
Bacteriological Quality of Refillable Drinking Water in the Simpang Tiga Area of Samarinda City

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ABSTRACT

Introduction: Refillable drinking water is widely used because of its accessibility and affordability. However, its bacteriological quality requires monitoring to identify potential microbial contamination.

Objective: To assess the bacteriological quality of refillable drinking water based on total coliform and *Escherichia coli* parameters in Simpang Tiga, Samarinda City.

Methods: This quantitative descriptive observational study included eight eligible refillable drinking water depots. Samples were examined at the Samarinda City Regional Health Laboratory between November 24 and December 3, 2025, using the three-tube, three-series Most Probable Number (MPN) method. Data were analyzed descriptively.

Results: Seven samples (87.5%) showed total coliform and *E. coli* results of <3.0 MPN/100 mL. One sample (12.5%), from Depot C, showed total coliform and *E. coli* values of 93 MPN/100 mL and 9.2 MPN/100 mL, respectively. Based on the laboratory's interpretation, seven samples met the requirements for both parameters, whereas one sample did not.

Conclusion: Most samples met the requirements for the two microbiological parameters examined. The source of contamination at Depot C could not be determined.

Keywords: *Escherichia coli*; MPN; refillable drinking water; total coliforms.

INTRODUCTION

Meeting domestic consumption needs and carrying out household activities depend on the availability of safe water. The security of this resource contributes to improved public health. Therefore, water used by humans must meet hygiene standards and be free from microbiological contamination and hazardous substances that could potentially cause health problems, particularly gastrointestinal

infections (Hargono *et al.*, 2022).

The safety of drinking water is determined by compliance with health quality requirements, including microbiological parameters. Based on Regulation of the Minister of Health of the Republic of Indonesia No. 2 of 2023 concerning Implementing Regulations for Government Regulation No. 66 of 2014 on Environmental Health, the maximum levels of *Escherichia coli* and total coliforms in drinking water are set at

0 CFU/100 mL. Meanwhile, testing using the Most Probable Number (MPN) method yields an estimated bacterial concentration expressed in MPN/100 mL. This difference in units must be taken into account when interpreting the results, as the two methods employ different measurement principles. Compliance with microbiological requirements is a critical component of quality control for Refillable Drinking Water Depot (DAMIU) products. This oversight has become increasingly relevant as the public increasingly turns to DAMIU as an alternative to bottled drinking water (AMDK) due to its relatively affordable price and ease of access. (Salsabila *et al.*, 2025)

The microbiological safety of refillable drinking water requires attention because product quality can be affected by processing conditions, storage, and sanitation at the refill stations. The risk of contamination may increase if the cleanliness of the facilities, filling equipment, and storage containers is not properly maintained (Foziyah *et al.*, 2018). Tests for total coliforms and *Escherichia coli* are used to assess the presence of indicator bacteria in drinking water. Total coliforms are a general indicator of microbiological quality that can reflect processing conditions and the possibility of recontamination, while *E. coli* is a more specific indicator of fecal contamination. Therefore, testing for

both parameters is necessary to obtain a picture of the bacteriological quality of refillable drinking water based on applicable microbiological requirements. (Foziyah *et al.*, 2018).

The sanitary conditions of the refill stations and the hygiene practices of the staff play a role in determining the bacteriological quality of refillable drinking water. This is supported by a study conducted in the service area of the Grogol Community Health Center (Puskesmas) in Sukoharjo Regency, which found that three out of ten DAMIU stations did not meet coliform standards. The study's findings also indicate a correlation between the quality of DAMIU stations and sanitation, as well as oversight by local health institutions. (Wijaya *et al.*, 2022).

Variations in the bacteriological quality of refillable drinking water across regions underscore the importance of testing tailored to local conditions. Yunus *et al.* (2018) reported that testing using the 3-3-3 Most Probable Number (MPN) method in Palopo City yielded coliform contamination levels of >1,100 MPN/100 mL, meaning the samples did not meet the requirements. Conversely, a study on the bacteriological quality of refillable drinking water was also conducted by Kurniawan (2024) in Demta District, Jayapura Regency, using the Most Probable Number

(MPN) method. Findings from various regions highlight the importance of bacteriological testing to determine the quality of refillable drinking water based on the parameters tested.

In addition to hygiene and sanitation conditions, water treatment technology can also affect the bacteriological quality of refillable drinking water. Research Tominik *et al.* (2018), compared Reverse Osmosis (RO) and Ultraviolet (UV) systems at DAMIU using the MPN method. The results showed that the RO system was more effective at reducing bacterial counts than the UV system. That study focused on comparing the effectiveness of treatment technologies, whereas this study does not compare types of treatment technologies but focuses on the bacteriological quality of refillable drinking water based on Total Coliform and *E. coli* parameters.

