Comparative Study on Test Methods of Total Coliforms in Domestic Drinking Water

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Abstract. Total coliforms are a group of facultative anaerobic Gram-negative sporeless bacteria that can ferment lactose, produce acid and gas, requiring oxygen. In this paper, it adopts three methods namely multi-tube fermentation method, membrane filter method and paper strip method to make analytic comparison on the total coliforms in drinking water. It also makes comparative study from aspects, i.e. test principle and procedure, scope of application and test result, discusses the advantages and disadvantages of the three methods. According to the result, compared with the traditional fermentation method and the membrane filter method, the paper strip method has the advantages such as simple operation, short test time and high accuracy, etc.

1. Introduction
Total coliforms are a group of facultative anaerobic Gram-negative sporeless bacteria that can ferment lactose, produce acid and gas, requiring oxygen. As the major standard for whether the water source has been polluted by excreta, the total coliforms are mainly from the human and animal excreta [1-3]. Currently, the total coliforms are the important index at home and abroad to test the pollution of domestic drinking water. As one of the important indexes evaluating the hygienic quality of drinking water, test of total coliforms has wide hygienic significances.

At present, the Chinese environmental protection departments mainly adopt the multi-tube fermentation method, membrane filter method and paper strip method to test the total coliforms [4]. In this study, it discusses the application of the three methods in testing the total coliforms in domestic drinking water by analyzing the experimental results of total coliforms in domestic drinking water through the multi-tube fermentation method, membrane filter method and paper strip method.

In this paper, through analytic comparison of the test methods of total coliforms, it can be discovered that the multi-tube fermentation method has great preparation workload, complicated operation and long cycle, so that it fails to meet the requirements for rapid sample test. Membrane filter method is a scientific feasible test method that applies to drinking water and water samples with smaller turbidity degree. Paper strip method has been listed into the standard test methods of domestic drinking water thanks to its simple operation and short test cycle; it is highly possible to become one of the rapid test methods evaluating the water quality pollution recently.
2. Reagents and materials
120 pieces of water samples are adopted in this experiment; all of which are from the running water from the urban area of the city and the domestic drinking water from suburban factories. The water samples are collected with sterilizing bottles. Rapid test paper strips for total coliforms, Guangdong Dayuan Oasis Food Safety Technology Co., Ltd.

3. Test methods of total coliforms

3.1. Multi-tube fermentation method (MTF)
The total coliforms in reproduction can ferment lactose, produce acid and gas, turn the culture medium of lactose peptone yellow and produce air bubbles. Primary fermentation experiment: mix the water samples completely and dilute them properly according to the pollution degree of water samples. Inoculate 1ml, 0.1ml and 0.01ml of each sample respectively into the fermentation tubes with culture medium of lactose peptone for cultivation for 24h at 37℃. Results of acid-producing and gas-producing fermentation tube experiments are suspicious microorganisms. Secondary fermentation experiment: inoculate the suspicious colonies into the EC culture medium for cultivation for 24h at 37℃, they can be identified as total coliforms if the colonies continue producing acid and gas during the cultivation. Look up the MPN table according to the quantity of positive fermentation tubes and calculate the most probable number of the quantity of the total coliforms in per liter of water sample [2,3,5].

3.2. Membrane filter method (MF)
Pour the water sample into the sterilized filter, adopt the 0.45-μ microporous filtering film to filter the water sample. Upon the vacuum filtration, the bacteria will be held back on the membrane. Stick the membrane filter on the culture medium of M-FC (Fuchsin sodium sulfite) for cultivation for 24h at 37℃. Total coliforms grow the yellow characteristic colonies on the membrane filter, count the quantity of the colonies and calculate the quantity of the total coliforms in per liter of water sample. A part of the difficult colonies can be selected for smear, dye and microscopy; the other part can be inoculated into the EC culture medium for cultivation for 24h at 37℃ to see whether there are gases; if there are gases produced, the difficult colonies are total coliforms [2,6]. Calculate the most probable number of the quantity of total coliforms in per liter of water sample.

