Inexpensive, Disposable Presence-Absence Test for Coliforms and \textit{Escherichia coli} in Water

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**ABSTRACT**

Six-ounce (151-g) Whirl-Pak® bags containing 3.05 g of dehydrated Presence-Absence (P-A) Broth and 5 mg of 4-methylumbelliferyl-β-D-glucuronide (MUG) were pasteurized with 10 kGy of gamma irradiation. To conduct a “bag” P-A test, 100 ml of water sample were added to a bag. The bag was closed, the medium was dissolved by massaging the bag for about 15 sec, and the bag was then placed in a rack for incubation. The bag method was compared with P-A tests conducted in 160-ml glass bottles and 200-ml polysulfone bottles, as well as with a 5-tube Lauryl Tryptose Broth (LTB) most-probable-number (MPN) method. Twenty-nine surface-water samples (11 streams, 7 rivers, and 11 lakes), 9 well-water samples, and 2 sewage effluents were examined. Chi-square analyses of the results revealed that no significant difference (P≤0.1) existed among the different P-A tests. The hydrolysis of MUG provided excellent \textit{Escherichia coli} detection and was easiest to determine in the bags.

The Presence-Absence (P-A) test for coliforms in water (2,4-8,13) is simple to perform, and results are easy to interpret. Because 100-ml samples are generally used in the P-A test, the sample volume is 3 times that of a standard 3-tube most-probable-number (MPN) test. Thus, its sensitivity is equivalent to membrane filtration of a 100-ml sample. Membrane filtration, however, is relatively expensive and complex. Because of these factors, the P-A test is becoming more widely used.

The purpose of the present study was to develop a convenient, disposable P-A test that is more economical than conventional P-A tests. In addition, 4-methylumbelliferyl-β-D-glucuronide (MUG) was added to the P-A medium to determine the presence of \textit{Escherichia coli} in P-A containers (9,10).

**MATERIALS AND METHODS**

Sterilization of dehydrated medium

In conventional P-A tests, 50-ml quantities of P-A Broth (4.575 g/50 ml) are sterilized in bottles, and 100-ml samples are added. The total volume is 150 ml (2). In the new P-A test, only 3.05 g of dehydrated P-A Broth is used, and the volume after sample addition is 100 ml.

The first problem in developing the new P-A test was pasteurization of the dehydrated P-A Broth. This was accomplished by gamma irradiation. To determine the dose of irradiation needed, 3.05 g of dehydrated P-A Broth (Difco) was placed in each of 20 6-oz (151-g) Whirl-Pak® bags (catalog B1062WA, Nasco, Fort Atkinson, WI). The bags were irradiated at the Iowa State University Nuclear Engineering Reactor. Half of the bags were given a dose of 5 kGy, and the other half, a dose of 10 kGy. Five bags of each irradiation level were inoculated with 100-ml sterile water and incubated at 30°C; the other five bags were inoculated and then incubated at 35°C. Control (unirradiated) bags were also inoculated and incubated. The bags were observed daily for bacterial growth for 40 d. In addition, total heterotrophic plate counts [Difco Tryptone Glucose Extract (TGE) Agar, (2)] and spore counts [TGE Agar after heating to 80°C for 10 min, (2)] were performed on 5 lots of Lactose Broth (Difco), 1 lot of Lauryl Tryptose Broth (LTB, Difco), and 1 lot of Presence-Absence (P-A) Broth (Difco) before and after 10 kGy of irradiation.

Sample collection and coliform counts

Twenty-nine surface-water samples (11 streams, 7 rivers, and 11 lakes), 9 well-water samples, and 2 sewage effluents were examined. The samples were stored overnight at 4°C while preliminary estimates of coliform content were made using a Modified Violet Red Bile Agar procedure (1). Dilutions were made in 0.1% peptone water to yield coliform levels of 10/100 ml and 1/100 ml and these were used to compare the different methods.

For the bag P-A test, 100 ml of each sample dilution was inoculated into each of 5 irradiated bags of P-A Broth. In some instances, 5 mg of 4-methylumbelliferyl-β-D-glucuronide (MUG, Hach Co., Loveland, CO) or 10 ml of a solution containing 0.5 mg/ml MUG was added to the bags. After inoculation, air was expelled from the bags, and the bags were sealed by rolling the opened end 3 times. Each bag was gently massaged for 15 sec to dissolve the medium. Each sample dilution was also inoculated into 5 glass prescription bottles (Fisher Scientific, Itaska, IL) and 5 polysulfone dilution bottles (Nalgene, Rochester, NY) containing 50 ml of sterile, triple-strength P-A broth, 7.5 mg of MUG, and a Durham tube; 10-, 1-, and 0.1-ml portions of the sample dilutions were also used to inoculate 5-tube MPN series of LTB with 50 μg/ml of added MUG (3,11). After incubation at 35°C for 24 and 48 h, bottles or tubes with acid production or acid and gas production were used to inoculate Difco Brilliant Green Bile (BGB) Broth (2). Gas production within 48 h at 35°C was indicative of the presence of coliforms (2). The plastic bags were not evaluated for gas production because
the plastic film was permeable to CO₂ and gas did not accumulate in the bag.

