The combination of filtration and ATP+ADP+AMP assay for the assessment of microbial quality of water

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1. Introduction

Water microorganisms are a useful indicator for the quality of drinking water, and for the maintenance of water-related facilities. Since culturing methods take several days, a rapid assay is required for quick response measures for incidents caused by microorganisms.

ATP swabbing tests have been examined for the assessment of microbial quality of water by the detection of ATP from organic debris including bacteria. However, there are two issues as below.

1. ATP from both microbial and non-microbial sources can be detected, and they cannot be differentiated.
2. The concentrations of ATP in microorganisms can vary during culture; ATP is predominant at the initial stages, but AMP becomes predominant at later time points *(1)*. Another previous study also showed that ATP+ADP+AMP (A3) in microorganisms is more stable than ATP, which is affected by nutritional conditions *(2)*.

Based on these points, a rapid and easy test for the assessment of the microbial quality of water was developed; the A3 test (Fig. 1) *(3)* is employed after sample pretreatment with syringe filter for the elimination of non-microbial A3 and concentration of microorganisms (Fig. 2). Moreover, the correlation between new A3 filtration assay and culturing method was evaluated using practical water samples.

Figure 1. The luminometer and the swab of the A3 test.

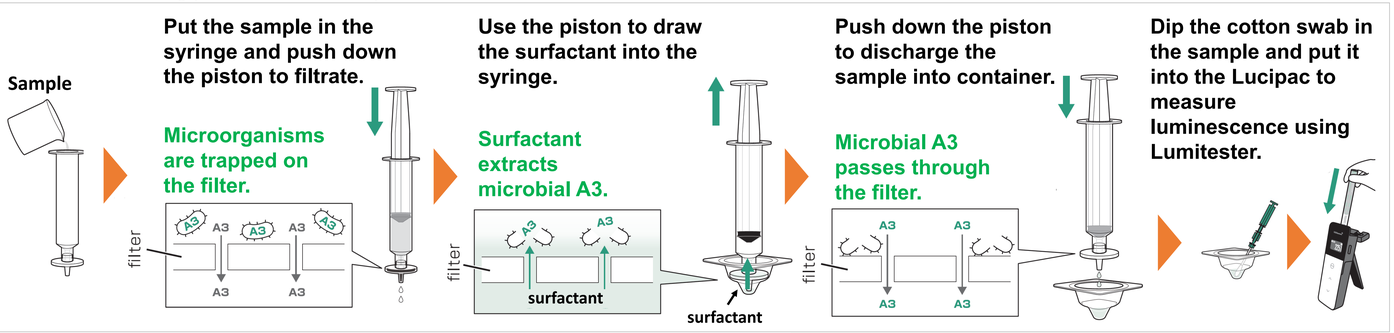

Figure 2. Concept and method of new A3 filtration assay

Total operation time for a sample is approximately 3 min.

2. Method

2-1. Sample

Eight kinds of water samples were collected. In order to obtain samples containing different microbial quality, a total of 29 water samples were prepared by storing at 25°C for 0-18 days (Table 1).

Table 1. Samples for assays

Sample	Storage term (days)
Well water	0, 5, 11, 18
Utility waste water	0, 5, 11, 18
Water from a water basin for hand washing of a shrine	2, 3, 4, 7, 11, 14
Pure water tank	0
Cooling tower water A	0, 1, 7
Cooling tower water B	0, 5, 11, 18
Tap water A	0, 1, 7, 14
Tap water B	0, 1, 7

2-2. Viable microbial count

Dilutions of water samples were plated on R2A agar and incubated at 30°C for 2-5 days. Viable microbial counts were measured as colony forming units (CFU).

