TETRA TECH

Executive Summary for Bacterial Challenge Work Conducted for Photonic Biosystems, Inc.
Week of February 7, 2011

An independent study of the Photonic Biosystems Coliform Analyzer (CA) for detection of *E. coli* and total coliform bacteria in drinking water matrices was conducted at Tetra Tech's Clancy Environmental laboratory, located in Saint Albans, VT, the week of February 7, 2011. Trials were designed to (i) differentiate $\log_{10}$ differences in the target population with shorter Time-to-Detection (TTD) as the organism concentration increased; (ii) evaluate the ability of the CA to detect low levels of the target organisms as the total number approaches 1 colony forming unit (cfu); (iii) evaluate the ability of the CA to detect low levels of the target organism in the presence of a high heterotrophic bacteria background, and; (iv) evaluate the flow rate and volume capacity of the CA filtration assembly to handle waters with increasing turbidities.

In the first trial, *E. coli* was serially diluted in de-chlorinated tap water yielding concentrations ranging from 10^6 to 10^0/100 mL. Confirmation of the concentration of *E. coli* at selected levels was determined using the standard membrane filtration and m-Endo agar method (Standard Methods, 9222B). Very good agreement was noted between the m-Endo counts, the counts on the CA membrane, and the CA calculated concentrations. At the lowest level tested the mean concentration of *E. coli* was 2 cfu/100 mL (n=2) and 2.3 cfu/100 mL (n=3) by the CA (both visual and software) and m-Endo methods, respectively. At the 10^1 level, the mean concentrations were 19 cfu/100 mL by m-Endo, 14.5 cfu/100 mL by visual counts on the CA membrane and 15 cfu/100 mL using the CA software. The TTD for the $\log_{10}$ concentration of E. *coli* ranged from 115 minutes for 10^6 cfu/100 mL to 511 minutes for 10^0 cfu/100 mL. The Saint Albans de-chlorinated tap water negative controls showed no response from the CA and were confirmed by the lack of colonies on the m-Endo plates and CA membranes.

In the second experiment, ten replicates at target *E. coli* concentrations of 1, 5 and 10 cfu/100 mL in de-chlorinated tap water were analyzed by the CA and using m-Endo agar. In addition, two replicates were tested at each level using Colilert with Quanti-Tray™ to yield an MPN result. Targeting 1 cfu/100 mL, the mean concentrations detected by each method were 1.0 ± 1.1 cfu/100 mL by the m-Endo method (n=10), 0.6 ± 0.5 cfu/100 mL by the CA (n=10) and 0.8 ± 1.0 cfu/100 mL visually counting the CA membranes. There were 4 negative results by m-Endo and CA and 6 negative results based on the visual inspection of the CA filters. The total number of *E. coli* detected at 1 cfu/100 mL ranged from 0 to 3 cfu on m-Endo agar and visually on the CA membranes while the calculated CA result was either <1 or 1 cfu/100 mL. At the 5 cfu/100 mL concentration, the m-Endo counts ranged from 1 to 8 cfu/100 mL with a mean concentration of 4.7 ± 2.0 cfu (n= 10). The CA calculated the mean concentration at 6.0 ± 4.9 cfu/100 mL (n=10) with results ranging from 1 to 15 cfu/100 mL. The visual CA counts ranged from 3 to 8 cfu/100 mL with a mean of 6.4 cfu/100 mL ± 1.5. At the 10 cfu/100 mL levels m-Endo counts ranged from 6 to 18 cfu/100 mL (n=10) and the mean concentration was determined to be 11.2 cfu/100 mL ± 4.2. The mean visual CA concentration was 13.6 ± 3.3 with counts ranging from 8 to 18 cfu/100 mL while the mean calculated CA concentration was 13.3 ± 7.2 with the counts reported ranging from 2 to 25 cfu/100 mL. The Colilert results yielded 1 MPN *E. coli* and <1 MPN *E. coli*/100 mL at the 1 cfu level; 5.1 MPN *E. coli* and 4.1 MPN *E. coli* /100 mL at the 5 cfu level and 8.4 MPN *E. coli* and 12.1 MPN *E. coli* /100 mL at the 10 cfu level.

