

Department of Medical Sciences, the drinking water samples were analyzed to determine the contamination of bacteria which include:

1. Sanitary indicators
 - 1.1 Coliforms
 - 1.2 *Escherichia coli* (*E. coli*)
2. Waterborne pathogens
 - 2.1 *Staphylococcus aureus* (*S. aureus*)
 - 2.2 *Clostridium perfringens* (*C. perfringens*)
 - 2.3 Salmonellae

The analysis of all samples was initiated on the same day of collection in order to prevent a significant change in number of bacteria.

2.1 The analytical methods

2.1.1 Sanitation indicators: Coliforms and *E. coli*

Coliforms were examined by multiple tube fermentation technique (Eaton, Clesceri, Rice, & Greenberg, 2005) using the presumptive medium, Lauryl sulfate tryptose broth (Oxoid, CM0451B) and the confirmed medium, brilliant green lactose bile broth (Merck, 1.05454.0500). The results of Coliforms were recorded as the most probable number (MPN) per 100 ml of water. *E. coli* was detected subsequently from the procedure of Coliforms using the EC medium (Merck, 1.10765.0500) with incubation at the elevated temperature of 44.5 °C. The results of *E. coli* were recorded as *E. coli* detected or not detected in a 100 milliliters (ml) water sample.

2.1.2 Waterborne pathogens: *S. aureus*, Salmonellae and *C. perfringens*

S. aureus, Salmonellae and *C. perfringens* were detected by membrane filter method using the particular culture media for each bacterium (Eaton, Clesceri, Rice, & Greenberg, 2005); and International Standard Organization [ISO], 1995). Confirmation identifying the types of bacteria was done by biochemical tests and serological tests. Particular enrichment media and selective agar media that were

used for examining bacteria were M-Staphylococcus broth (Difco 264920) and Lipovitellenin-salt-mannitol agar (Oxoid, CM0085) for *S. aureus*, Buffered peptone water (prepared from mixed compositions by laboratory), Tetrathionate broth (Oxoid, CM1048), Xylose lysine desoxycholate agar (Oxoid, CM0469), Bismuth sulfite agar (Merck, 1.05418.0500) and Brilliant green agar (Merck, 1.07232.0500) for Salmonellae, and Cooked meat medium (Difco, 226730) and Tryptose-sulfite-cycloserine (Merck, 1.11972.0500) for *C. perfringens*. The results of *S. aureus*, Salmonellae and *C. perfringens* were recorded as the particular bacterium detected or not detected in a 100 ml water sample.

Contamination of bacteria in drinking water was assessed according to the standard limits of the Food Act issued by the Ministry of Public Health (No.61, B.E. 2524) (สำนักงานคณะกรรมการอาหารและยา, 2545) as shown in Table 2.

Table 2. Standard Limits of the Ministry of Public Health No. 61 (B.E. 2524)

Determination	Limits
Coliforms, MPN/100 ml	must be less than 2.2
<i>E. coli</i>	must not be detected
<i>Staphylococcus aureus</i>	must not be detected
Salmonellae	must not be detected
<i>Clostridium perfringens</i>	must not be detected

Stage II. Intervention

After monitoring the pretest, the intervention was done with school administrators or/and staff of the targeted schools in order to provide knowledge of sanitary conditions of the water supply and good sanitation practices for maintaining good quality and improving the quality of drinking water for students. Published materials and a compact disc (CD) of such knowledge were given to the schools, along with discussions between the researcher and the school administrators or responsible staff. In addition, a simple test kit for self-examination of the contamination of Coliforms in drinking water was provided. The intervention period took three months.

Stage III. Posttest (After intervention)

After the intervention stage, the examination of indicator microbes and inspection of sanitary conditions of water supply was conducted by using the same procedures as the pretest (before intervention) stated above. A total of 65 samples were collected and analyzed in the posttest period. The number of the posttest water samples was higher than the pretest because four additional drinking water samples were taken from newly installed water dispensing points in a few targeted school. As planned in this study, the drinking water was collected from all water points in each school.

3.4 DATA ANALYSIS

The data was entered into a database. The analytical results of bacterial contamination in drinking water samples obtained from the pretest and posttest were analyzed and displayed for frequencies of the contamination in samples as the percentage. The mean value (\bar{X}) of Coliforms count was calculated to represent the average number. The difference or association between the two sample groups with qualitative data, such as frequency of bacterial contaminated samples, was determined with Chi square (χ^2) test with the confidence level of 95% ($p = 0.05$) (ศิริชัย พงศ์วิชัย, 2543). Moreover, water samples of poor quality were evaluated along with the sanitary conditions of the water supply (water cooler tanks and multiple-faucet basins)

In summary, this chapter has shown the methodology of this study. In the next chapter, the findings will be presented.