Observations on September 27, 2025, indicated that the DAMIU in the Simpang Tiga area of Samarinda City uses PDAM water as its primary source. However, the implementation of sanitation and hygiene practices is not yet fully optimal, as evidenced by the poor maintenance of the cleanliness of filling equipment and storage containers. These conditions highlight the importance of microbiological testing to assess the

bacteriological quality of refillable drinking water based on the presence of Total Coliforms and *Escherichia coli*. Testing these two parameters can provide information on the microbiological quality of the product, but it is not sufficient to ensure the overall safety of the drinking water.

Bacteriological testing of refillable drinking water has been conducted in various regions with diverse research characteristics, including variations in sampling times, RO and UV treatment technologies, and testing locations. However, information regarding the bacteriological quality of DAMIU in Simpang Tiga, Samarinda City, remains limited, particularly regarding Total Coliform and *E. coli* parameters using the 3-3-3 MPN method. This information gap prompted this study to identify the bacteriological conditions of refillable drinking water in that area more specifically.

The presence of Total Coliforms and *E. coli* in refillable drinking water can be determined using the 3-3-3 MPN method. The test results were then compared with Indonesian Ministry of Health Regulation No. 02 of 2023. Based on this, the study was conducted to evaluate the bacteriological quality of DAMIU in Simpang Tiga, Samarinda City..

METHOD

This study employed a quantitative approach with a descriptive observational design. Based on preliminary data collection, there were 13 Refillable Drinking Water Depots (DAMIU) using ultraviolet (UV) technology in Simpang Tiga Village, Loa Janan Ilir Subdistrict, Samarinda City. A total of eight depots met the study criteria and were selected as the sample. Inclusion criteria included depots that were still actively operating, used a UV treatment system, were located within the study area, and were willing to participate. Samples were collected directly from the depot's filling faucets from November 24 to December 3, 2025. Sample testing was conducted at the Samarinda City Regional Health Laboratory (UPTD).

Bacteriological quality testing was conducted using the 3-3-3 Most Probable Number (MPN) method to determine the levels of total coliforms and *Escherichia coli*. During the presumptive test phase, samples were aseptically inoculated into Lauryl Tryptose Broth (LTB) medium supplemented with Durham tubes. The test used three series of inoculum volumes—10 mL, 1 mL, and 0.1 mL—with each series consisting of three tubes. All tubes were incubated at 35–37 °C for 24–48 hours. A positive result was determined by the formation of gas in the Durham tubes.

Tubes that tested positive in the screening test were subjected to a confirmatory test using Brilliant Green Lactose Bile Broth (BGLB) medium. The cultures were incubated at 35–37 °C for 24–48 hours. Gas formation in the Durham tube is used as an indicator of Total Coliforms. A follow-up test recorded for the identification of *E. coli* is the indole test using Tryptone Water medium. Cultures are incubated at 44–45 °C for 24–48 hours, after which 3–5 drops of Kovac's reagent are added. The formation of a red ring on the surface of the medium indicates a positive indole reaction. However, a positive indole reaction alone does not definitively confirm the species identity of *E. coli*; therefore, final identification must be based on the confirmation criteria specified in the laboratory procedure being used.

The Most Probable Number (MPN) method is a statistical estimation technique used to estimate the concentration of microorganisms based on a combination of the number of tubes showing positive and negative reactions at specific inoculum volumes. The value is determined using an MPN index table corresponding to a design of three series of tubes, each consisting of three tubes with inoculum volumes of 10 mL, 1 mL, and 0.1 mL. Based on the test summary, the Depot C sample showed a positive

tube combination of 3-2-0 for Total Coliforms and 2-0-0 in the test reported as *Escherichia coli*, with values of 93 MPN/100 mL and 9.2 MPN/100 mL, respectively. Meanwhile, the other seven samples showed a 0-0-0 tube combination in the screening test. According to the test results report from the Samarinda City Regional Health Laboratory (UPTD), all seven samples were reported to have results of <3.0 MPN/100 mL. This value indicates that the results are below the method's reporting limit, not that the bacterial count is exactly zero. The laboratory report also states that results of <3.0 MPN/100 mL are considered equivalent to zero for assessment purposes..

When interpreting these values, it is important to consider the method's detection limit and reporting criteria, as the MPN is a statistical estimate rather than a direct count of bacteria. For guidance on interpreting MPN tables, refer to Blodgett (2023); however, the tables and reporting limits used in actual testing must be verified against the laboratory's documentation.

