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Effect of blood agar storage duration at 2–8°C on bacterial growth from rectal swabs

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Abstract

Reliable microbiological testing depends in part on the ability of culture media to support bacterial isolation and the observation of colony characteristics. This study aimed to evaluate differences in bacterial growth from rectal swab specimens stored on Blood Agar Plates at 2–8 °C for varying durations. A laboratory-based experimental study was conducted using 30 Blood Agar Plates divided into three storage-duration groups: 0, 7, and 14 days. Thirty rectal swab specimens were inoculated onto the plates and incubated at 35–37°C for 24 hours. The number of bacterial colonies was assessed as the primary quantitative outcome, while colony size, distribution, color, and hemolytic pattern were evaluated descriptively. Data were analyzed using One-Way ANOVA followed by Tukey's test. The number of bacterial colonies differed significantly among the three storage-duration groups. The highest mean colony count was observed in the 7-day group, while the 14-day group showed the lowest mean count, with significant differences across all pairwise comparisons. The findings indicate a nonlinear relationship between storage duration and bacterial recovery. The substantially lower bacterial recovery after 14 days highlights the importance of controlling the duration of culture medium storage as part of laboratory quality assurance. Further research using standardized bacterial inocula and physicochemical assessments is needed to establish validated storage limits.

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1. Introduction

Reliable microbiological examination also depends on the quality of the culture media used to recover and characterize microorganisms. Culture media provide the nutrients and environmental conditions required for bacterial growth, and deterioration in their performance can reduce bacterial recovery or alter observable characteristics important for laboratory identification. Consequently, the preparation, quality control, and storage of culture media are important components of laboratory quality assurance because compromised media performance may affect the reliability of microbiological results and subsequent clinical decision-making (Forbes et al., 2018; Orekan et al., 2021).

Blood Agar Plate (BAP) is widely used as an enriched and differential culture medium in clinical microbiology. Its nutrient-rich composition supports the growth of a broad range of bacteria, including relatively fastidious organisms, while the presence of blood allows hemolytic reactions to be observed during culture. These characteristics make BAP useful for the primary isolation and preliminary characterization of clinically relevant microorganisms, including Streptococcus spp. (Krihariyani et al.,

2016). Maintaining the quality of BAP is consequently important, as changes in the medium's physical or nutritional properties may affect its capacity to support bacterial recovery.

In routine laboratory practice, BAP is often prepared in batches and refrigerated at 2–8°C until required for examination. Refrigerated storage is intended to maintain the stability of prepared media and reduce deterioration before use (World Health Organization [WHO], 2016). The duration of storage, however, is also an important consideration. Prolonged storage may be accompanied by changes in pH, moisture, and nutrient availability, which can gradually impair the ability of culture media to support bacterial growth (Clinical and Laboratory Standards Institute [CLSI], 2017; Orekan et al., 2021). Thus, maintaining an appropriate storage period is not merely a matter of convenience; it is essential to ensuring that a prepared medium remains suitable for microbiological examination.

Evidence from previous studies indicates that storage duration can influence the performance of different microbiological culture media. Rohmah (2024), for example, reported a significant reduction in the number of Escherichia coli colonies recovered from MacConkey agar following prolonged storage at 2–8°C. Moreno et al. (2017) similarly observed changes in bacterial growth consistency and hemolytic reactions in Blood Agar Plates following extended refrigerated storage. Findings from Adetoye et al. (2018) also indicated that aged culture media could produce lower bacterial recovery than freshly prepared media, while Tuttle et al. (2021) reported reduced *Escherichia coli* viability on stored Mueller–Hinton agar. These findings indicate that storage duration can influence the microbiological performance of solid culture media, although the magnitude and nature of the effect may differ according to the type and intended use of the medium.

