontiers in Immunology, 11*, 3–5. https://doi.org/10.3389/fimmu.2020.605273