In experiment 3 the objective was to show that the presence of non-target heterotrophic organisms did not trigger a false positive CA response nor cause a false negative response when *E. coli* were present. *Pseudomonas aeruginosa* ATCC 35422 and *Aeromonas hydrophila* ATCC 7965 were the heterotrophic

bacteria chosen for these trials. They are commonly found in water, *Pseudomonas can* grow on m-Endo agar and appears as a non-coliform, and *Aeromonas* is well documented to give false positive total coliform reactions in some chromogenic substrate tests. Initially three replicates at each seeding level for each heterotrophic bacterium were tested and yielded no response using the CA, either by visual inspection of the membranes or the calculated CA result. On m-Endo agar, small pinpoint, clear colonies were noted on the *Pseudomonas aeruginosa* plates (approximately 41 on the 10^4 plates and 500 on the 10^5 plates) and none was observed on the *Aeromonas hydrophila* plates. In this trial 10 cfu *E. coli* in 100 mL was the targeted seed level; however, the actual counts ranged from 8 to18 cfu/ 100 mL in samples containing only *E. coli* (n=3) as detected using m-Endo agar, 15 to 21 cfu/ 100 mL using the visual CA membrane count and 12 to 52 cfu/ 100 mL using the calculated CA count. When 10^4 *P. aeruginosa* was added to the sample, the *E. coli* count ranged from 13 to 20 cfu/100 mL using the visual CA counts and 12 to 34 cfu/100 mL as calculated by the CA. When the concentration of *P. aeruginosa* was increased to 10^5 cfu/mL, seeded *E. coli* were detected at concentrations ranging from 16 to 20 cfu/100 mL by visually inspecting the CA filters and calculated *E. coli* were determined to be 18 to 23 cfu/100 mL by the CA. A single replicate of *E. coli* in 10^5 *P. aeruginosa* was analyzed on m-Endo agar and yielded a count of 16 cfu *E. coli* and approximately 550 clear, pinpoint colonies, considered non-coliform bacteria. Similar observations were noted using *Aeromonas hydrophila* as the heterotrophic bacterium. In replicates where approximately 10 cfu *E. coli* were added to 10^4 *A. hydrophila* the range of *E. coli* detected by visual CA count was 12 to 22 cfu while the calculated CA count was 5 to 15 cfu. When the concentration of *A. hydrophila* was increased to 10^5 the *E. coli* counts ranged from 13 to 22 cfu, and 6 to12 cfu by the visual and calculated CA count, respectively. A single replicate was also analyzed at this concentration on m-Endo agar and yielded 12 cfu *E. coli*.

In the final experiment, flow rate and water filtration-capacity assessments were evaluated for volume throughput using different water matrices. A water matrix was created using sediment from surface water to yield three samples with turbidity values of 5.4, 4.5 and 3 ntu. This sediment contained silt and fine particles and has proven to be difficult to filter in other assays. The 0.8 µm CA membrane was able to filter the entire 100 mL matrix at the two lower turbidity levels; however, the time required to filter the 100 mL volume increased from 47 seconds (3 ntu) to 56 seconds (4.5 ntu) and finally 2:43 minutes for 94 mL of the 5.4 ntu water. Using the same matrix two samples were created with turbidities of 3.2 ntu and 1.7 ntu. Using the 0.45 µm CA membrane the time required to filter 100 mL was 2:48 minutes for the 3.2 ntu water and 1:57 minutes for the 1.7 ntu water. A high quality surface water (approximately 1 ntu) was tested to determine the maximum volume that could pass through the 0.8 µm CA membrane. After 8 minutes approximately 300 mL volume had been filtered. The membrane surface was darkened due to captured particles and no evaluation was made to determine if the sediment would inhibit target bacteria growth.

In conclusion, these trials show encouraging results for a new rapid detection method for total coliform and *E. coli*. In terms of organism-recovery efficiency, there was strong agreement between the traditional detection methods for coliform bacteria and *E.coli,* Colilert and m-Endo plate counts, compared to the CA results (visual and calculated) even at concentrations targeting 1 cfu/100 mL. A positive feature of this technology is the ability to visually confirm the calculated CA result by examining the plate after incubation in situations where users wish to record counts (i.e., surface waters, recreational waters). Another positive attribute is the ability to pick isolated colonies from the CA membrane for traditional biochemical confirmation and identification assays where users wish to study coliform ecology of a system.