A Novel Approach for the Detection of Coliforms and Other Pathogenic Bacteria in Drinking Water

Bhattacharyya Sayan*

Professor, Department of Microbiology, AIIH&PH, Kolkata, India

ABSTRACT

Drinking water is an essential requirement for human health, but contamination by microorganisms can create a serious public-health risk. Among the most important microbiological indicators of drinking-water quality are total coliform bacteria and *Escherichia coli* (E. coli), which can indicate contamination from environmental sources or, particularly in the case of E. coli, recent fecal pollution. Conventional microbiological methods are useful but may require considerable time, multiple culture steps, and specialized laboratory facilities. This study proposes the application of a rapid membrane-filtration and differential detection approach for investigating coliforms and selected pathogenic bacteria in 50 drinking-water samples. The proposed approach combines membrane filtration with selective and differential culture media, allowing the detection and enumeration of indicator organisms and facilitating subsequent identification of suspected pathogens. EPA Method 1604, for example, uses membrane filtration and MI medium to simultaneously detect total coliforms and E. coli through enzyme-based reactions and can provide results within approximately 24 hours.

The study design includes the collection of 50 drinking-water samples from different sources, microbiological examination, appropriate positive and negative controls, colony enumeration, and confirmatory identification of suspected bacterial isolates. The principal objective is to determine the microbiological quality of the sampled drinking water and evaluate whether the proposed rapid testing approach can provide useful information about fecal contamination and the potential presence of pathogenic bacteria. The study may also help identify differences in microbiological quality between treated, household-stored, bottled, groundwater, or other drinking-water sources.

Keywords: Drinking water; Coliforms; Escherichia coli; Pathogenic bacteria; Membrane filtration; Microbiological quality; Rapid detection; Water contamination

***Correspondence to**: Dr. Bhattacharyya Sayan, UHU&TC Chetla under AIIH & PH Kolkata, India

Received: Oct 03, 2026; **Accepted**: Oct 16, 2026; **Published**: Oct 30, 2026

Citation: Sayan B (2026) A Novel Approach for the Detection of Coliforms and Other Pathogenic Bacteria in Drinking Water. Research J Infect Dis Ther 1:102. **DOI**: https://doi.org/10.61309/rjidt.1000102

Copyright: ©2026 Sayan B. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

INTRODUCTION

Safe drinking water is the cornerstone of public health and is also essential for life. Safe and readily available water is important for public health, whether it is used for drinking, domestic use, food production or for recreational purposes [1]. Although water may appear clear, colorless, and odorless, it can contain microorganisms that are capable of causing gastrointestinal and other infectious diseases. Bacteria, viruses, dissolved heavy metals, pesticides and certain industrial pollutants may be invisible and

cannot necessarily be identified through taste, smell or color [2]. Microbial contamination may enter water through sewage leakage, creation of vacuum and intermittent water supply, inadequate sanitation, animal waste, contaminated groundwater, damaged distribution systems, poor household storage, or insufficient treatment [3].

Microbiological examination of drinking water therefore plays an important role in determining whether water is suitable for human consumption or not. Direct examination for every possible pathogen is impractical because drinking water can potentially contain numerous bacterial, viral, and protozoan pathogens. For this reason, microbiological monitoring commonly relies on indicator organisms, especially *E. coli* and coliform bacteria. Total and fecal coliforms are good indicators of recent contamination of drinking water [4].

The World Health Organization identifies *E. coli* as the preferred indicator of fecal contamination of drinking water. For water directly intended for drinking, its guideline value is that *E. coli* or thermotolerant coliform bacteria should not be detectable in a 100 mL sample.

Total coliforms are a broader group. Their detection does not necessarily prove fecal contamination because some members like *Klebsiella* spp. and *Enterobacter* spp. can occur naturally in soil, vegetation, and water. Nevertheless, an elevated coliform count can indicate inadequate treatment, contamination after treatment, or problems within the water-distribution or storage system. Faeces can contain a wide range of pathogens, which when introduced to the environment may be able to persist for variable amounts of time, often at concentrations that are too low for reliable detection but nevertheless hazardous to human health [5]. *E. coli* provides a more specific indication of fecal pollution of drinking water because it is strongly associated with the intestinal tract of humans and other warm-blooded animals.

