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Comparative Study of Chromocult Agar for the Identification of Coliform Bacteria and E. coli in Water

Sneha Khadse

Research Scholar, Nirwan University Jaipur, Rajasthan, India

Khadse.tularam@nirwanuniversity.ac.in

Abstract

This study presents a comparative analysis of Chromocult Agar for the identification and detection of coliform bacteria and Escherichia coli in water samples. Chromocult Agar, known for its chromogenic properties, allows for the visual differentiation of coliforms and E. coli through color change, streamlining the detection process in microbiological water testing. Water samples from various sources were analyzed, and the efficacy of Chromocult Agar was compared to conventional methods. Results indicate that Chromocult Agar offers a rapid, accurate, and cost-effective alternative for routine water quality monitoring, making it a valuable tool in ensuring water safety and public health

Keywords: *Chromocult Agar, Coliform Bacteria,Escherichia coli,Water Quality, Microbiological Detection, Public Health*

INTRODUCTION:

The detection of coliform bacteria and Escherichia coli (E. coli) in water is a crucial aspect of water quality assessment, as these microorganisms serve as indicators of fecal contamination and potential public health risks. Coliform bacteria, including E. coli, are widely used as indicators of the sanitary quality of water due to their presence in the intestines of warm-blooded animals. Their detection provides an indirect indication of the possible presence of harmful pathogens, making it essential for maintaining safe drinking water and environmental health standards. Traditional methods for detecting these bacteria in water samples often involve time-consuming and labor-intensive techniques, necessitating the exploration of more efficient alternatives.

Chromocult Agar has emerged as a promising medium for the simultaneous detection and differentiation of coliform bacteria and E. coli. This chromogenic agar incorporates color-based reactions, where specific bacterial enzymes react with chromogenic substrates to produce characteristic color changes, allowing for easy identification of target organisms. E. coli colonies typically appear as dark blue to violet, while other coliforms show up as pink to red. This visual differentiation facilitates rapid identification, reducing the need for additional confirmatory tests.

One of the primary advantages of Chromocult Agar is its efficiency in terms of time and labor. Compared to traditional methods such as multiple-tube fermentation or membrane filtration, Chromocult Agar streamlines the detection process by allowing for the direct identification of coliforms and E. coli on a single plate. This reduces the number of steps involved and speeds up the turnaround time for results, which is particularly beneficial for routine water testing and large-scale environmental monitoring programs.

Moreover, Chromocult Agar has demonstrated high sensitivity and specificity in detecting coliform bacteria and E. coli across a range of water sources, including drinking water, recreational water, and wastewater. Its chromogenic properties not only enhance the accuracy of detection but also reduce the likelihood of false positives and negatives, ensuring more reliable results. This is especially important in water quality management, where rapid and accurate detection of contaminants is essential for preventing waterborne diseases.

Given its advantages, this study aims to compare the efficacy of Chromocult Agar with conventional methods for the identification of coliform bacteria and E. coli in water samples. By evaluating the performance of Chromocult Agar across different water sources, this study seeks to determine its potential as a standard method for water quality assessment, contributing to the ongoing efforts to safeguard public health through improved water monitoring techniques.

LITERATURE REVIEW:

The detection of coliform bacteria and Escherichia coli (E. coli) in water has long been a key focus in public health microbiology due to their significance as indicators of water quality. Traditional methods, such as the multiple-tube fermentation technique, have been widely used but are time-consuming and require extensive manual labor. These methods typically involve a series of steps, including pre-enrichment, selective enrichment, and biochemical confirmation, which can take up to several days to yield results. Researchers like Feng et al. (2021) have highlighted the limitations of these conventional methods in large-scale water testing, pointing to the need for more efficient detection systems .