The timely collection, handling, and processing of clinical specimens can also influence the reliability of microbiological testing (Hartadi & Sudarma, 2025; Haliza et al., 2025). The issue is particularly relevant when culture media are used for bacterial recovery from rectal swab specimens. Rectal swabs are routinely collected for the detection of enteric pathogens in clinical settings, including the evaluation of gastrointestinal infections (Walker et al., 2019). The culture approach and the characteristics and performance of the culture medium may influence the recovery of bacteria from these specimens. Enrichment-based culture approaches, for example, have been shown to improve the detection of bacterial pathogens from rectal swab specimens compared with direct plating (Blanc, 2025). In addition, culture media provide the nutrients and growth factors required for bacterial growth and isolation, making their composition and performance important considerations in microbiological examination (Bonnet et al., 2019). Any reduction in BAP's ability to support bacterial growth could consequently affect the recovery of organisms from clinical specimens and, in turn, the reliability of microbiological examination. Despite evidence on the effect of storage duration on several types of culture media, the available literature provides less direct evidence on the performance of BAP after refrigerated storage for bacterial recovery from rectal swab specimens. Previous research has addressed media such as MacConkey agar and Mueller–Hinton agar, while evidence specifically linking BAP storage duration at 2–8°C with bacterial growth from clinical rectal swab specimens remains limited. Therefore, this study aimed to evaluate the effect of BAP storage duration at 2–8°C on bacterial growth from rectal swab specimens. The findings provide evidence to support appropriate BAP storage practices, strengthen internal laboratory quality assurance, and enhance the reliability of microbiological examinations.

2. Method

2.1 Research Design and Experimental Units

This study employed an experimental design to evaluate whether the duration of Blood Agar Plate (BAP) storage at 2–8°C affected bacterial growth recovered from rectal swab specimens (Fraenkel et al., 2019). Three storage-duration groups were compared: freshly prepared BAP (0 days), BAP stored for 7 days, and BAP stored for 14 days. Each group consisted of 10 BAPs, resulting in 30 experimental units. The 0-day group served as the control. The study was conducted at the Bina Medika Public Health Laboratory, Denpasar, Indonesia, in May 2026. The BAPs used in the experiment were prepared from a single production batch to minimize variation arising from differences in medium preparation. A total of 40 BAPs were prepared. Thirty plates were allocated to the three experimental groups, while the remaining 10 plates were retained as backup media in case of damage during preparation or failure to meet the predefined initial quality-control criteria. BAPs were allocated to the storage-duration groups according to predefined laboratory criteria, and all experimental plates were maintained under the same storage conditions at 2–8°C. The storage duration was defined as the period from completion of BAP preparation and initial quality

control to the day the corresponding group was removed from refrigerated storage for inoculation. Plates assigned to the 0-day group were used as freshly prepared media without a 7- or 14-day storage period. Rectal swab specimens were obtained from routinely received specimens and used only when they met the predefined eligibility criteria. Specimens were included if collected using appropriate transport procedures, received within 24 hours of collection, showed no evidence of external contamination, and could be processed in accordance with the laboratory's standard operating procedures. Specimens with incomplete identification, inappropriate collection or transportation, or other deviations from the laboratory requirements were excluded.

2.2 Instruments and Data Collection

The study used an incubator maintained at 35–37°C, a laboratory refrigerator maintained at 2–8°C, a thermometer/thermohygrometer for monitoring storage conditions, a colony counter, sterile inoculating loops, sterile cotton swabs, waterproof laboratory markers, and a Class II Biological Safety Cabinet. The materials included 40 BAPs, 30 eligible rectal swab specimens, sterile defibrinated sheep blood, physiological saline (0.9% NaCl), 70% ethanol, and appropriate personal protective equipment. BAPs were prepared according to the Standard Operating Procedure of the Bina Medika Public Health Laboratory (2024). The culture medium was sterilized at 121°C for 15 minutes, then cooled to approximately 45–50°C before 5% sterile defibrinated sheep blood was added. Approximately 15–20 mL of the prepared medium was aseptically dispensed into sterile Petri dishes. After solidification, the plates were stored in an inverted position at 2–8°C under refrigerated conditions, according to their assigned storage-duration groups. Before allocation to the experimental groups, the prepared BAPs underwent the laboratory's initial quality-control procedures.

Plates that did not meet the predefined requirements were excluded and replaced using the reserved backup plates. The quality-control assessment included the laboratory criteria routinely applied to verify the suitability of the prepared medium before microbiological use. At the designated storage period, the corresponding BAPs were removed from refrigeration and used for culture examination. Eligible rectal swab specimens were inoculated onto the BAPs using aseptic techniques in accordance with the laboratory's standard operating procedures. Following inoculation, all culture plates were incubated at 35–37°C for 24 hours under the same incubation conditions. Bacterial growth was assessed after incubation using a standardized observation form. Colony count was treated as the primary quantitative outcome. Colony size, morphology, color, and hemolytic pattern were also recorded as descriptive characteristics of the recovered colonies. Observations were performed using standardized laboratory procedures to maintain consistency across the three storage-duration groups. Bacterial growth characteristics were recorded according to predefined laboratory observation criteria. The categories of good growth, poor growth, and no growth were applied only when the corresponding operational definitions established by the laboratory were met.

