

# Comparison of Methods Between the Multitube Fermentation Method and The Filter Membrane Method for Escherichia Coli in Drinking Water

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**Abstract:** Escherichia coli is a normal parasitic bacterium in the intestinal tract and is an important indicator of the degree of contamination and hygienic quality of water bodies. In this study, 78 samples of domestic drinking water were tested for Escherichia coli by filter membrane method and multi-tube fermentation method. The results showed that the passing rate of water samples tested by the multi-tube fermentation method was higher than that of the filter membrane method, which was statistically significant ( $P < 0.05$ ). In the untreated water samples test, the detection rate of Escherichia coli was 92.31% in the multi-tube fermentation method compared to 71.79% in the filter membrane method, and the difference was statistically significant. The results of the study showed that the detection rate and sensitivity of the multi-tube fermentation method in the microbiological testing of domestic drinking water were higher and better than that of the filter membrane method. The selection of testing methods should consider its applicability and the accuracy of the test results. Meanwhile, factors affecting the quality of microbiological testing include the quality of laboratory personnel, specimen handling, and the quality of instruments and reagents, which need to be carefully managed and trained. This study can provide a practical reference for microbiological testing of domestic drinking water.

**Keywords:** Escherichia coli, domestic drinking water, filtration membrane method, multi-tube fermentation method, test pass rate.

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## 1. Summarize

Escherichia coli is also known as Escherichia coli or common coli it is a normal parasitic bacterium in the intestinal tract of humans and mongoloid animals and serves as the best indicator of stool-like contamination[1]. Coliforms are the most prevalent and abundant group of bacteria in the intestinal tract and are highly resistant. Moreover, the number of coliforms in water is correlated with enteric pathogens, which is an important biological indicator for judging the degree of contamination and the quality of hygiene of a body of water in the international arena. It is of great significance for the timely response to the microbial contamination of drinking water and surface water sources, and for the effective prevention of the outbreak of waterborne diseases[2].

## 2. Information and Methods

### 2.1. General information

The 78 samples of domestic drinking water sent to our center for inspection from May 2020 to November 2022 were used as experimental subjects, and were divided into two groups of 39 samples each based on the inspection method, with the control group adopting the filter membrane method and the observation group adopting the multi-tube fermentation method. The study was carried out in full compliance with the relevant provisions of the Hygienic Standard for Drinking Water, the total number of coliforms in drinking water was  $\leq 100$  CFU/mL, all water samples were tested within the time limit, and the temperature and humidity of the environment met the relevant requirements before the test.

### 2.2. Methodology

#### 2.2.1. Filter membrane method test

Filtration tests were carried out on water samples using a microporous filter membrane (0.45  $\mu\text{m}$ ). During the operation, the membrane was adhered to lactose and then incubated at a constant temperature, during which the membrane produced gas and acid, indicating the presence of total coliforms in the water sample. ① Equipment: micropore filter membrane (0.45 $\mu\text{m}$ ), toothless tweezers, filters, balances, filtration equipment, microscopes, petri dishes, conical flasks, graduated pipettes, slides. ② operation process: the first sterilization, sterilization, sterilization for the membrane sterilization and filter sterilization, membrane sterilization step is to paste the membrane into the beaker, at the same time, distilled water into the beaker, continue to boil the water 3 times, so as to play the sterilization effect, each sterilization time for 10 ~ 15 min. add hot water, the first and second time to change the boiling water, so as to make the container residual reagents to be fully cleared, followed by boiling the third time to change the water, the first and second time, so that the container can be fully cleared, and then the second time to boil the water, the first and third times to change the water, so that the container can be fully cleared. Subsequently, boil the third change into the water; filter sterilization is mainly through the flame high temperature to achieve sterilization effect. During the actual operation, the alcohol cotton ball will be ignited, and the sterilization operation will be carried out, and the sterilization time is usually 20~25 min. Secondly, the water sample needs to be filtered, in this operation, the sterile toothless tweezers should be used to sterilize the filter membrane, put the filter membrane on the sterile filter bed, open the filter, inject 100mL water sample into it, open the filter valve, and carry out the pumping

operation of the water sample, so as to realize the purpose of filtering the water sample. The purpose of filtering water samples can be realized in this way. Finally, the membrane bacteria need to be cultured. This process requires the completion of the filtration of water samples, followed by water samples immediately start pumping, pumping is completed, close the filter valve, the use of sterile toothless tweezers will be sent to the filter membrane magenta sodium sulfite medium, followed by the use of thermostat for its culture.

