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The Effect of Vacuum Pressure in Membrane Filtration Systems for the Efficient Detection of Bacteria from Natural Mineral Water

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Abstract The procedures currently used for determining microbiological quality of natural mineral water recommend filtration through membrane filters. In this study, we evaluated the effect of vacuum pressure for the accurate detection of bacteria from water samples seeded with *Escherichia coli*. We observed that the number of *E. coli* detected increased with increasing vacuum pressure. In order to examine the retention rate of bacteria in the holes of the membranes under the different pressures, the membrane filters were removed after filtration, washed with sterile water by vortexing, and placed on m-Endo agar plates. With all the filters tested, the number of *E. coli* retained within the filters at negative 600 mmHg was approximately 10 to 20% higher than that obtained with 100 mmHg. These results demonstrate that the vacuum pressure exerted during the filtration procedure may affect the fixation of bacteria into some portions of openings in the membrane filter.

Key words: Vacuum pressure, membrane filtration, *Escherichia coli*, pore size, mineral water

In recent years, sales of commercially available mineral water in Korea have rapidly expanded. Natural mineral water may not be subject to any chemical treatment or addition; it must be pumped to the surface through stainless-steel pipes and tapped directly from the source for packaging in bottles. Therefore, at the mineral water source and at any time in the production line, the water must be free from all possible pathogenic organisms. The quality of mineral water with regard to its microbial content is currently determined by monitoring for *Escherichia coli*, *Enterococcus*, *Pseudomonas*, *Salmonella* and parasites, whose presence is considered to indicate the fecal contamination of water [1]. Membrane filtration or the multiple-tube fermentation technique are the most widely used methods in Korea for the detection of these bacteria. However, since the multiple-tube fermentation technique is time-consuming, taking about one week to complete, and a complex procedure, membrane filtration has been generally recommended.

It is known that the accurate recovery of bacteria from water samples by using the membrane filter method depends on several factors which are related to the filters used. These factors have been evaluated by many investigators who report that wrinkles, brittleness, hydrophobicity or nonwetting areas, as well as material composition or inhibitory compounds of the filter yield lower levels of bacteria recovery [3, 5, 6, 12, 18]. In addition, some investigators have suggested that blocked pores, abnormal pore structure, and electrostatic interactions might also inhibit bacterial recovery [2].

Variations in filters may occur because of differences in manufacturing procedures, materials, storage conditions, and the degree of quality control [4, 9, 15]. It has been reported that filters of poor quality may have an adverse effect on counting, recovery, and bacterial colony morphology [7, 8].

Although many studies have been performed to evaluate factors that are connected with types, pore size, composition, material, and quality of membrane filters for the effective recovery of bacteria from water samples [13, 14, 19, 20], the effect of vacuum pressure during the membrane filtration of water samples on bacteria recovery had not been systematically examined prior to this report. The purpose of this study was to determine whether different vacuum pressures could affect bacterial detection using membrane filters.

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MATERIALS AND METHODS

Bacterial Sample

Escherichia coli KCTC (Korea Collection for Type Culture) 1045 was used as an indicator organism throughout the study. *E. coli* KCTC 1045 was cultured in 5 ml LB broth for 24 h at 35°C. One milliliter of culture ($1,85 \times 10^9$ cells/ml) was centrifuged at 3,000 rpm for 5 min to harvest the cells. The resulting cell pellet was washed with 1 ml sterile water and centrifuged for 5 min at 3,000 rpm to remove the residual broth. The pellet was resuspended in 1 ml sterile water, and 10 μl of the suspension was transferred to 10 ml sterile water. 0.5 ml of the diluted cell suspension was transferred to 1,000 ml natural mineral water which had been sterilized by 0.22 μm filtration in a sterilized 2,000 ml bottle with a magnetic bar. After mixing, the water sample was stored at 4°C for a week prior to experiments. The number of viable cells was determined by spreading 100 μl of the water sample on m-Endo agar plates and incubating at 35°C.

Membrane Filters and Filtration System

We used the disposable filter membranes with two different pore sizes, 0.45 μm pore size filter of 5 different materials and 0.22 μm pore size filter of one material (Table 1). For filtration, we used a 6-valve stainless steel filter assembly (Sartorius Co.) with vacuum device in such a way that differential pressure could be exerted. The membrane filter apparatus was aseptically assembled on a clean bench and connected to the vacuum system (Fig. 1). First, sterile water (250 ml) was applied to the funnel and then the 1 ml water sample stored at 4°C was applied to the funnel. Different vacuum pressures were applied to the filtering apparatus and the liquid was allowed to pass completely through the filter. Subsequently, the filters were removed and placed on m-Endo agar plates to detect the viable *E. coli* cells. The procedure was repeated under each vacuum pressure. All plates were cultured for 24 h in a 37°C incubator and the colonies were counted. For each treatment, experiments were performed at least three times and the numbers were averaged.