The microbiological tests documented in this study included a preliminary test using Lauryl Tryptose Broth (LTB), a confirmatory test for total coliforms using Brilliant Green Lactose Bile Broth (BGLB), and an indole test using Tryptone Water as a follow-up

test for bacteria suspected to be *Escherichia coli*. The conduct of public health laboratory testing adheres to service and quality assurance standards as stipulated in the Decree of the Minister of Health of the Republic of Indonesia No. HK.01.07/MENKES/1801/2024.

The test results were analyzed descriptively by presenting the values of both parameters for each sample and the percentage distribution of samples that met or did not meet the microbiological requirements for drinking water. The assessment is based on Regulation of the Minister of Health of the Republic of Indonesia No. 2 of 2023, taking into account the differences in principles and units of reporting between MPN results and quality standards expressed in CFU/100 mL.

RESULT

Bacteriological quality testing was conducted on eight samples of refillable drinking water from a depot in Simpang Tiga Village, Samarinda City, from November 24 to December 3, 2025. The testing used the 3-3-3 Most Probable Number (MPN) method to assess the parameters of Total Coliforms and *Escherichia coli*. Test results were reported in MPN/100 mL units in accordance with the laboratory report. The evaluation was conducted in accordance with the microbiological requirements for drinking water as stipulated in Regulation of the Minister of Health of the Republic of Indonesia No. 2 of 2023, which sets the maximum

levels for both parameters at 0 CFU/100 mL

Table 1. Total Coliform (MPN/100 mL)

Sample Code	Total Coliform (MPN/100 mL)	Category
Depot A	<3,0	Eligible
Depot B	<3,0	Eligible
Depot C	93	Ineligible
Depot D	<3,0	Eligible
Depot E	<3,0	Eligible
Depot F	<3,0	Eligible
Depot G	<3,0	Eligible
Depot H	<3,0	Eligible

Source: (Primary data from laboratory tests, 2025)

Based on Table 1, seven of the eight refillable drinking water samples namely Depot A, B, D, E, F, G, and H showed Total Coliform results of <3.0 MPN/100 mL, as stated in the laboratory test report. Meanwhile, the sample from Depot C showed a Total Coliform value of 93

MPN/100 mL, thus failing to meet the microbiological requirements for that parameter. This result indicates the presence of coliform bacterial contamination in the Depot C sample. However, the source of the contamination could not be determined based on the tests conducted.

Table 2. Results of Escherichia coli Testing at DAMIU.

Sample Code	<i>E. coli</i> (MPN/100 mL)	Category
Depot A	<3,0	Eligible
Depot B	<3,0	Eligible
Depot C	9,2	Ineligible
Depot D	<3,0	Eligible
Depot E	<3,0	Eligible
Depot F	<3,0	Eligible
Depot G	<3,0	Eligible
Depot H	<3,0	Eligible

Source: (Primary data from laboratory tests, 2025)

Based on Table 2, seven refilled drinking water samples namely, Depot A, B, D, E, F, G, and H showed Escherichia

coli levels of <3.0 MPN/100 mL. In contrast, the sample from Depot C showed a value of 9.2 MPN/100 mL, thus failing to meet the microbiological requirements for

the E. coli parameter. These findings indicate the presence of fecal contamination in the Depot C sample based on the identification reported by the

laboratory. However, the source and mechanism of contamination could not be determined from these test results.

Table 3. Distribution of Bacteriological Quality of DAMIU Based on the Parameters Tested

Category	Result	
	Sample	%
Eligible	7	87,5%
Ineligible	1	12,5%
Total	8	100%

Source: (Primary data from laboratory tests, 2025)

Based on Table 3, seven out of eight refilled drinking water samples (87.5%) were categorized as meeting the requirements based on the Total Coliform and Escherichia coli parameters tested. Meanwhile, one sample (12.5%), namely Depot C, did not meet the requirements because it showed a Total Coliform value of 93 MPN/100 mL and an E. coli value of 9.2 MPN/100 mL. This classification

follows the interpretation in the laboratory report, which states that results <3.0 MPN/100 mL are considered equivalent to zero for assessment purposes. These findings only reflect the bacteriological quality based on these two parameters at the time of testing and cannot yet be used to draw conclusions regarding the overall safety of the drinking water

DISCUSSION

The test results showed that most of the refilled drinking water samples had indicator bacteria levels below the laboratory reporting threshold, while one sample tested positive for both parameters examined. These differences indicate variations in the bacteriological quality of refilled drinking water in the Simpang Tiga area of Samarinda City. These findings suggest that the use of the same treatment system does not always result in uniform microbiological test results. However, these differences cannot yet be directly attributed to the treatment or sanitation conditions at the refill stations, as these factors were not specifically examined.