The P-A and MPN containers were also examined for fluorescence when held under a long-wave ultraviolet lamp [15-watt blacklight blue lamp. (10)]. MUG-positive samples were streaked on Difco Plate Count Agar containing 50 μg/ml of MUG, and the plates were incubated for 24 h. Isolated MUG-positive colonies were identified by using API-20E strips (Analytab Products, Plainview, NY).

Statistical analysis

Containers that contained BGB Broth-positive coliforms were counted for each of the 3 container methods. Samples that resulted in all positive or all negative containers were eliminated from the data set. Chi-square analyses (12) were used to determine if a significant difference (P<0.1) existed between the different methods.

RESULTS

Radiation threshold for pasteurization

If the P-A Broth was not pasteurized, bacterial growth was present in 2 of 5 bags inoculated with sterile water and incubated for 2 d at 30°C, and in 3 of 5 bags inoculated with sterile water and incubated for 2 d at 35°C. After 7 d of incubation, growth was present in all unirradiated bags. When a dose of 5 kGy was used, growth was observed in only 1 of 10 bags; this bag had growth after incubation at 30°C for 2 d. When a dose of 10 kGy was used, no growth was observed until d 7 when 1 bag incubated at 30°C became turbid. Therefore, all bags used in subsequent experiments were treated with 10 kGy. Before irradiation, both heterotrophic plate counts and spore counts ranged up to 10⁷/g. After irradiation, no bacteria could be recovered when 1-g samples were plated.

P-A methods comparison

Because the results from rivers, lakes, wells, and sewage effluents exhibited similar trends, the data were pooled for Chi-square analysis (Table 1). The bag method yielded 166-positive containers, compared with 162 and 140 for the glass and polysulfone containers, respectively. However, no significant difference (P<0.1) existed among the three methods.

MUG-positive bacteria

When MUG was used in P-A tests, fluorescence was easiest to determine in the bags, followed by the glass bottles and tubes, and was difficult to determine in polysulfone bottles. All 47 of the MUG-positive strains isolated from different sample sources and containers were E. coli.

| TABLE 1. Comparison of three P-A methods at two coliform levels. |
|---------------|---------------|---------------|---------------|
| Coefficient level/100 ml | Number of confirmed coliform containers | | |
| | Bag | Polysulfone | Glass | |
| 10⁻⁶ | 94 | 80 | 89 |
| 1⁰ | 72 | 60 | 73 |
| Totala | 166 | 140 | 162 |

[aNo significant differences existed between the three P-A methods at P<0.1 (12).]

DISCUSSION

The P-A test is intended for use on routine sample submissions collected at water-treatment plants and distribution systems (2,4,6-8). The P-A test is based on the premise that no coliform should be present in 100 ml of a potable water sample (2,4).

Because potable water samples containing coliforms were difficult to obtain, other sample sources were diluted and tested. The bag method yielded the greatest number of positive containers, but no significant differences (P<0.1) existed among the 3 P-A methods. Thus, the bag method is as efficient as the standard method for conducting the P-A test. The bag method would be appropriate for routine P-A tests because the bags are flexible, compact and easy to handle. Time and labor required to clean containers would be saved because the bags are disposable. When MUG is used in P-A tests, fluorescence is easiest to determine in the bags and is indicative of the presence of E. coli. Also, the bags containing dehydrated P-A Broth have a long shelf life when compared with liquid medium in bottles. The shelf life of dehydrated P-A medium in bags at -20, 4, 23, and 55°C was at least 13 months, which was the longest storage period tested (data not shown). Because dehydrated P-A Broth is hygroscopic, the bags must be stored in closed containers. Calcium chloride was used as a desiccant when the bags were stored at room temperature. The incidence of bag leakage was minimal (2 of 600 bags, both near the beginning of the study). Bag P-A tests are inexpensive. The total cost of materials for a bag P-A test is only $0.26 without MUG and $0.71 if MUG is added. Costs of the media alone for the glass- and polysulfone-bottle P-A tests would be $0.29 without MUG and $0.97 if MUG is used. Of course, these are minimal costs; additional costs would be involved in the manufacture, irradiation, and marketing of P-A bags. However, even greater costs would be involved in buying containers, media preparation and sterilizing and washing used containers for glass- and polysulfone-bottle P-A tests.

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REFERENCES