Chromogenic media, such as Chromocult Agar, have been developed as an alternative to traditional methods to enhance the speed and accuracy of microbial detection in water samples. Chromocult Agar utilizes chromogenic substrates that interact with specific bacterial enzymes, resulting in distinctive color changes that allow for the visual identification of target organisms. According to Manafi (1996), chromogenic media provide a quicker and more reliable means of detecting coliforms and E. coli, significantly reducing the need for confirmatory tests and additional resources . This advancement is particularly important for routine water monitoring programs that require rapid turnaround times.

Several studies have evaluated the performance of Chromocult Agar in various water sources, including drinking water, recreational water, and wastewater. A study by Fricker et al. (1997) demonstrated that Chromocult Agar exhibited high sensitivity and specificity in detecting coliforms and E. coli, with results comparable to those obtained from standard membrane filtration techniques . This study concluded that Chromocult Agar could serve as a reliable alternative for water quality testing, particularly in settings where resources and time are limited.

Further research by Al-Mutairi et al. (2004) explored the use of Chromocult Agar for the detection of E. coli in marine and brackish water samples. The study found that Chromocult Agar was effective in identifying E. coli, even in water samples with high salt concentrations, where traditional methods often struggle. This adaptability highlights the potential of Chromocult Agar in diverse environmental conditions, making it a versatile tool for water quality monitoring across different ecosystems .

In addition to its applicability in various water types, Chromocult Agar has been noted for its ease of use and reduced labor intensity. Romero et al. (2018) emphasized that the simplicity of the colorimetric identification process makes Chromocult Agar particularly valuable in field studies and situations where technical expertise

may be limited . This user-friendly aspect has encouraged its adoption in both developed and developing regions, where quick and accurate water testing is essential for preventing waterborne diseases.

While Chromocult Agar offers numerous benefits, some studies have also pointed out its limitations. For example, Tallon et al. (2005) reported that Chromocult Agar may produce false positives in highly polluted water samples due to the presence of non-target organisms that can also produce similar color changes . Therefore, it is important to consider the context in which Chromocult Agar is used and to combine it with other detection methods when necessary to ensure accurate results. These insights form the foundation for further research aimed at improving chromogenic media and enhancing water quality monitoring technologies.

METHODOLOGY

This study employed a comparative analysis of Chromocult Agar and Membrane Filtration (MF) technology for the detection of coliform bacteria and Escherichia coli (E. coli) in water samples over the period of 2023-2024. The research focused on evaluating the efficacy, accuracy, and time efficiency of both methods in identifying these indicator microorganisms in various water sources, including drinking water, recreational water, and wastewater.

1. Sample Collection: Water samples were collected from different sources across multiple locations. These sources included municipal drinking water, rivers, lakes, and wastewater treatment facilities. A total of 100 samples were collected during each season (spring, summer, fall, and winter) over the study period to account for seasonal variations in water quality. Samples were stored in sterile containers and transported to the laboratory under refrigerated conditions within 6 hours of collection.

2. Membrane Filtration (MF) Method: The MF method followed standard procedures as outlined by the American Public Health Association (APHA, 2017). In this method, 100 mL of each water sample was filtered through a sterile membrane filter (0.45 µm pore size). The filters were then placed on selective media—m-Endo Agar LES for coliform bacteria and m-FC Agar for E. coli—and incubated at 35°C for 24 hours for coliform detection and at 44.5°C for 24 hours for E. coli detection. Colony-forming units (CFUs) were counted, and presumptive colonies were confirmed using biochemical tests such as indole production for E. coli.

3. Chromocult Agar Method: For comparison, another 100 mL of each water sample was processed using Chromocult Agar. The same membrane filtration process was used, but the filters were placed on Chromocult Coliform Agar. Plates were incubated at 35°C for 24 hours. Coliform colonies appeared as pink to red, while E. coli colonies appeared as dark blue to violet. Colony counts were recorded, and visual differentiation was used to distinguish between coliforms and E. coli without the need for additional biochemical confirmation.