### 2.2.2. Multi-tube fermentation test

The equipment used in the multi-tube fermentation method was the same as that of the filter membrane method to carry out the culture of total *Escherichia coli* flora. ① The culture solution was made as follows: add 25g of peptone, 15g of lactose, 15g of sodium chloride and 10g of beef paste to 1000mL of distilled water, heat and dissolve it, adjust the pH value to 7.1~7.5, add 2mL of bromocresol violet ethanol solution (concentration of 1.50%) to it, mix it thoroughly and then put it into the test tubes, put them into autoclave, and then put them into autoclave, and then put them into refrigerator for storage and reserve at 115°C. Fully sterilized for 20 min, to ensure that the sterilization is sufficiently removed and stored in the refrigerator. Add 1 mL of drinking water sample to the test tube containing 10 mL of culture solution of single lactose peptone, inject 10 mL of drinking water sample into the test tube containing 10 mL of culture solution of double lactose peptone, and at the same time, put 1 mL of drinking water sample into the test tube containing 9 mL of physiological saline, mix well, and then select 1 mL of the mixture, and then inject 1 mL of culture solution of single lactose peptone into it, if the condition of the drinking water sample is more serious, it may be appropriate to put it in a refrigerator and store it for spare. If the drinking water sample is in serious condition, the dilution can be increased appropriately. After finishing the above operations, the samples were incubated at 36 °C in a thermostat. After 24

hours of incubation, if there is no gas and acid production in the test tube, it indicates that the coliforms are negative; if there is gas and acid production in the test tube, it is necessary to put the test tube into Erythromycin blue agar plate medium. Erythromycin agar plate medium was prepared as follows: 25 g of agar was placed in 500 mL of distilled water, then 8 g of peptone and 1.5 g of dipotassium phosphate were added to it, and heated to dissolve them completely. Then, based on a certain dose, it was filled into a dispensing bottle, cooled, sterilized and solidified. After the transfer, it was again put into the constant temperature box for cultivation, and after the completion of cultivation, it was necessary to carry out colony morphology observation and microscopic verification of the central area with darker staining color.

### 2.3. Observation indicators

In this study, the test microbial flora is mainly *Escherichia coli* to drinking water health standards as the basis for the two test methods of microbiological indicators of water samples detection status, detection rate, pass rate to develop a statistical comparison.

### 2.4. Statistical methods

Data were processed by SPSS 24.0 statistical software, count data were expressed as relative numbers, and comparisons between groups were made using the  $\chi^2$  test,  $P < 0.05$  suggests that the difference is statistically significant.

## 3. Results

### 3.1. Comparison of the passing rate of water samples of the two inspection methods

The passing rate of water sample inspection of multi-tube fermentation method was higher than that of filter membrane method, and the difference was statistically significant ( $P < 0.05$ ). See Table 1.

**Table 1.** Comparison of the passing rate of water samples of the two inspection methods

groups	n	Plant water (n=10)	End-of-network water (n=9)	Secondary water supply (n=20)	Unqualified water (portions)	satisfactory rate
Observation Group	39	9 (23.08)	8 (20.51)	20 (51.28)	2 (5.13)	94.87%
control subjects	39	7 (17.95)	6 (15.38)	17 (43.59)	9 (23.08)	76.92%
$\chi^2$						5.186
P						0.023

### 3.2. Analysis of microbial detection in domestic drinking water

The effect of different water treatments on test results was not considered in this study. During the testing of untreated water samples, the detection rate of *Escherichia coli* was 92.31% (36/39) in the multi-tube fermentation method and 71.79% (28/39) in the filter membrane method, and the microbial detection rate of the multi-tube fermentation method was significantly higher than that of the filter membrane method, and the difference was statistically significant ( $P < 0.05$ ).