Table 1. List of membrane filters tested in this study.

Material	Filter type	Pore size (μm)
Cellulose ester	Millipore	0.45
Cellulose acetate	Sartorius	0.22
Cellulose acetate	Sartorius	0.45
Cellulose nitrate	Nalgene	0.45
Polycarbonate	Nuclepore	0.45
Polysulfone	Gelman	0.45

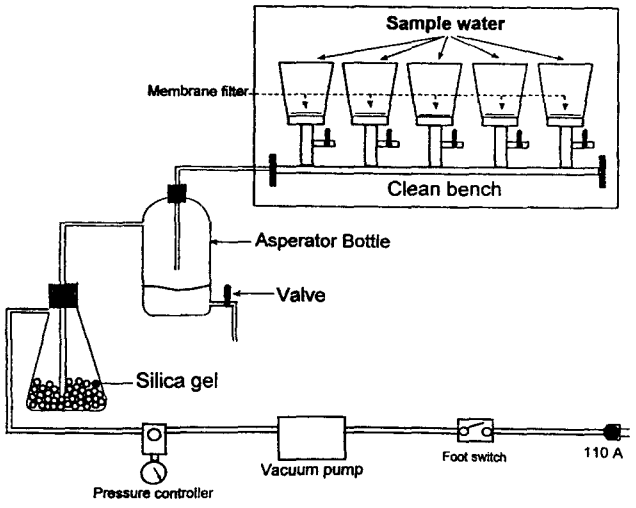

Fig. 1. 6-valve stainless steel filter assembly with vacuum device.

Retention Effect

The effect of vacuum pressure on the retention of *E. coli* in pores of the filter membranes was examined as follows. After filtration under the different vacuum pressures, the membrane filters were removed and placed in sterile 250 ml beakers. Fifty milliliters of sterile water were added to each beaker. The loosely bound *E. coli* was eluted from the membrane by agitating with a vortex mixer for 1 min at maximum speed. Afterward, the filter was removed and placed on m-Endo agar plates to detect unremoved (i. e. tightly bound) *E. coli*.

RESULTS AND DISCUSSION

Effect of Different Vaccum Pressures on the Detection of Bacteria

We examined the effect of different vacuum pressures on the bacterial detection of water samples using the filtration method. Figure 2 shows the relationship between the number of *E. coli* detected and vacuum pressure with 0.22 and 0.45 μm pore size membrane filters. The time required for the filtration of 250 ml water sample with increasing pressure gradually decreased; the filtration time with the 0.45 μm filter was shorter than that with the 0.22 μm pore size membrane filter over the entire negative pressure range. The maximum *E. coli* detection was obtained under the highest vacuum pressure on both 0.22 and 0.45 μm pore size filters. Also, we found that the smaller pore-size (0.22 μm) filter yielded lower numbers of *E. coli* than the 0.45 μm filter. This could be explained by the possible retention of the *E. coli* in the holes of the membrane filter. Indeed, when we measured the number of *E. coli* retained after washing the filters, higher numbers of bacteria were detected with 0.45 μm filter membranes. This result seems to suggest

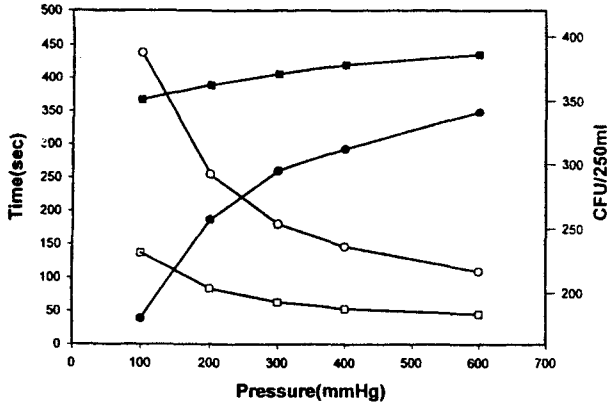

Fig. 2. Filtration time and *E. coli* detection of 0.22 and 0.45 μm cellulose acetate membrane filters under different vaccum pressures.

