



# Case Study

## Frisco Lakes by Dell Web

### Summary

The study was performed on January 30<sup>th</sup>, 2020 at Frisco Lake community centre. The study involved water testing from 28 sampling points. The water samples were tested for total microbial count using Heterotrophic Plate Count (HPC) method APHA9215C, total microbial count using the ExactBlue system, and 16s rRNA sequencing. In addition, each sample was screened for free chlorine levels using a Milwaukee colorimeter and MI526-25 reagent.

HPC and ExactBlue testing, as well as free chlorine testing was done on-site, within the same day the samples were collected. HPC plates were then shipped to University of Waterloo, ON, for incubation, and results were read after 7 days of incubation.

16s rRNA testing was performed offsite- samples collected were preserved and shipped to Metagenom Bio Inc, in Waterloo, ON, Canada.

### Study Results

Samples were collected in 500 ml sterile sampling bags, and stored at room temperature before testing. All samples were tested within 4 hours of sampling.

#### 1. HPC Results

The HPC plating was performed on site, as follows. Each sampling bag was shaken then open in a manner to minimize air exposure and reduce the risk of contamination. Using a pipette and a sterile, single use tip, 100 microliters of samples were removed from the middle of the bag, about 1” below the surface. The sample removed was transferred aseptically to the plate, and spread using a sterile, single use cell spreader.

The plates were then marked with sample number and placed in an insulated box for transportation.

For determining the number of colony-forming units (CFU), the colonies on each plate were counted and multiplied by 10 to obtain the CFU/ml result. For plates where the colonies were not countable

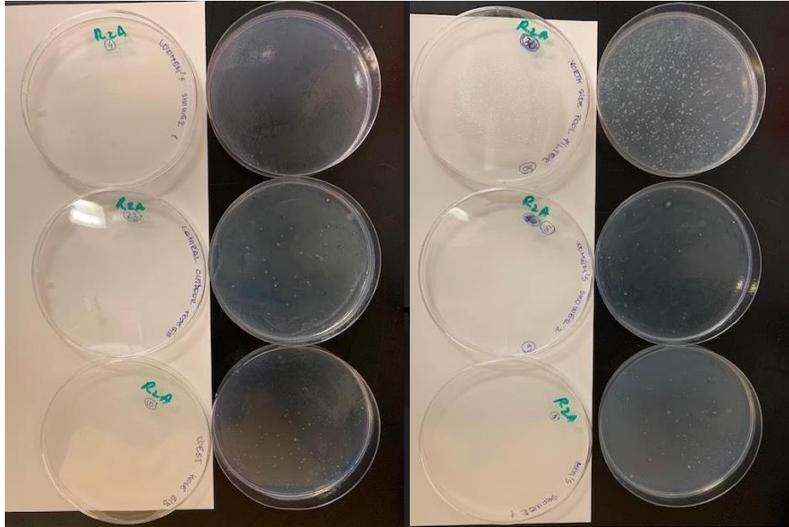


Figure 1: Examples of plates read after 7 days of incubation

(colonies merged, or were covered by biofilm) the results were market as TNTC (too numerous to count)

## 2. ExactBlue Test Results

ExactBlue testing was performed immediately after the HPC sample was collected. For the test, 20 ml of sample were concentrated using the filter cartridges and syringes included with the kit, backwashed with 4 ml of sterile saline solution, and 1ml of cell suspension in sterile saline solution was transferred to the test cuvette and mixed with the test reagent. The cuvette was then shaken and introduced back into the ExactBlue device for measurement. The measurement followed the steps as described in the ExactBlue app.

The reports generated were then saved as pdf files.