2.3 Data Analysis

Data were analyzed using IBM SPSS Statistics version 27. Descriptive analysis was performed to summarise bacterial growth over storage duration. Colony counts were summarized using the mean and standard deviation when the data met the assumptions for parametric analysis. Categorical observations, including colony characteristics and growth categories, were presented as frequencies and percentages. The primary inferential analysis was a one-way analysis of variance (One-Way ANOVA) to determine whether mean bacterial colony counts differed significantly among the three BAP storage-duration groups: 0, 7, and 14 days. Before the ANOVA was performed, the distribution of colony-count data was assessed for normality using the Shapiro–Wilk test, and homogeneity of variance among groups was assessed using Levene's test. When the assumptions for One-Way ANOVA were met, the analysis was performed at $\alpha = 0.05$. A statistically significant overall F-test indicated that at least one storage-duration group differed from another. When a significant overall difference was identified, Tukey's Honestly Significant Difference (HSD) post hoc test was used to determine which pairs of storage-duration groups differed significantly.

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2.4 Ethical Considerations

The study was conducted in accordance with institutional regulations, laboratory biosafety procedures, and applicable ethical requirements for research involving human-derived biological specimens. All rectal swab specimens were processed according to the Standard Operating Procedure of the Bina Medika Public Health Laboratory. Specimen information was coded and handled confidentially,

and no personally identifying information was included in the research dataset. Ethical approval was obtained from the STIKES Wira Medika Bali Health Research Ethics Committee under approval number 796?E1STIKESWIKA/E/C/V/2026.

3. Results and Discussion

3.1 Results

A total of 30 rectal swab specimens were cultured using BAPs assigned to three storage-duration groups: 0, 7, and 14 days, with 10 experimental units in each group. Bacterial colony count was treated as the primary quantitative outcome. The descriptive results are presented in Table 1.

Table 1. Bacterial colony counts according to Blood Agar Plate storage duration

Storage duration	n	Mean	Minimum	Maximum	SD
0 days	10	617.90	250	880	218.88
7 days	10	1282.80	325	1801	401.17
14 days	10	166.60	7	341	124.95

Table 1 shows that the highest mean bacterial colony count was observed in the BAPs stored for 7 days, with a mean of 1282.80 ± 401.17 colonies. The freshly prepared BAPs (0 days) yielded a lower mean count of 617.90 ± 218.88 colonies. In contrast, the 14-day group showed the lowest mean colony count, at 166.60 ± 124.95 colonies. Thus, the descriptive pattern was not linear across storage duration: colony counts increased from day 0 to day 7 but declined markedly by day 14. In addition to colony count, colony characteristics were assessed descriptively for size, distribution, colour, and hemolytic pattern. The results are presented in Table 2.

Table 2. Descriptive characteristics of bacterial colonies according to Blood Agar Plate storage duration

Variable	Category	0 days, n (%)	7 days, n (%)	14 days, n (%)
Colony size	Small	0 (0)	2 (20)	7 (70)
	Medium	6 (60)	8 (80)	2 (20)
	Large	4 (40)	0 (0)	1 (10)
Colony distribution	Uneven	1 (10)	1 (10)	7 (70)
	Uniform	9 (90)	9 (90)	3 (30)
Colony color	White	7 (70)	0 (0)	9 (90)
	Grayish white	2 (20)	10 (100)	1 (10)
	Gray	1 (10)	0 (0)	0 (0)
Hemolysis pattern	Alpha	7 (70)	3 (30)	5 (50)
	Beta	3 (30)	6 (60)	2 (20)
	Gamma	0 (0)	1 (10)	3 (30)

Table 2 shows that the colony characteristics varied across the three storage-duration groups. The 0-day BAPs were predominantly associated with medium-sized colonies (60%), uniform distribution (90%), white coloration (70%), and alpha hemolysis (70%). In the 7-day group, medium-sized colonies remained predominant (80%), with uniform distribution in 90% of observations, grayish-white coloration in all observations (100%), and beta hemolysis in 60% of observations. The 14-day group showed a different pattern, with small colonies predominating (70%) and uneven distribution observed in 70% of the observations. White colonies were most frequent in this group (90%), while alpha hemolysis remained the most common hemolytic pattern (50%). Before conducting the One-Way ANOVA, the normality of bacterial colony counts within each storage-duration group was assessed using the Shapiro–Wilk test. The results are presented in Table 3.