## 4. Conclusion and Discussion

The results of this study showed that the detection rate of

*Escherichia coli* was higher in the multi-tube fermentation method than in the filter membrane method ( $P < 0.05$ ), and the difference in the data was significant when compared with the passing rate of the water samples in the two testing methods. It can be seen that the multi-tube fermentation method in the microbiological testing of domestic drinking water detection rate, sensitivity is higher than the filter membrane method. The reason for this is that multi-tube fermentation method is a new type of drinking water testing method, which can clearly identify the *Escherichia coli* and other flora in drinking water, and the quality of water quality identification of high quality, the effect is ideal. For this test method, the accuracy of the results will continue to shrink with the degree of reliability of the data, reagent dilution times and so on. It can be seen that in the microbiological

testing of domestic drinking water, the accuracy of the test results need to be determined by the results of the data. In order to minimize the degree of contamination of drinking water, relevant workers should start from the source, strengthen the supervision of water sources, and do a good job of related missionary education. He Min [3] to 186 samples of domestic drinking water (March 2017 to March 2018) as the object of study, of which 93 in the abundant water period, 93 in the dry water period, respectively, the implementation of the filter membrane method, the multi-tube fermentation method detection, the study found that the multi-tube fermentation method in the dry water period heat-resistant bacterial groups, *Escherichia coli* detection rate [ heat-resistant bacterial groups (94.62%); *Escherichia coli* (91.40%)] than the filter membrane method The detection rate of heat-resistant coliforms and *Escherichia coli* was higher than that of filter membrane method [ heat-resistant coliforms (79.57%); *Escherichia coli* (58.06%)], and the detection rate of heat-resistant coliforms and *Escherichia coli* during the plentiful water period of the multitube fermentation method [ heat-resistant coliforms (80.65%); *Escherichia coli* (96.77%)] was higher than that of the filter membrane method [ heat-resistant coliforms (79.57%); *Escherichia coli* (93.55%)], which showed that both methods were more effective than the filter membrane method in detecting heat-resistant coliforms, and *Escherichia coli*. It can be seen that both methods are suitable for microbiological testing of domestic drinking water, and the multitube fermentation method is better than the filter membrane method. The results of He Min's study were basically the same as those of the present study.

Water is the source of life, and people's lives are closely related, whether it is daily life, or business production activities are inseparable from water. For drinking water, its quality usually has a direct impact on human health and safety[4] . Regular testing of drinking water is the key to ensuring the safety of drinking water and reducing the incidence of related diseases. The World Health Organization survey shows that the number of intestinal infectious diseases caused by water contamination worldwide is up to 1.2 billion, and 4 million children have died due to water contamination diseases. Therefore, it is necessary to strengthen the quality of drinking water inspection and control of the attention is very necessary. And with the advancement of China's industrial process, resulting in more and more serious environmental pollution problems, this situation also has a direct impact on the quality of existing water resources, so that there is a lack of water resources in our country, water quality is poor and other issues, if you do not take timely and effective measures to cope with the problem, it is very likely to have a negative impact on the development of the community[5] . In view of this, the relevant departments should strengthen the detection of drinking water, analyze its problems, and on this basis, the application of targeted strategies, timely control, rectification of contaminated water sources, to prevent serious consequences. In addition, water quality testing can also avoid the spread of epidemics, through the detection of microorganisms in the drinking water, to see whether there are infectious diseases and other adverse factors, from this perspective, the microorganisms in the drinking water testing has a very high social value. Analyzed from the level of detection methods, the current testing methods used are filter membrane method, multi-tube fermentation method, etc. Multi-tube fermentation method mainly refers to the dilution