⊖: Filtration time of 0.45 μm pore size, ⊖: Filtration time of 0.22 μm pore size, ■: *E. coli* detection of 0.45 μm pore size, ●: *E. coli* detection of 0.22 μm pore size.

that the retention of *E. coli* is closely related with *E. coli* detection in membrane filters. This is in line with previous reports [13, 17] that the recovery rates (opposite in meaning to the retention of bacteria in membrane filter) of bacteria with 0.45 μm filters were fairly low compared with the recovery rates for the 0.22 μm filters that were of the same material as the 0.45 μm filters. In these reports, the recovery rate of bacteria was determined as follows. After filtration, the filtered organisms on the membrane filter were eluted with sterile water by either vortexing or sonication, and 100 μl of eluted cell suspension was plated onto media. The percentage of recovery was then determined by counting the colonies after incubation and comparing with the initial number of bacteria.

Detection of Bacteria Using the Various Membrane Filters

The effect of different membrane materials on bacterial detection was examined with the five types of 0.45 μm pore size filters. Although the five types of filters had the same pore size, their filtration times were different under the same negative pressure (Table 2). The filtration with the cellulose ester filters was the fastest among all the filters tested. However, as shown in Fig. 2, the continuous decrease of filtration time with increasing negative pressure was observed in all membrane filters. Interestingly, at all negative pressures exerted, the largest number of viable *E. coli* cells were detected on cellulose ester filter. In addition, the number of bacteria detected with all five different filter membranes was positively correlated with increasing negative pressure (Table 3).

Retention Rate of Bacteria Using the Various Membrane Filters

We examined the possibility that bacteria embedded in the filter pores could affect bacterial detection as described in Materials and Methods. As expected, when the filter membranes were washed before being placed onto m-Endo agar plates, the retention rate of *E. coli* on all tested filters was higher at 600 mmHg than at 100 mmHg (Table 4). Also, the retention rate of the 0.22 μm cellulose acetate filter was slightly lower than that of the 0.45 μm filter of the same material. The largest number of *E. coli* was also detected with the cellulose ester filter membrane among the 5 types of filters. Noticeably, the number of *E. coli* detected after washing was markedly reduced with polysulfone and polycarbonate type filter membranes.

Table 2. Filtration time (sec/250 ml distilled water) of five kinds of 0.45 μm pore size filters under different vacuum pressures.

Filters \ Pressures	-150 (mmHg)	-300 (mmHg)	-450 (mmHg)	-600 (mmHg)	-750 (mmHg)
Cellulose ester	91.27	47.20	33.34	25.96	21.63
Cellulose acetate	124.53	63.12	46.48	35.82	31.41
Polycarbonate	158.02	80.48	59.31	44.57	40.49
Cellulose nitrate	141.48	71.17	51.30	39.36	36.04
Polysulfone	99.97	50.21	36.40	28.73	23.10

Table 3. *E. coli* detection effect (detection percentage for pour plating count) of five kinds of 0.45 μm pore size filters under different vacuum pressures.

Filters \ Pressures	-150 (mmHg)	-300 (mmHg)	-450 (mmHg)	-600 (mmHg)	-750 (mmHg)
Cellulose ester	79 (%)	84 (%)	87 (%)	90 (%)	91 (%)
Cellulose acetate	48 (%)	52 (%)	54 (%)	57 (%)	59 (%)
Polycarbonate	65 (%)	71 (%)	72 (%)	74 (%)	76 (%)
Cellulose nitrate	76 (%)	82 (%)	85 (%)	87 (%)	89 (%)
Polysulfone	76 (%)	81 (%)	83 (%)	86 (%)	87 (%)

Table 4. Retention effect (percentage of retention effect for pour plating count) of 0.22 μm and 0.45 μm filters at 100 and 600 mmHg negative pressure.

Filters \ Pressures	-100 (mmHg)	-600 (mmHg)
Cellulose ester (0.45 μm)	60 (%)	71 (%)
Cellulose acetate (0.22 μm)	32 (%)	46 (%)
Cellulose acetate (0.45 μm)	35 (%)	49 (%)
Polycarbonate (0.45 μm)	32 (%)	52 (%)
Cellulose nitrate (0.45 μm)	57 (%)	62 (%)
Polysulfone (0.45 μm)	37 (%)	52 (%)