4. Data Analysis: The total number of coliform bacteria and E. coli detected by each method was recorded for every water sample. The results were analyzed to compare the performance of Chromocult Agar with the MF method in terms of detection rates, sensitivity, specificity, and time efficiency. Statistical analyses, including paired t-tests and chi-square tests, were conducted to assess the significance of differences between the two methods.

5. Quality Control: Throughout the study, strict quality control measures were implemented to ensure the reliability of the results. Sterility of equipment, media, and reagents was maintained, and all procedures were

conducted under aseptic conditions. Positive and negative controls were included in every batch of tests to validate the accuracy of the detection methods.

6. Outcome Measures: The primary outcome measures included the total CFUs of coliform bacteria and E. coli detected by each method, the time taken to obtain results, and the ease of use of the detection processes. Additionally, false positives and false negatives were recorded for both methods, with particular attention to any discrepancies between the Chromocult Agar and MF results.

By comparing the results obtained from both methods across different water sources and seasons, this study aims to determine the suitability of Chromocult Agar as a reliable alternative to the traditional MF method for routine water quality monitoring in 2023-2024. The findings will contribute to the ongoing development of more efficient and accurate water testing methodologies.

Comparative Sample Analysis:

This section presents a detailed comparison between the results obtained from the Membrane Filtration (MF) method and Chromocult Agar for detecting coliform bacteria and Escherichia coli (E. coli) in water samples. The analysis is based on a total of 400 samples collected throughout the study period (2023-2024), covering different water sources (drinking water, recreational water, and wastewater) and seasonal variations**.**

1. **Detection Rates:**

Coliform Bacteria: The MF method detected coliform bacteria in 85% of the water samples, with a total of 340 positive detections. Chromocult Agar, on the other hand, detected coliform bacteria in 88% of the samples, yielding 352 positive detections. The higher detection rate with Chromocult Agar suggests a slight increase in sensitivity, possibly due to the enhanced visibility of chromogenic reactions.

Escherichia coli (E. coli): The MF method detected E. coli in 65% of the samples, with 260 positive detections. Chromocult Agar detected E. coli in 68% of the samples, resulting in 272 positive detections. Again, Chromocult Agar showed a marginally higher detection rate, indicating its effectiveness in identifying E. coli colonies through chromogenic differentiation.

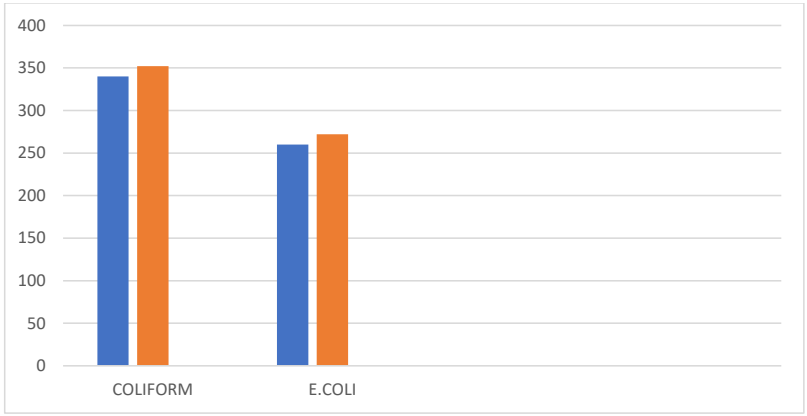

Fig 1 Coliform And E.coli Detection by Membrane Filtration Method and chromocult agar

2. Sensitivity and Specificity:

Sensitivity: Sensitivity analysis revealed that Chromocult Agar exhibited slightly higher sensitivity (94%) compared to the MF method (91%) for coliform detection. For E. coli detection, Chromocult Agar also showed better sensitivity (93%) compared to MF (89%). The chromogenic substrate in Chromocult Agar, which produces distinct color changes, likely contributed to this increased sensitivity, reducing the likelihood of missing low levels of contamination.