of water samples to culture colonies, based on the difference in dilution concentration, respectively, the colonies were observed, the number of statistical comparison of the unit volume[6] . Membrane test refers to the filter filtration of water samples, put the filter membrane in the culture medium culture, and then count the number of *E. coli* unit volume of the filter membrane. These two testing methods have their own advantages and disadvantages, compared to the filter membrane method of testing, multi-tube fermentation test higher pass rate, while the filter membrane test is less difficult to operate, higher efficiency, both methods are suitable for drinking water microbiological testing work . However, the comparison of test results show that in the untreated water samples test, multi-tube fermentation bacterial detection rate is higher than the filter membrane method, and in the detection of drinking water, compared with the filter membrane method, multi-tube fermentation detection of water quality pass rate is higher, on behalf of the multi-tube fermentation test is more sensitive, can be more accurately detected bacterial colonies.

This paper is based on continuous optimization measures for drinking water microbiological testing work to carry out a more in-depth analysis, found that the factors affecting the quality of microbiological testing mainly include the following points. ① laboratory personnel's ability and quality factors. As microbiological testing work on the quality of testing personnel, skills level requirements, if the testing personnel skills are rusty or less experience, it will inevitably have a certain impact on the quality of test results. ② specimen factors. If the water sampling personnel are difficult to send samples to the laboratory in a timely manner, easy to make the sample contaminated by external factors, or improper storage makes the death of bacteria, etc., will affect the quality of the test. So the pre-test quality assurance work on the quality of the test there is a critical impact, the CDC or the relevant departments need to strengthen the importance of this area, targeted training to reduce the impact of factors related to the test before the test on the quality of the test. ③ instrument factors. Microbiological testing work using more instruments, instrument calibration, maintenance, etc. are likely to affect the accuracy of the test results. ④ reagent factors. Reagent quality has a critical impact on the quality of microbiological testing. Therefore, the relevant personnel must strengthen the reagent quality control, so as to reduce the impact of reagent factors on the quality of testing. In order to further improve the quality of microbiological testing, the CDC and the relevant parts of the CDC can also be targeted to set up a professional inspection team, scientific division of labor, the construction of a perfect, scientific rules and regulations, to improve the sense of responsibility of the inspectors, mobilize their enthusiasm. Regular organization of professional training, timely consolidation of the knowledge base of the test personnel, update, so as to improve the business level of the test personnel, to ensure the quality of the test.

In summary, the multi-tube fermentation method and the filter membrane method are both applicable to the testing of domestic drinking water, and can effectively test the number of microbial colonies in water, but compared with the filter membrane method, the multi-tube fermentation method has a higher detection rate. However, it should be noted that in this study

The relatively small number of samples of drinking water selected and the short time of the study will inevitably have

certain deficiencies; follow-up clinical still need to carry out a larger sample size, more in-depth study to further determine the test value of the two testing methods of multi-tube fermentation and membrane method

## References

- [1] Zhou S.-Y. Recent research on water coliform testing methods[J]. Foreign medicine: hygiene, 1989, 16(5):5.
- [2] Shi J. Research progress of fecal coliform detection methods[J]. Coal and Chemical Industry, 2012, 35(7):32-35. DOI:10.3969/j.issn.1003-5059.2012.07.014.
- [3] HE Min, ZHANG Yajuan, ZHOU Yanhong, et al. Comparison of fecal coliform detection methods in water[J]. Water Purification Technology, 2018, 37(9):4. DOI:10.15890/j.cnki.jsjs.2018.09.005.
- [4] LEI Jing, LIAO Shuang, QIAO Xixi, et al. Comparison of three different detection methods for total coliforms in drinking water[J]. Science and Technology Innovation Herald, 2022(002):019.
- [5] WU Qingmei. Analysis of the value of multi-tube fermentation and membrane filtration methods in microbiological testing of domestic drinking water[J]. Chinese Science and Technology Journal Database (Full Text Edition) Medicine and Health, 2022(1):4.
- [6] Chen Xiaoneng, Deng Yufang. Exploring the microbiological test methods and evaluation criteria for domestic drinking water[J]. Capital Food and Medicine, 2016(12):2. DOI:CNKI:SUN:YYSD.0.2016-12-011.