ABSTRACT

Industrial cooling towers and waters require constant monitoring for bacterial presence to avoid or mitigate issues, such as corrosion and health threats, caused by biologic organisms. The current analysis methods require up to 5 days for results. Customers are required to take 6 one-liter samples, from the water system and concentrate them prior to analysis. We analyzed four concentration methods: centrifugation, disposable filtration, glass filtration and concentrating pipet. Each method was analyzed for time to answer, recovery, and per trial and capital cost. Staining procedures were optimized with E. coli and S. aureus and improved using DuraClone dried reagents. The stained samples were then analyzed on the CytoFLEX VSSC for total aerobic bacteria counts (TAB). Industrial samples from cooling towers and biochemical protein production processes were analyzed.

Results: The TAB recovery was highest with the CP (71%) with lower recovery values for DF>GF-Cent. The time required for a 1 L sample to answer was fastest (16 min) CP>GPC>GF-Cent. Total cost per test was DF>GPF>GFCent. The TO and PI staining provided the highest signal to noise for live/dead on bacteria.

Conclusion: Presented here is an optimized sample to answer method for counting TAB via flow cytometry. The CytoFLEX provides effective detection of bacteria and can be easily adapted into a mobile testing unit with DuraClone reagents. The assay measured a detection limit lower than 45 colony forming units per ml and reduced assay time from 5 days to 16 minutes. Combining the sample concentration method, DuraClone reagents and the CytoFLEX flow cytometer, customers are able to quickly respond to bacteria contamination.

MATERIALS AND METHOD

Sample concentration:

Recovery was first tested with Polysciences, 700 nm YG beads in 1 L DI H2O, then E. coli and S. aureus. Method 1: Centrifugation (Centri) in the Beckman Coulter Allegra 30-x required a slow spin at 500 g, 20 min and then a capture spin at 3750 g for 20 min. Method 2: Pall Sentino pump with disposable microfilter (20 or 50 µM). Method 3: glass filtration (Pall, Steriffeq) was used with 20 µM PCTE pre-filter and 0.45 µm PCTE capture filter (GF). Method 4: a concentrating pipet (CP, Innovaprep) with a 330 µm pipet sleeve and 0.45 µm polystyrene fiber pipet with PBS elution buffer were used with a 2 days elution. Staining: Bacteria were stiled with Syto9 (TO), PI, CFSE, Thiazole orange (Sigma), SYBR Green (Thermo), Propidium Iodide (PI), 7AAD, and HO33342 with a PBS, 1 mM EDTA and 0.1% Tween 20 solution. DuraClone dried reagent TO and PI were tested in the final analysis with Beckman Coulter flow count beads. Flow cytometry: The samples were analyzed on a CytoFLEX with a VSSC trigger, fluorescence detection for TO, PI and Flow count beads.

Viability and bacteria staining:

Stock solution: E. Coli or S. aureus were suspended in PBS so that the suspension was slightly turbid. Half of the stock solution volumes were heated in a 70°C water bath for 20 minutes to create non-viable bacteria populations. The heat killed bacteria were allowed to cool to room temperature and remixed with the viable bacteria. The mixed bacteria solutions were diluted 1:10 in PBS

A 0.1 mL aliquot of the diluted bacteria was incubated each individual viability dye and every possible combination of either 7-AAD or Propidium iodide with the remaining 6 permeable dyes for 30 minutes. All FDA and CFSE incubations were done in a 37°C water bath. Incubations and staining were done in the following combinations shown here: Carboxyfluorescein diacetate succinimidyl ester (CFSE) at 5µM; Syto 9 at 10 µM; Thiazole Orange (TO) at 3.4 µM; SYBR Green at 100X; Hoechst 33342 at 88.5 µM; Fluorescein diacetate (FDA) at 10 µM; Propidium iodide at 136 µM; and 7-AAD at 300 µM. After 30 min, 0.9 mL of PBS was added to the stained bacteria. Bacteria suspensions were analyzed on the CytoFLEX flow cytometer.

Limit of Detection:

Stock solution: E. Coli were suspended in PBS so that the suspension was slightly turbid. The stock solutions were diluted 1:10 in PBS working solution. A 0.1 mL aliquot of the diluted bacteria was incubated with Syto 9 for 30 minutes at the following bacteria dilutions: Working solution, a 1:10 of working solution, and serial dilution to a 1:1280 dilution of bacteria in PBS. All dilutions were brought up to 1mL analysis volume including the addition of FlowCount beads before being analyzed on a CytoFLEX instrument equipped with Violet Side Scatter detection. 10 µL of the three lowest dilution samples were plated on agar plate as predicate to determine viable bacteria count.

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Figure 1 Recovery work flow of different concentration and filtration methods for total aerobic bacteria count (TAB)