Traditional drinking water-testing approaches include multiple-tube test, membrane filtration, and enzyme-substrate methods [6]. Modern differential methods attempt to reduce the time and labor required to identify indicator organisms. One example is EPA Method 1604, which uses a membrane filter and MI medium to detect total coliforms and *E. coli* simultaneously on the basis of specific enzyme activities. The present study is designed around 50 drinking-water samples and focuses on the development or application of a rapid, practical microbiological screening strategy of drinking water. In addition to coliforms and *E. coli*, suspected pathogenic bacterial isolates can be subjected to appropriate confirmatory identification using established microbiological or molecular methods. Newer methods are necessary, since old methods may be time-consuming or expensive, besides being non-specific.

A major challenge in drinking-water microbiology is obtaining reliable information quickly enough to allow corrective action. A method that can simultaneously screen for indicator organisms and provide presumptive differentiation of important bacterial groups may be particularly useful for routine surveillance.

Membrane filtration is attractive for drinking-water analysis because relatively large volumes of water can be examined. Multiple tube test, also called presumptive coliform test, and multiple tube fermentation method (MTFM) , is an old test that detects total coliforms but is laborious and time consuming [7]. EPA Method 1604 describes filtration

of an appropriate water volume, commonly 100 mL for drinking water, through a 0.45-µm membrane, followed by incubation on MI medium. The method detects total coliforms and *E. coli* using different enzyme-associated reactions. A study involving 50 samples provides an opportunity to compare microbiological quality among samples and to assess the practical performance of the testing strategy. The results could be expressed as colony-forming units per 100 mL (CFU/100 mL) for organisms that are quantitatively enumerated.

Importantly, the presence of an indicator organism should not automatically be interpreted as proof that a specific pathogen is present. Indicator organisms provide evidence about water quality and possible contamination. Suspected pathogenic bacteria require appropriate confirmatory testing.

The main objective of this study was to investigate the microbiological quality of 50 drinking-water samples using a rapid culture-based detection approach for coliforms and selected bacterial contaminants and compare with conventional multiple tube test. It was done as an initial observational study or pilot study.

The specific objectives were:

I. To detect total coliform bacteria in 50 drinking-water samples.
II. To detect and enumerate the lactose fermenters where present.
III. To screen for suspected bacterial pathogens using appropriate selective and differential microbiological techniques.
IV. To compare the conventional tests with the new test
V. To determine the proportion of samples showing evidence of microbiological contamination.
VI. To evaluate the usefulness of the rapid tube-based method approach for routine water-quality screening.

MATERIALS AND METHOD

Study Design: A laboratory-based cross-sectional study was conducted using 50 drinking-water samples. Study period was from May 2025 to February 2026. The samples were selected from different sources, such as municipal supplies, household storage containers, groundwater, bottled water, or other locally relevant drinking-water sources. They came to the laboratory for routine testing for total and fecal coliforms.

For a scientifically stronger study, the source of each sample should be recorded along with the collection date, location code, type of water source, treatment status, and storage conditions.

Each sample received a unique identification number, such as W01 through W50. This prevents confusion during laboratory processing and allows results to be traced back to the relevant sampling point.

Sample Collection: Samples should be collected using sterile, appropriate microbiological sampling containers and transported to the laboratory under validated conditions. The sampling procedure should follow the laboratory's approved drinking-water microbiology protocol.

Care should be taken to avoid contamination of the sample during collection, transportation, and processing. Sample holding time and temperature should be controlled according to the selected standard method because delays or inappropriate storage can alter bacterial numbers and therefore affect the validity of results.

Primary Detection Method: The primary method used was multiple tube test. This approach is well

established for microbiological examination of water. EPA Method 1604 describes the simultaneous detection of total coliforms and *E. coli* using MI medium.