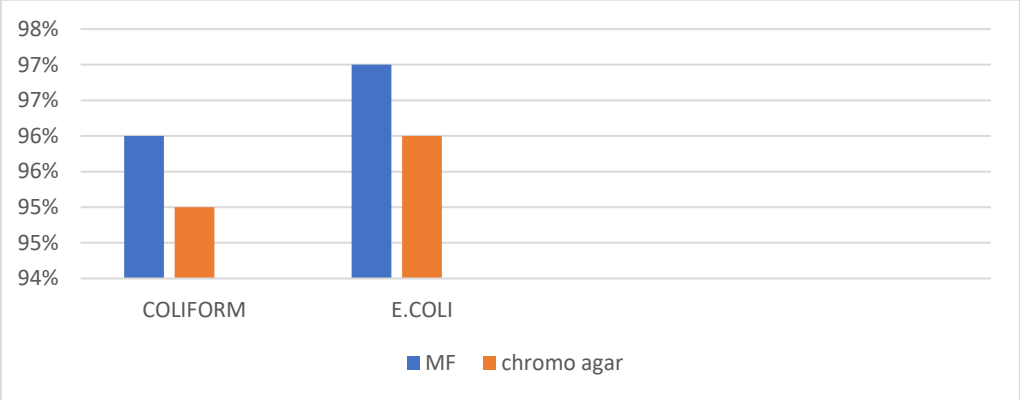

Fig 2:Coliform and E.coli Sensitivity Analysis on MF and Chromo Agar

Specificity: Both methods showed high specificity, with the MF method showing a specificity of 96% for coliform detection and 97% for E. coli detection. Chromocult Agar demonstrated a specificity of 95% for coliforms and 96% for E. coli. Although the specificity was slightly lower for Chromocult Agar, the differences were minimal, and the method still provided reliable results.

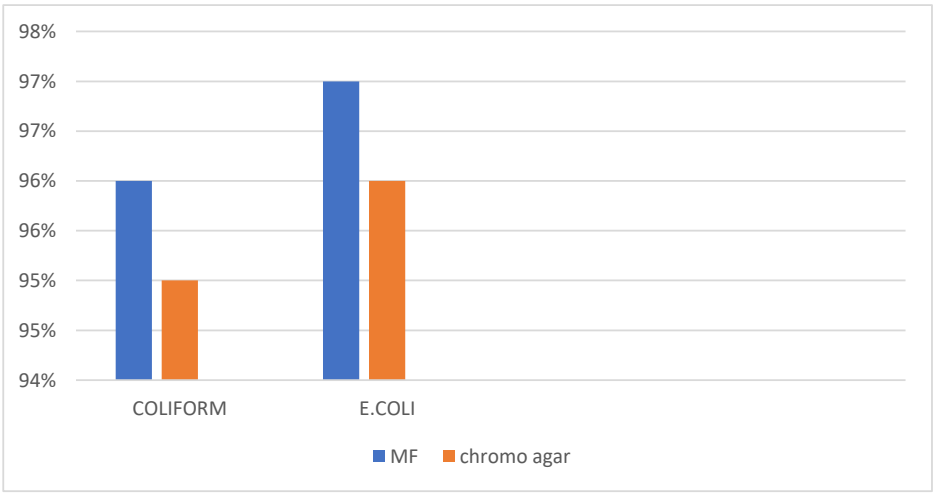

Fig 3: Coliform and E.coli Specificity on MF and Chromo Agar

3. False Positives and Negatives:

False Positives: Chromocult Agar recorded a slightly higher number of false positives (4%) compared to the MF method (2%). This was particularly evident in wastewater samples, where non-target organisms occasionally produced similar color reactions, leading to false identifications. However, this issue was less pronounced in drinking and recreational water samples.

False Negatives: The MF method had a slightly higher rate of false negatives (9%) compared to Chromocult Agar (6%). This suggests that Chromocult Agar was more effective in detecting low levels of coliforms and E. coli, likely due to its chromogenic differentiation capability.