Surface Structures of the Various Membrane Filters

The electron microscopic analysis was performed to observe the structure of different filter membranes more closely. As shown in Fig. 3, the cellulose acetate, cellulose ester, and polysulfone membranes have similar matrix structures while the two other filters (polycarbonate and cellulose nitrate) have a planar structure. Scanning electron microscope photographs of the surface of filter membranes reveal the irregular nature of the surface pores. It also shows that the distribution of the membrane filter pore sizes is quite widespread except for the polycarbonate membrane filter. It is known that commercial cellulose acetate membrane filters of 0.45 μm pore size have a nominal pore size ratings of 0.22~0.80 μm [10]. If filter membranes are used to filter bacteria whose diameter is similar to the membrane's pore size rating, some bacteria might be expected to penetrate through the surface of the membrane, and become trapped within the tortuous flow paths within the membrane. Where such depth filtration occurs, the surface charge of the membrane can be very important, since particles with opposite charge to the membrane surface will be attracted to the surface and adsorbed within the membrane. If bacteria locate on the surface of the membrane, the growth of bacteria will be poor because growth on the surface of a membrane filter is itself a stressful situation, due to differential drying on the top of the filter. Therefore, it has been shown that the optimal structure for a membrane filter is one in which the organisms are not retained precisely at the surface of the filter, but are allowed to penetrate into the pores a little way. Therefore, we considered the possibility that the differences in bacterial retention may have been caused by the material and structural differences, and pore size distributions of the membranes. In 1979, Zierdt evaluated pore diameters and membrane filter materials in a study of filtration of bacteria. He successfully detected certain bacteria when he used filters with large pore sizes [20]. Sladek *et al.* (1975) demonstrated that fecal coliform counts showed a dramatic increase when the pore size opening of the filter was larger than 0.45 μm. There is a remarkable increase in detection when surface-opening pore diameters are between 1.0 and 2.0 μm, followed by a decrease at still larger surface openings. The latter decrease is due to poor retention of coliforms by the filter, but the former increase is due to other factors. Also, they carried out extensive tests and showed that neither the chemical composition of the filter nor the method of sterilization had any role to play in differential detection. Rather, the effect was due to the size of the pore itself. They concluded that optimal detection of fecal coliforms occurred when the bacteria could fit part way into openings in the membrane surface, where they could be cradled and thus more completely surrounded by nutrient. It was visualized that nutrient for bacterial growth must pass up through the membrane structure by capillary action, and because of surface evaporation, an incompletely surrounded bacterium might be subjected to locally hypertonic conditions, resulting in plasmolysis and death [16]. This was thought to be an especially significant factor for stressed organisms.

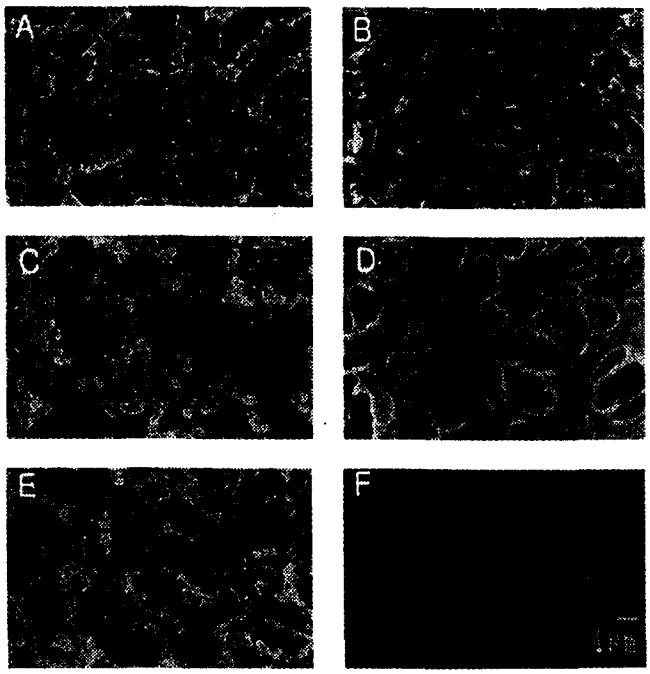

Fig. 3. Scanning electron micrographs of pore structures of membrane filters (×5000).
A: 0.45 μm cellulose acetate, B: 0.22 μm cellulose acetate, C: 0.45 μm cellulose ester, D: cellulose nitrate, E: polysulfone, F: 0.45 μm polycarbonate.

We suggest that high vacuum pressure exerted during the filtration procedure could affect the fixation of bacteria into the holes or some portions of the openings in the membrane filter. Therefore, we consider that vacuum pressure plays an important role in the detection of bacteria from water samples using membrane filtration systems.

In conclusion, we recommend that all laboratories using the membrane filtration for analysis of the microbiological quality of drinking water samples

determine the optimal vacuum pressure for each testing bacterium as well as *E. coli* to ensure the accuracy of their data.

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