Rapid Method of Measuring Bacteria with the Use of a Flow Cytometer

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1. Introduction

Regarding bacterial examinations of tap water, the drinking water quality standards include common bacteria and *Escherichia coli*. Additional items for determining water quality include heterotrophic bacteria counts. Since the detection of these bacteria requires a long cultivation time, it is difficult to rapidly and precisely determine whether tap water contains bacterial contaminants.

Flow cytometers (FCMs) are now available for the determination of bacterial counts within approximately 15 minutes. Since the method is capable of detecting all types of bacteria, alive or dead (total bacteria), FCMs may enable the rapid and precise detection of bacterial contaminants in tap water.

We examined the feasibility of using a FCM for detecting contamination in mixtures of tap water with water from various sources, which were used to model cross-connections. We also investigated the use of a FCM for monitoring increases or decreases in the number of bacteria in the process of water transmission and distribution.

2. Research

(1) Detecting contamination due to cross-connections

We examined whether we could detect in tap water the addition of a small amount of water from sources likely to be contaminated. For contaminating the tap water, we used well water, rainwater treated water, reclaimed water, and industrial water. We mixed these contaminating water samples with tap water at various ratios and measured the free residual chlorine, conductivity, and the number of total bacteria using an FCM.

(2) Monitoring increases or decreases in the number of bacteria in the process of water transmission and distribution

We also studied whether we could measure the number of total bacteria in purified water at WPPs (water purification plants) as well as tap water from faucets, and whether we could monitor increases or decreases in the number of bacteria in the process of transmitting and distributing water. We collected samples of purified water from four purification plants (Asaka, Misono, Kanamachi, and Misato) and samples of tap water from faucets at 47 locations within the 23 wards of central Tokyo. We measured the numbers of common bacteria, heterotrophic bacteria, and total bacteria.

3. Results

(1) Detecting contamination due to cross-connections

In terms of free residual chlorine and conductivity, there were no differences detected between some samples of contaminating water and the tap water itself, so it was difficult to detect the addition of a small amount of contaminating water. However, the number of total bacteria in the

contaminating water samples was detected to be 10 to 870 times higher than that in tap water. We were able to detect the addition of the contaminating water into the tap water at concentrations of as low as 10% contaminating water. Our research demonstrated that we could precisely detect whether contaminating water had been mixed into the tap water due to cross-connections by measuring the total number of bacteria using the FCM.

(2) Monitoring increases or decreases in the number of bacteria in the process of water transmission and distribution

While the number of common bacteria was below the limit of quantification in all samples of purified water and tap water, heterotrophic bacteria and total bacteria were detected in tap water taken from faucets at some locations, although each of these numbers was also below the limit of quantification in purified water. In water from faucets at certain locations, the number of total bacteria was detected to be approximately 100 times higher than the number of heterotrophic bacteria, and total bacteria was detected at more locations than heterotrophic bacteria. These findings show that it is easier to detect an increase in total bacteria than an increase in other types of bacteria. Therefore, measurement with the FCM enables us to precisely and rapidly monitor increases or decreases in the number of bacteria as well as water quality abnormalities in the process of water transmission and distribution.