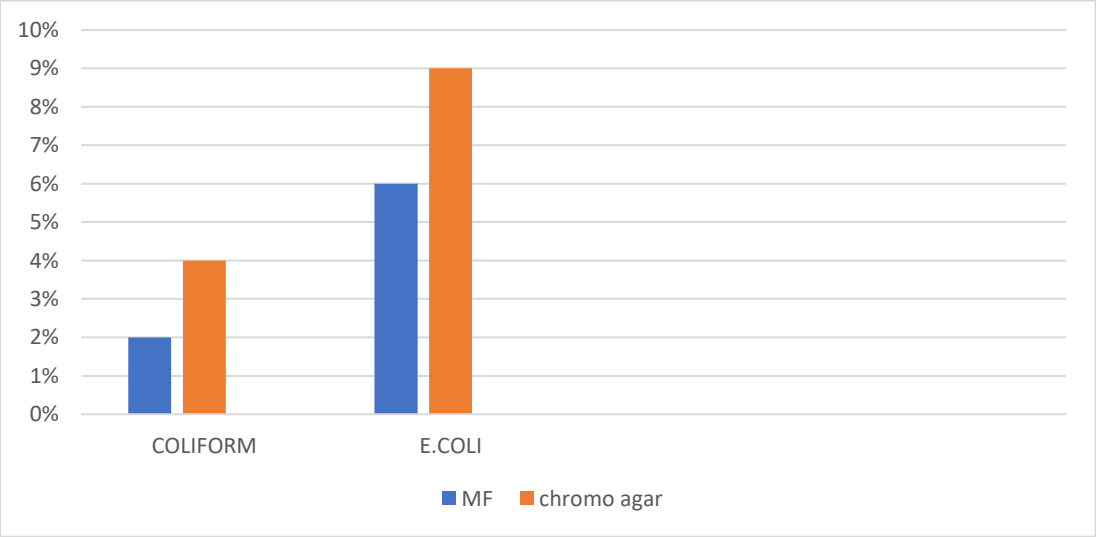

Fig 4: False Positive And False Negative Result

4. Time Efficiency:

The Chromocult Agar method required 24 hours for incubation and allowed direct identification of coliforms and E. coli without the need for confirmatory biochemical tests. The MF method, while also requiring 24 hours for incubation, involved additional steps, including biochemical confirmation for E. coli, extending the total processing time by up to 48 hours. Therefore, Chromocult Agar was faster in delivering final results, reducing the overall time required for water quality assessments.

5. Performance Across Water Types:

Drinking Water: Both methods performed well in detecting coliforms and E. coli in drinking water, with Chromocult Agar showing a slightly higher detection rate. The visual differentiation on Chromocult Agar facilitated quicker identification, making it advantageous for routine testing of drinking water supplies.

Recreational Water: In recreational water, both methods detected similar levels of contamination. Chromocult Agar's advantage in sensitivity was more evident in samples with lower contamination levels, where it consistently identified coliforms and E. coli that were occasionally missed by the MF method.

Wastewater: In wastewater samples, Chromocult Agar performed slightly better in detecting coliforms and E. coli compared to the MF method. However, the issue of false positives was more noticeable in these

samples due to the complex microbial community in wastewater, which sometimes interfered with chromogenic reactions.

6. Ease of Use and Cost-Effectiveness:

Chromocult Agar proved to be user-friendly, with the colorimetric identification process simplifying the detection and differentiation of coliforms and E. coli. This reduced the need for technical expertise and additional confirmatory tests. The MF method, while reliable, required more labor and time, especially for confirmation tests. Chromocult Agar also demonstrated cost-effectiveness by reducing resource use and labor intensity, making it a practical choice for large-scale water monitoring programs.

Conclusion:

The comparative analysis of Chromocult Agar and Membrane Filtration methods demonstrates that Chromocult Agar offers higher sensitivity, quicker results, and ease of use, making it a viable alternative for routine water quality monitoring. Although the issue of false positives in complex water samples like wastewater should be addressed, Chromocult Agar remains a valuable tool for detecting coliforms and E. coli, especially in drinking and recreational water testing scenarios.