3.3. Paper strip method
Paper strip method is to take the paper strip as the culture medium carrier and adhere the nutrient substances and indicators required during the total coliform growth as well as the substances inhibiting gram-positive bacterium to the paper strip. The bacteria in reproduction will be cultivated for 24h at 37℃ to observe the results. During the experiment, each water sample shall be inoculated by three 10-time concentration gradients, 15 paper strips are inoculated. In which 5 large paper strips are inoculated with 10mL of water sample, 5 small paper strips are inoculated with 1mL of water sample and the other 5 small paper strips are inoculated with 1mL of 1:10 diluted water sample [7]. The water sample shall be positive if there are red spots or red colonies on the paper strip and it turns yellow around. Look up the MPN value table on according to the quantity of the positive paper strips and calculate the most probable number of the quantity of total coliforms in each liter of water sample.

4. Comparative analysis on the three test methods
Fermentation method and membrane filter method are the traditional methods testing coliforms. They have the disadvantages such as complicated operation procedures, long test cycle and more interference factors; verification experiment is necessary. However, these test methods require simple operation skills and lower test cost, especially the fermentation method, which remains the most important test method of water pollution monitoring. The paper strip method has overcome the shortcomings of the previous two test methods and optimized the test process. Requiring even simpler
operations and shorter test time without any verification experiment, paper strip method can judge the pollution of total coliforms in water sample correctly; what’s more, the method has been listed as the national standard test method [3].

Through the statistical comparison of the test results of fermentation method, membrane method and paper strip method, in the 120 water samples, 23 total coliforms are detected by the fermentation method, 21 total coliforms are detected by the membrane filter method and 25 total coliforms are detected by the paper strip method. Upon comparing the three methods, the test results have no significant difference ($P > 0.05$). The comparison of advantages and disadvantages of the three test methods is shown in Table 1.

| Test method           | Test procedure | Verification experiment | Test cost | Test time | detectable rate |
|-----------------------|----------------|--------------------------|-----------|-----------|----------------|
| Fermentation method   | Complicated    | Necessary                | Low       | 48-72h    | 19.2%          |
| Membrane filter method| Complicated    | Necessary                | Low       | 48-72h    | 17.5%          |
| Paper strip method    | Simple         | Unnecessary              | Higher    | 24h       | 20.8%          |

Table 1 is the comparative analysis on the application of fermentation method, membrane filter method and paper strip method in the total coliform test. Among the total coliform test methods, fermentation method is a traditional test method that has been widely applied all over the world. The test method has the advantage of simple test principle, the tester can operate with basic microbiology training. As fermentation method is low-cost and easy to popularize, it remains the major test method in the routine test. However, this method requires complicated test procedures, more interference factors and identification experiment; therefore, the test cycle is relatively long. Fermentation method is applied to the total coliform test; due to the longer test time and complicated test procedures, it is not applied to analyze the large number of samples or make rapid evaluation on the hygiene situation of water [2,6].

Membrane filter method has lower cost to test the total coliforms; the operation procedures are rather simple; the test cycle is short as 24h without verification experiment. In this experiment, compared with the other two test methods, the result of membrane filter is lower, for there are interferences during colony identification as the colonies are not evenly distributed on the membrane filter.

Paper strip method can make up the shortcomings of the traditional methods such as fermentation method and membrane filter method. The method operation is simple, the test time is shorter than that of the other two methods, no culture medium is required in advance. Although paper strip method has higher cost to test the total coliforms, it has the simplest operations in the three test methods without verification test. In addition to the above advantages, the test results are reliable to judge the pollution of water correctly. According to the test results, the three test results have no significant difference; however, the paper strip method has higher detectable rate and positive rate than the fermentation method and membrane filter method; therefore, the sensibility of paper strip method is concluded slightly higher than that of the fermentation method and membrane filter method.

In conclusion, any of the fermentation method, membrane filter method and paper strip method can be applied to the total coliform test to evaluate how the water samples are polluted by the total coliforms. However, each of them has its advantages and disadvantages, so that we shall select the proper test method in the actual test according to the specific situation.

5. Conclusion

Paper strip method has been favored by the testers at present for its advantages, i.e. simple operation and short test cycle. The test technology has wide application prospect in the future. Fermentation method and membrane filter method have been recognized by laboratories, organizations and institutions all over the world; the three methods have been identified as the national standard.
methods.

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