In another approach, bacteria present in a measured water volume are concentrated onto a membrane filter. The membrane is then transferred to an appropriate selective/differential culture medium and incubated under the conditions specified by the validated method. Other tests like tests using ONPG can also be used.

The medium contains substrates that react with enzymes associated with coliform bacteria and *E. coli*. According to EPA Method 1604, fluorescence associated with β-galactosidase activity is also used for total-coliform detection, while a color reaction associated with β-glucuronidase activity helps identify *E. coli*. Here, if lactose fermenters and late lactose-fermenters are present, they will break the ONPG into ortho-nitro phenol, which gives the yellow colour.

The important advantage is that the method provides simultaneous information about two major indicators rather than requiring completely separate primary tests.

New Method: In new method, in small sterile test tube, 2 ml peptone water with and without 1 ONPG disk and Lead acetate paper strip (floating above water in between cotton and tube) was used. 2 ml of the drinking water sample was added to the test tube. Tubes were kept at both 37 ◦C and room temperature. Time required was 6 to 16 hours. In 12 of these tubes, ONPG was used. In the rest, only lead acetate and peptone water were used. Subculture was done after the initial incubation, on MacConkey agar, and later the colonies were identified thence.



Figure 1: Conventional test using multiple tube test.

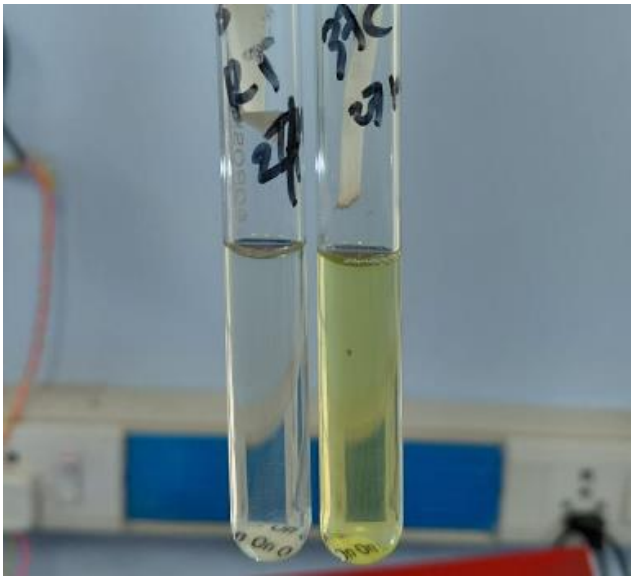


Figure 2: New method with ONPG (showing positive yellow color) and lead acetate paper disk.

ENUMERATION

After incubation, colonies showing the characteristic reactions specified by the selected conventional method were counted. Results were calculated and reported as CFU per 100 mL, depending on the sample volume analyzed.

In the new method, the yellow color formation on tube with ONPG disk and blackening by Lead acetate

paper was calculated. Results correlated well with multiple tube test. Those samples that were found to be not fit by multiple tube test, were also positive in the new test. Thus, the specificity was high. Sensitivity of the new test was also high. Many samples that were negative by MTT also came out to be positive in new method. In new test, turbidity correlated well with MPN of conventional test.

DETECTION OF OTHER BACTERIAL PATHOGENS

New method detected accurately many pathogens like *P. aeruginosa* and H_2S producers (like *Proteus* spp. and *Salmonella* spp.), which were not picked up well by other methods.

The study may include additional screening for bacterial groups such as *Salmonella*, *Shigella*, *Vibrio*, enterococci, or other organisms relevant to the local epidemiology and study objectives. However, these should not be identified solely on the basis of colony appearance.

Suspected isolates should undergo appropriate confirmatory testing in a qualified microbiology laboratory. Confirmation may involve biochemical identification, validated commercial identification systems, MALDI-TOF MS, or molecular assays such as PCR, depending on laboratory capacity and the organism being investigated.

In the new method, the appearance of turbidity was also noted. The grade of turbidity correlated well and directly with the MPN. Mostly there was no variation in color formation at both temperatures. Turbidity, if appearing in 1 tube, came in the other tube also.