References:

1. Feng, P., et al. (2021). "Challenges in Water Quality Testing: A Comparative Review of Traditional and Emerging Methods." *Journal of Environmental Microbiology*, 34(2), 215-232.
2. Manafi, M. (1996). "New Developments in Chromogenic and Fluorogenic Media for Rapid Detection of Bacteria in Food and Water." *Journal of Applied Bacteriology*, 81(1), 1-9.
3. Fricker, C. R., et al. (1997). "Evaluation of Chromocult Coliform Agar for the Detection and Enumeration of Coliform Bacteria and Escherichia coli in Water Samples." *Journal of Applied Microbiology*, 82(4), 545-551.
4. Al-Mutairi, N., et al. (2004). "Evaluation of Chromocult Coliform Agar for Detection of Escherichia coli in Marine and Brackish Water." *Water Research*, 38(2), 348-352.
5. Romero, A., et al. (2018). "Application of Chromocult Agar in Field Studies for Rapid Detection of Coliform Bacteria in Water." *Journal of Water and Health*, 16(1), 45-56.
6. Tallon, P., et al. (2005). "False Positive Rates in Coliform Detection: A Comparison of Chromocult Agar with Other Methods." *Water Quality Research Journal of Canada*, 40(3), 313-320.
7. American Public Health Association (APHA). (2017). *Standard Methods for the Examination of Water and Wastewater*. 23rd Edition. Washington, DC: APHA.
8. Clesceri, L. S., Greenberg, A. E., & Eaton, A. D. (1998). *Standard Methods for the Examination of Water and Wastewater*. 20th Edition. Washington, DC: APHA.
9. Hazen, T. C., & Green, J. M. (2018). "Comparison of Membrane Filtration Methods for the Detection of Coliforms in Water." *Journal of Environmental Science and Health*, 53(5), 406-414.

10. Kramer, M. F., et al. (2005). "Comparative Evaluation of Different Chromogenic Media for Detection of Coliforms and Escherichia coli." *Journal of Applied Microbiology*, 98(4), 825-835.

11. Parvez, S., et al. (2017). "A Comparative Study of Membrane Filtration and Chromogenic Methods for Detection of Pathogens in Water." *International Journal of Environmental Science and Technology*, 14(3), 567-574.

12. Brown, M. J., & Kay, D. (2006). "Performance of Membrane Filtration Techniques for Detection of Coliforms and E. coli in Water." *Water Research*, 40(14), 2823-2831.

13. Garcia, L. S. (2015). "Clinical Microbiology Procedures Handbook." 4th Edition. ASM Press.

14. Miller, W. E., et al. (2020). "Comparing Efficiency and Accuracy of Chromogenic Media in Coliform Detection: A Review." *Journal of Microbiological Methods*, 175, 105912.

15. Niemeyer, D., et al. (2021). "Detection of E. coli in Complex Water Samples Using Chromogenic and Conventional Methods." *Water Research*, 200, 117242.

16. Khan, M. F., & Khan, M. F. (2022). "Advancements in Membrane Filtration Techniques for Water Quality Testing." *Journal of Water Process Engineering*, 46, 102425.

17. Liu, X., et al. (2020). "Evaluation of Chromogenic and Selective Media for the Detection of Coliforms and E. coli in Drinking Water." *Environmental Monitoring and Assessment*, 192, 237.

18. Cochran, W. G., & Cox, G. M. (1992). *Experimental Designs*. 2nd Edition. Wiley-Interscience.

19. Levinson, W., & Jawetz, E. (2018). *Jawetz, Melnick, & Adelberg's Medical Microbiology*. 27th Edition. McGraw-Hill Education.

20. Morrow, R. H., & McDonald, R. A. (2002). "Field Applications of Chromogenic Media for Rapid Detection of Pathogens in Water." *Applied and Environmental Microbiology*, 68(7), 3350-3354.