2-3. Filtration and A3 test

Each sample (10 mL) was filtered through a syringe filter (pore size; 0.8 µm, material; cellulose acetate) to eliminate extracellular A3 and trap microorganisms. Successively, the surfactant was drawn into the syringe using the piston to extract microorganism-derived A3 by the treatment of the filter with the surfactant. Since the extracted A3 passes through the filter, the syringe piston was pushed down to collect the extracted A3 in a new container. The extracted A3 was measured by the A3 test (LuciPac™ A3 Surface/Lumitester™ Smart, Kikkoman Biochemifa Company), and the measurement output was relative light units (RLU) (Fig. 2).

3. Result

3-1. Correlation between RLU and CFU

The correlation between luminescence intensity (RLU) based on microbial A3 and viable microbial count (CFU/mL) for each sample was evaluated.

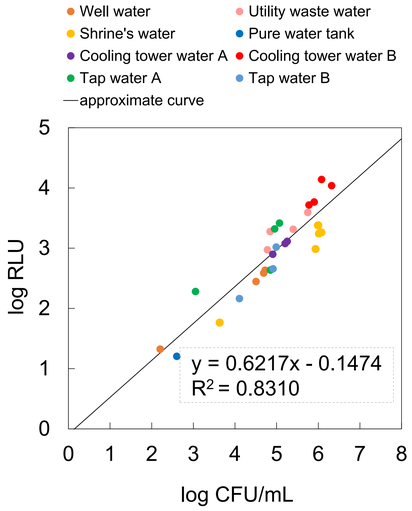

Figure 3. Correlation between RLU and CFU/mL in logarithmic scale

The measurement values for all samples were 1.2-4.1 log RLU and 2.2-6.3 log CFU/mL. A positive correlation was found between RLU and CFU/mL in logarithmic scale ($y = 0.622x - 0.147$, $r^2 = 0.831$).

3-2. Monitoring RLU and CFU over time

The quality of water samples over time was evaluated by luminescence unit (RLU) and microbial count (CFU/mL). Fig. 4 shows the RLU ( ) and CFU/mL( ) for each sample.

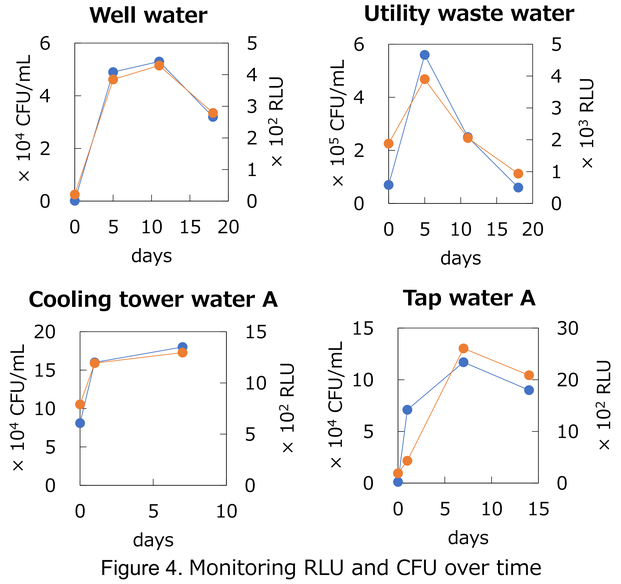

Figure 4. Monitoring RLU and CFU over time

During sample storage, the RLU measurements and log CFU numbers increased over time in sample of cooling tower water A, In the other samples, the values increased and then decreased. The trend of consistency between changes of RLU and CFU was confirmed.

4. Summary

- **A test with quick and simple procedure was developed by combining the filtration process with the A3 test.**
- **A positive correlation was found between the new A3-filtration assay and the culturing method.**
- **Changes in viable counts over time could be monitored with the combination of filtration and A3 test.**
- **This method is useful for rapid hygiene control of water and water-related facilities.**

(1) Smith, N. W., Sindelar, J. J., and Rankin, S. A., *J. Food Prot.* 2019; 82: 2088–2093.
(2) Sakakibara, T., Murakami, S., and Imai, K., *Anal. Biochem.* 2003; 312: 48–56.
(3) Bakke, M., and Suzuki, S., *J. Food Prot.* 2018; 81: 729–737.