INTERPRETATION OF RESULTS

The interpretation of the results could distinguish between total coliform contamination and fecal contamination.

A sample containing total coliforms but no detectable *E. coli* may indicate environmental contamination, inadequate treatment, contamination during storage, or deterioration of the distribution system. It does not necessarily demonstrate recent fecal contamination.

Detection of *E. coli* is more significant because it provides stronger evidence of fecal pollution. The WHO guideline for water directly intended for drinking is that *E. coli* or thermotolerant coliform bacteria should not be detectable in any 100-mL sample.

If *E. coli* is detected, further investigation should be considered, including examination of the source, treatment process, distribution system, and storage practices.

The detection of a suspected pathogen should be treated separately from an indicator-organism result. Confirmatory identification is necessary before concluding that a particular pathogen is present. Here the new test can prove to be helpful. Lactose breakdown is indicated by presence of yellow colour formation by ONPG disk, and Hydrogen sulfide production by blackening of the lead acetate paper. Hydrogen sulfide producers like *Citrobacter freundii* could be identified by the new tube-based method, while conventional multiple tube test failed to identify them. Additionally, results of the new test were comparable at 37 dehrees C as well as room temperature, indicating that this new method can be used in the field settings.

DISCUSSION

Microbiology of drinking water is an important subject. Unsafe drinking water claims many lives every year across the world. Approximately 1.6 million death cases are reported per year because of

waterborne pathogens in the world [8]. Common indicator bacteria for fecal contamination of drinking water are coliforms, *E. coli* and *P. Aeruginosa* [9].

Our proposed new approach has some advantages which are as follows. Firstly, MTT fails to detect bacteria like *Pseudomonas aeruginosa* and *Salmonella* Typhi that are also capable of infecting man by means of drinking water. The n89ew test was able to identify them. *P. aeruginosa* could be identified by diffusible blue-green pigment in upper part of the tube. *Moraxella* spp. could also be identified by subculture. Even *Citrobacter freundii* can be identified by breakdown on ONPG and blackening of the lead acetate paper strip, together. The new test detects them very well. This new test can be commercialized, too. Also, the MPN values of conventional test correlated well with the MacFarland turbidity values in the liquid media of the new test.

Second, differential media can furnish information about more than one group of microorganisms in the same analytical procedure. So, this new test can safely be used as adjunct to existing methods like multiple tube test.

Thirdly, the approach can provide relatively rapid results and can also be commercialized. Enumeration and identification can be done within 24 hours or less under the specified conditions.

Fourthly, numerical results can be reported, like lactose fermenter and Hydrogen sulfide producer is present or not, this making samples easier to compare. Also, since it produces results by 6-16 hours, it can also predict or indicate the presence of possible pathogens which can be detectable by multiple tube test. Fifthly, results were comparable at room temperature as well as at 37°C, indicating that this test can be used in the field settings where incubators may not be available. Finally, the approach can function as a useful screening platform, with suspicious samples subsequently undergoing more specific identification.

LIMITATIONS

Despite its advantages, the method has limitations. Culture-based testing detects organisms that can grow under the selected conditions and therefore may not detect all viable bacteria. Some microorganisms may be stressed, injured, slow-growing, or present in a viable-but-non culturable state. Also, this technique may need subculture for proper identification of the bacteria, so it may necessitate extra time of at least 1 day after the initial procedure.

Coliform detection also does not provide a complete picture of water safety. Viruses, protozoan parasites, and chemical contaminants require different analytical methods. Therefore, a sample that is negative for coliforms should not automatically be considered free from every possible hazard.

Another limitation is that presumptive identification of a bacterial colony is not equivalent to definitive species identification. Confirmatory testing should be performed whenever a clinically or epidemiologically important pathogen is suspected.

The quality of the final result also depends heavily on sample collection, transportation, laboratory technique, controls, and adherence to the validated method.