Test Type: BACTERIA

| Date                 | Water Source | Location | Test Output   | Confidence | Notes     |
|----------------------|--------------|----------|---|------------|-----------|
| 2020-01-31, 05:15 PM | Sample 17    |          | Bacteria level: Below detection limit (< 5000 CFU/ml) | 60         | Fresno tx |

Device ID: DB:BE:08:D3:76:55  
IP-based location (approx.): Toronto CA (43.6653 -79.4343)



Test Type: BACTERIA

| Date                 | Water Source      | Location  | Test Output                         | Confidence | Notes |
|----------------------|-------------------|-----------|-------------------------------------|------------|-------|
| 2020-01-31, 09:01 PM | Sample 23, site 1 | Fresno tx | Bacteria level: High (>5000 CFU/ml) | 97         |       |

Device ID: C2:81:D4:38:95:E5  
IP-based location (approx.): Toronto CA (43.6653 -79.4343)



Test Type: BACTERIA

| Date                 | Water Source | Location  | Test Output                         | Confidence | Notes |
|----------------------|--------------|-----------|-------------------------------------|------------|-------|
| 2020-02-01, 12:43 AM | Sample 30    | Fresno tx | Bacteria level: High (>5000 CFU/ml) | 92         |       |

Device ID: C2:81:D4:38:95:E5  
IP-based location (approx.): Toronto CA (43.6653 -79.4343)



Test Type: BACTERIA

| Date                 | Water Source     | Location | Test Output                         | Confidence | Notes     |
|----------------------|------------------|----------|-------------------------------------|------------|-----------|
| 2020-01-31, 05:24 PM | Sample 27 site 1 |          | Bacteria level: High (>5000 CFU/ml) | 97         | Fresno tx |

Device ID: 9D004A2F-E4A6-09A5-3DFA-3C1923B5AD15  
IP-based location (approx.): Toronto CA (43.6653 -79.4343)

Figure 2: Sample of ExactBlue reports

## PCR- 16s rRNA Test Results

The samples sent for PCR testing were collected following the same sample preparation as for ExactBlue, immediately after ExactBlue test, for each sample. 20 ml of sample was concentrated using the same syringe and filter cartridge used for ExactBlue testing, and the cell are transferred to a 2 ml cryo-vial using RNA preservation solution provided by Metagenom Bio.

The samples were then shipped to Metagenom Bio for testing. The results provided by Metagenom Bio as a summary spreadsheet, and visualized using the QIIME 2 Viewer.

## Analysis and Interpretation

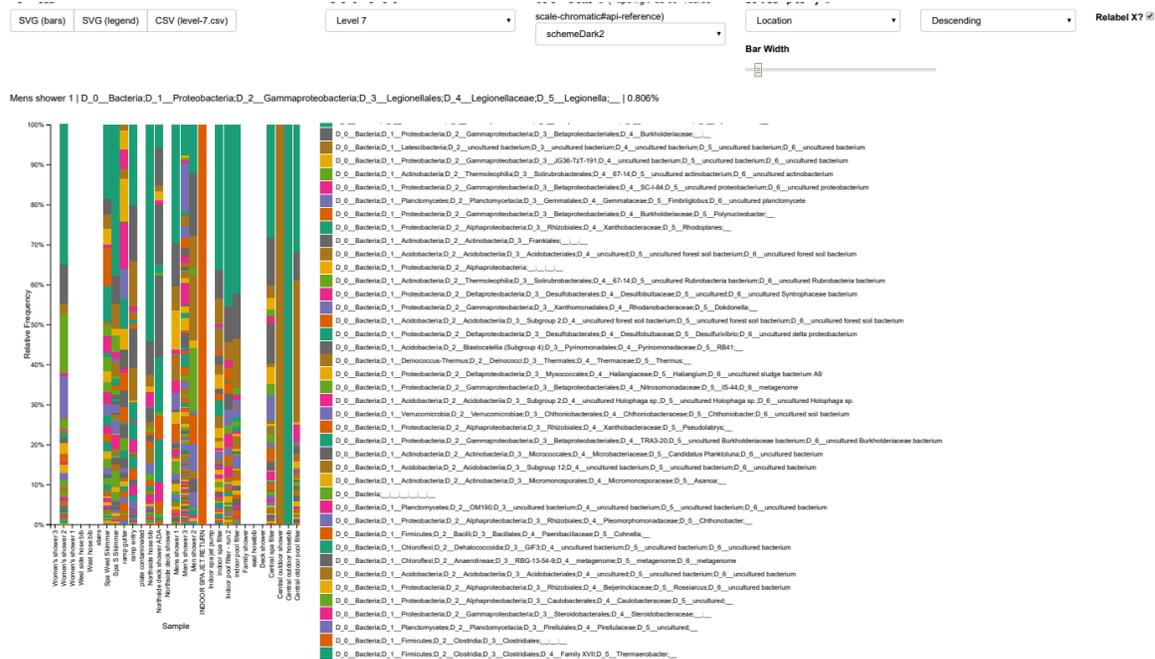


Figure 3: Stacked bar plot representation of microbial population in each sample- each color represents a specific microorganisms. The height of each bar represents %of the RNA of the total sample