Table 3. Results of the Shapiro–Wilk normality test

Storage duration	p-value	Interpretation
0 days	0.295	No significant departure from normality
7 days	0.097	No significant departure from normality
14 days	0.226	No significant departure from normality

The Shapiro–Wilk test did not indicate a significant departure from normality in any of the three groups, with p-values of 0.295, 0.097, and 0.226 for the 0-, 7-, and 14-day groups, respectively. All values exceeded 0.05, supporting the normality assumption for the planned parametric analysis. Homogeneity of variance was subsequently assessed using Levene's test. Levene's test yielded a p-value of 0.108, indicating no statistically significant difference in variance among the three storage-duration groups. The assumption of homogeneity of variance was therefore considered satisfied. Because the assumptions for parametric analysis were satisfied, a One-Way ANOVA was performed to examine whether mean bacterial colony counts differed among the three BAP storage-duration groups.

Table 4. One-Way ANOVA of bacterial colony counts according to Blood Agar Plate storage duration

Variable	F (df1, df2)	p-value	Interpretation
Bacterial colony count	42.138 (2, 27)	<0.001	Significant difference

Table 4 demonstrated a statistically significant difference in mean bacterial colony counts among the 0-, 7-, and 14-day storage groups ($F(2,27) = 42.138$, $p < 0.001$). The result indicates that at least one storage-duration group differed significantly from the others in terms of bacterial colony count. The descriptive results in Table 1 show that the highest mean colony count occurred after 7 days of storage, whereas the lowest occurred after 14 days. Therefore, Tukey's HSD post hoc analysis was subsequently performed to identify the specific group comparisons responsible for the overall difference. Pairwise comparisons using Tukey's HSD test are presented in Table 5.

Table 5. Tukey's HSD post hoc comparisons of bacterial colony counts

Group comparison	Mean difference	p-value	Interpretation
0 days vs. 7 days	−664.900	<0.001	Significant difference
0 days vs. 14 days	451.300	0.003	Significant difference
7 days vs. 14 days	1116.200	<0.001	Significant difference

Table 5 demonstrated statistically significant differences in bacterial colony counts across all three pairwise comparisons. The 7-day group had a significantly higher mean colony count than the 0-day group (mean difference = −664.900, $p < 0.001$). The 0-day group also had a significantly higher mean colony count than the 14-day group (mean difference = 451.300, $p = 0.003$). The largest difference was observed between the 7-day and 14-day groups, with the 7-day group showing a significantly higher mean colony count (mean difference = 1116.200, $p < 0.001$). The results demonstrate significant variation in bacterial colony counts according to BAP storage duration. The pattern was characterized by an increase in mean colony count from 0 to 7 days, followed by a substantial decline after 14 days of storage.

3.2 Discussion

The present study demonstrates that the duration of refrigerated BAP storage was associated with significant differences in bacterial colony counts obtained from rectal swab specimens. The pattern, however, was not linear. BAPs stored for 7 days produced the highest mean colony count, whereas those stored for 14 days produced the lowest count. The significant differences observed across all pairwise comparisons indicate that storage duration is an important consideration in maintaining the performance of BAPs used for bacterial culture. At the same time, the higher colony count observed in the 7-day group should not be interpreted as evidence that stored BAPs are inherently superior to freshly prepared media.

The unexpectedly higher colony count after 7 days of storage warrants careful interpretation. Freshly prepared media are likely to provide favorable conditions for bacterial recovery, particularly because the components of the medium have undergone fewer storage-related changes. The higher count in the 7-day group may instead reflect variation in the bacterial load of individual rectal swab specimens, differences in inoculation efficiency, or other experimental factors not fully controlled. Thus, the present findings demonstrate a difference in observed bacterial recovery between the storage groups, but they do not establish that seven days of storage enhances bacterial proliferation. Reports by Watanabe et al. (2022) and Komkaew and Saoha (2024) similarly indicate that appropriately stored culture media can retain their capacity to support bacterial growth over a defined period. However, preservation of growth-supporting capacity does not necessarily imply improved performance compared with freshly prepared media.