PUBLIC HEALTH SIGNIFICANCE

Microbiological monitoring of drinking water is important because contamination can occur at multiple stages between the original water source and the consumer. A water treatment plant may produce microbiologically safe water, but contamination can

occur later through damaged pipes, cross-connections, inadequate storage, or unhygienic handling. Outbreaks of waterborne epidemics have occurred in various countries, primarily attributed to pathogenic bacteria [10].

Regular monitoring of indicator organisms can therefore help identify failures before widespread illness occurs.

The WHO emphasizes the importance of *E. coli* as an indicator of fecal contamination and recommends that it not be detectable in 100 ml of drinking water.

A 50-sample investigation can provide a useful snapshot of microbiological quality. However, larger and repeated sampling programs and more studies are needed to establish long-term water-quality patterns. Sampling across different seasons can be especially valuable because rainfall, flooding, temperature, groundwater movement, and changes in water use may influence microbial contamination.

The proposed study of 50 drinking-water samples provides a practical framework for investigating coliform contamination and screening for other bacterial hazards. A rapid tube-based detection strategy can simultaneously provide valuable information about total coliforms and *E. coli*.

The most important advantage of the new approach is its ability to transform an apparently simple water sample into measurable microbiological information. Results can be compared among water sources and used to identify samples requiring further investigation.

However, coliform testing should be understood as an indicator-based assessment rather than a complete examination for every pathogen. Detection of *E. coli* is particularly important because it indicates possible fecal contamination, while total coliforms have a broader environmental significance. Suspected pathogenic bacteria should be confirmed using appropriate laboratory identification techniques.

Overall, a well-controlled investigation involving 50 samples can provide useful evidence about drinking-water quality and can serve as a foundation for a larger surveillance programme.

CONCLUSION

This test is rapid, sensitive and specific and is low-cost also. Combining rapid screening with confirmatory microbiological or molecular identification offers a stronger strategy than relying on a single test. Such approaches can contribute to early detection of contamination, improved water-quality management, and better protection of public health. More studies and more research is required to arrive at any logical or definitive conclusion. This test can act as adjunct to concentional tests.

REFERENCES

1. https://www.who.int/news-room/fact-sheets/detail/drinking-water
2. https://www.firstpost.com/health/clear-water-may-not-be-safe-to-drink-what-tds-ph-and-hidden-contaminants-really-tell-you-ws-e-14040097.html. Last accessed 01/9/26
3. Murei A, Kamika I (2026) Water quality challenges and microbial risk in rural areas: water source, pollution pathways, microbial risk, surveillance gaps, and integrated risk management. Front Water 8:1803894.
4. Holcomb DA, Stewart JR (2020) Microbial Indicators of Fecal Pollution: Recent Progress and Challenges in Assessing Water Quality. Curr Environ Health Rep 7: 311-324.

5. Korajkic A, Wanjugi P, Brooks L, Cao Y, Harwood VJ (2019) Persistence and decay of fecal microbiota in aquatic habitats. Microbiol Mol Biol Rev 83.
6. Rompre A, Servais P, Baudart J, de-Roubin MR, Laurent P (2002) Detection and enumeration of coliforms in drinking water: current methods and emerging approaches. J Microbiol Methods 49: 31-54.
7. Gunawardane MM, Priyadarshini KPR (2025) Decoding the multiple tube fermentation method used in coliform testing. J Multidisciplinary Translational Res 10: 42-49.
8. Zahedi A, Monis P, Deere D, Ryan U (2021) Wastewater-based epidemiology Surveillance and early detection of waterborne pathogens with a focus on SARS-CoV-2 Cryptosporidium and Giardia. Parasitology Res 120: 4167-4188.
9. Oon YL, Oon YS, Ayaz M, Deng M, Li L, et al. (2023) Waterborne pathogens detection technologies: advances, challenges, and future perspectives. Front Microbial. 14:1286923.
10. Ambili A, Sebastian D (2021) Evaluation of sensitivity and cost-effectiveness of molecular methods for the co-detection of waterborne pathogens in India Mar Biotechnol 23: 955-963.