These findings can be discussed in conjunction with the study by Wijaya and Wijayanti (2022) in the service area of the Grogol Community Health Center, Sukoharjo Regency, which also reported the presence of refillable drinking water depots that did not meet bacteriological requirements. Yunus (2018) also reported the presence of coliform contamination in refillable drinking water in Palopo City based on testing using the Most Probable Number (MPN) method. Although the locations and characteristics of the tests differ, these findings indicate that the bacteriological quality of refillable drinking water products still requires attention. This comparison is descriptive in nature because differences in sample size,

station conditions, and testing procedures can influence results across locations.

The presence of Total Coliforms is a general indicator that can provide insight into the microbiological condition of drinking water. A positive result for this parameter can serve as a basis for further investigation into the treatment process, equipment cleanliness, and the possibility of recontamination after treatment. However, total coliforms cannot be used as direct evidence of fecal contamination because this group of bacteria can also be found in non-fecal environments. Therefore, their presence is more appropriately interpreted as an indicator of the need for attention to the microbiological quality control of the product.

Unlike Total Coliforms, *Escherichia coli* is a more specific indicator of potential fecal contamination. A positive result reported in one of the samples is a cause for concern because it indicates a potential problem with the microbiological quality of drinking water. However, the interpretation of *E. coli* identification must still take into account laboratory confirmation procedures. The available documentation lists the indole test as a follow-up test, whereas a positive indole reaction alone is not sufficient to confirm the species identity of *E. coli*. Therefore, the interpretation of results must be based on the identification procedures used by the testing laboratory. Results that fall below the laboratory's reporting limit also require proper interpretation. The MPN method is a statistical technique for estimating the concentration of microorganisms based on a combination of tubes showing positive and negative reactions. Therefore, results

reported below the reporting limit cannot be interpreted as the absolute absence of bacteria. This interpretation is important to avoid stating that a sample is completely free of microorganisms based solely on the results of testing these two parameters.

All water refilling stations in this study used an ultraviolet (UV) treatment system. A study by Tominik et al. (2018) examined the bacteriological quality of refilled drinking water in reverse osmosis (RO) and UV treatment systems. However, this study did not compare water quality before and after treatment nor examine the operational parameters of the UV system. Therefore, variations in bacteriological results among water refilling stations cannot be used to determine the effectiveness or failure of the UV technology employed.

The source of contamination in samples that tested positive remains unconfirmed. Theoretically, raw water conditions, the treatment process, equipment cleanliness, operator hygiene, and the possibility of recontamination may be related to the bacteriological quality of refillable drinking water. However, these relationships cannot be proven based solely on the results of testing just two microbiological parameters. Additional examinations of treatment conditions and sanitation hygiene are needed to obtain more complete information regarding the factors that may play a role..

Overall, these findings highlight the importance of regularly monitoring the bacteriological quality of refillable drinking water. Testing for total coliforms and *E. coli* can provide information on the presence of indicator bacteria in the final product, but does not fully reflect all aspects of drinking water safety. Repeated

monitoring, testing of other water quality parameters, and evaluation of the management conditions at the distribution centers are necessary to support a more comprehensive oversight of drinking water quality.

LIMITATIONS OF THE RESEARCH

This study was limited to testing for total coliforms and *Escherichia coli* in eight samples of refillable drinking water collected during a single sampling period. Repeated sampling, raw water testing, measurement of UV operational parameters, and standardized assessments of depot hygiene and sanitation were not conducted. Furthermore, documentation regarding specific *E. coli* confirmation procedures, the performance of follow-up tests, and laboratory quality control is not yet fully available. Although the reporting thresholds are listed in the laboratory report, the details of the standard operating procedures (SOPs) and the MPN index tables used still require verification. These limitations should be taken into account when interpreting and generalizing the findings.

AUTHOR CONTRIBUTION STATEMENT

Anggie Putri Setiawan was fully responsible for the conceptualization of the research, the development of the methodology, sample data collection, laboratory testing, and the writing of the initial draft of the manuscript. Tiara Dini Harlita served as the primary advisor, validated the methodology, and supervised the conduct of the research. Sresta Azahra and Dr. Agus Evendi served as examiners and co-advisors, providing critical feedback, validating the final data, and making substantive revisions to the

manuscript. All authors have read and approved the final published version of the manuscript.

CONFLICT OF INTEREST

The authors declare that there is no potential conflict of interest whether financial, institutional, or personal with any party that could influence the results or objectivity of this study..

STATEMENT ON THE USE OF AI AND AI-BASED TECHNOLOGY

The author uses artificial intelligence (AI) tools solely to assist with grammar, improve writing structure, and edit drafts. The author bears full responsibility for the accuracy of the content, the validity of the empirical data, and the scientific integrity of the entire manuscript.

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