A substantially different pattern was observed after 14 days of storage. The marked reduction in bacterial colony counts suggests that extended refrigeration may compromise BAPs' capacity to support bacterial recovery. One possible explanation is the gradual alteration of the medium's physical and chemical characteristics during storage. Changes in moisture content, nutrient availability, or other physicochemical properties may influence the environment encountered by bacterial cells during inoculation and subsequent growth. These mechanisms, however, were not directly measured in the present study and should consequently be regarded as plausible explanations rather than demonstrated causes. Previous work by Komkaew and Saoha (2024) likewise indicated that prolonged storage can reduce culture medium performance, while Forbes et al. (2018) emphasized that the quality of culture media is fundamental to successful bacterial isolation and the reliable interpretation of colony characteristics.

Descriptive changes in colony characteristics accompanied the reduction in colony count after 14 days. Smaller colonies and less uniform distribution were more frequently observed in the 14-day group, while differences in colony color and hemolytic patterns were also apparent across the storage groups. Such observations are relevant because colony morphology and hemolysis are commonly used as preliminary characteristics during the recognition and differentiation of clinically important bacteria. Nevertheless, the morphological findings in this study were descriptive and were not subjected to inferential statistical testing. They should consequently be interpreted as observed patterns rather than statistical evidence that storage duration independently altered bacterial phenotype. Watanabe et al. (2022) noted that environmental conditions within culture media, including factors related to nutrient availability and moisture, can influence colony development and morphology, providing a plausible context for the variation observed in the present study.

The observed variation in colony counts was not confined to a single pair of storage conditions. Each storage-duration group differed significantly from the others. The largest difference occurred between the 7-day and 14-day groups, which is consistent with the pronounced decline in colony recovery after the longer storage period. These findings are broadly compatible with previous observations that refrigerated storage can preserve the usability of culture media for a limited period, whereas extended storage may reduce the consistency of bacterial growth and the reliability of observable colony characteristics (Komkaew & Saoha, 2024; Forbes et al., 2018). The present study adds a more specific observation by demonstrating that the response in bacterial recovery across the tested storage periods was not a simple progressive decline, as the 7-day group produced a higher mean colony count than the freshly prepared group.

The findings suggest that storage duration should be considered when preparing BAPs in batches for routine microbiological examination. The markedly lower bacterial recovery observed after 14 days indicates that prolonged storage under the conditions examined may be undesirable when reliable bacterial recovery is required. In contrast, the 7-day BAPs continued to support substantial bacterial growth; however, the present evidence is insufficient to establish seven days as an optimal or universally applicable storage limit. Such a recommendation would require additional quality-control parameters and validation under the specific conditions of each laboratory. Forbes et al. (2018) emphasized the importance of culture medium quality in microbiological isolation and identification, underscoring the need to integrate storage control into routine laboratory quality-assurance practices. The findings indicate that BAP storage duration at 2–8°C is associated with significant variation in bacterial colony recovery from rectal swab specimens. The particularly low recovery observed after 14 days supports the need for careful control of storage duration, while the higher count observed after 7 days should be interpreted cautiously rather than as evidence of improved medium performance. The contribution of this study is the demonstration of a nonlinear pattern across the three storage conditions, highlighting that the relationship between storage duration and bacterial recovery cannot be adequately described as a simple progressive decline. Further controlled studies are needed to determine the physicochemical mechanisms underlying this pattern and to establish storage limits that can be confidently applied in routine clinical microbiology laboratories.

4. Conclusion

This study demonstrated that the storage duration of Blood Agar Plates at 2–8°C was associated with significant differences in bacterial colony counts from rectal swab specimens, with the highest mean colony count observed after 7 days and the lowest after 14 days. Significant differences were identified across all pairwise comparisons, indicating that storage duration should be considered in the quality management of Blood Agar Plates used for routine microbiological examination. The findings provide

practical evidence for medical laboratories to incorporate storage duration into internal quality-control procedures, particularly because prolonged storage for 14 days was associated with substantially lower bacterial recovery under the conditions examined. However, the study was limited by the relatively small number of specimens, the use of only three storage durations and a single storage temperature, the absence of physicochemical assessments of the culture medium, and potential variation in bacterial load among rectal swab specimens. Future research should use larger samples and standardized bacterial inocula, include additional storage intervals and physicochemical quality parameters, and evaluate different bacterial species and culture media. The objectives are to clarify the mechanisms underlying the observed nonlinear pattern and establish storage limits that can be validated for routine medical laboratory practice.

Conflict of Interest

The authors declare no conflict of interest.

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