Given the limitations in regards to testing time and conditions, HPC testing was done in one replicate, and with no serial dilutions to confirm the cell count- which is the standard procedure in a lab setting. Also, no quality control procedures were put in place to determine the presence of contaminants or determine potential false positive/false negative results. As the intention of the study was to determine the capacity of ExactBlue system to collect comparable data to HPC, we believe that the study has achieved its intended purpose.

| Number | Location                    | Sampling Time | HPC (R2A)<br>Probable CFU/ml | ExactBlue Result<br>(MPN) | Chlorine value<br>(ppm) |
|--------|-----------------------------|---------------|------------------------------|---------------------------|-------------------------|
| 1      | Mens shower 1               | 9:50 AM       | 2400                         | 5906                      | 1.01                    |
| 2      | Men shower 2                | 9:50 AM       | >1000                        | 8259                      | 1.03                    |
| 3      | Men's shower 3              | 9:50 AM       | >1000                        | 5573                      | 1.6                     |
| 4      | Women's shower 1            | 10:00 AM      | >1000                        | 4614                      | 1.39                    |
| 5      | Women's shower 2            | 10:00 AM      | 430                          | 934                       | 0.19                    |
| 6      | Women's shower 3            | 10:00 AM      | 360                          | 2943                      | 1.69 ppm                |
| 7      | Spa S Skimmer               | 9:40 AM       | 390                          | less than 500             | over 2.5                |
| 8      | Spa West Skimmer            | 9:36 AM       | 350                          | 801                       | over 2.5                |
| 9      | Deck shower                 | 9:32 AM       | 40                           | less than 500             | 0.15                    |
| 10     | West hose bib               | 9:30 AM       | 1470                         | less than 500             | 0                       |
| 11     | Ramp entry                  | 9:19 AM       | >1000                        | about 1500                | over 2.5                |
| 12     | Ramp gutter                 | 9:19 AM       | 240                          | less than 500             | over 2.5                |
| 13     | East hosebib                | 9:27 AM       | >1000                        | about 6000                | 0.42                    |
| 14     | Indoor pool filter          | 9:34 AM       | 310                          | less than 500             | 1.28                    |
| 15     | Indoor spa filter           | 9:26 AM       | 410                          | 1267                      | 2.03                    |
| 16     | Indoor spa jet pump         | 9:37 AM       | 350                          | less than 500             | Over 2.50               |
| 17     | Indoor spa jet return       | 9:41 AM       | 490                          | less than 500             | over 2.5                |
| 18     | Stairs                      | 9:53 AM       | 470                          | less than 500             | over 2.5                |
| 19     | Family shower               | 11:00 AM      | 350                          | LESS than 500             | 0.35                    |
| 20     | Indoor pool filter - run 2  | 11:00 AM      | 0                            | LESS than 500             | 2.32                    |
| 21     | Central outdoor pool filter | 10:55 AM      | 420                          | 2742                      | 1.44                    |
| 22     | Central outdoor shower      | 11:02 AM      | >1000                        | 7392                      | 0.02                    |
| 23     | Central outdoor hosebib     | 11:02 AM      | 310                          | 12996                     | 0.95                    |
| 24     | Northside deck shower       | 11:38 AM      | 360                          | less than 500             | 0.01                    |
| 25     | Northside deck shower ADA   | 11:21 AM      | 300                          | 9361                      | 0.02                    |
| 26     | Northside hose bib          | 11:41 AM      | 580                          | 1441                      | 0                       |
| 27     | Central spa filter          | 10:55 AM      | 470                          | 8140                      | over 2.5                |
| 29     | West side hose bib          | 12:08 PM      | 390                          | less than 500             | 0                       |
| 30     | North side pool filter      | 11:45 AM      | 6710                         | 10272                     | 0.46                    |

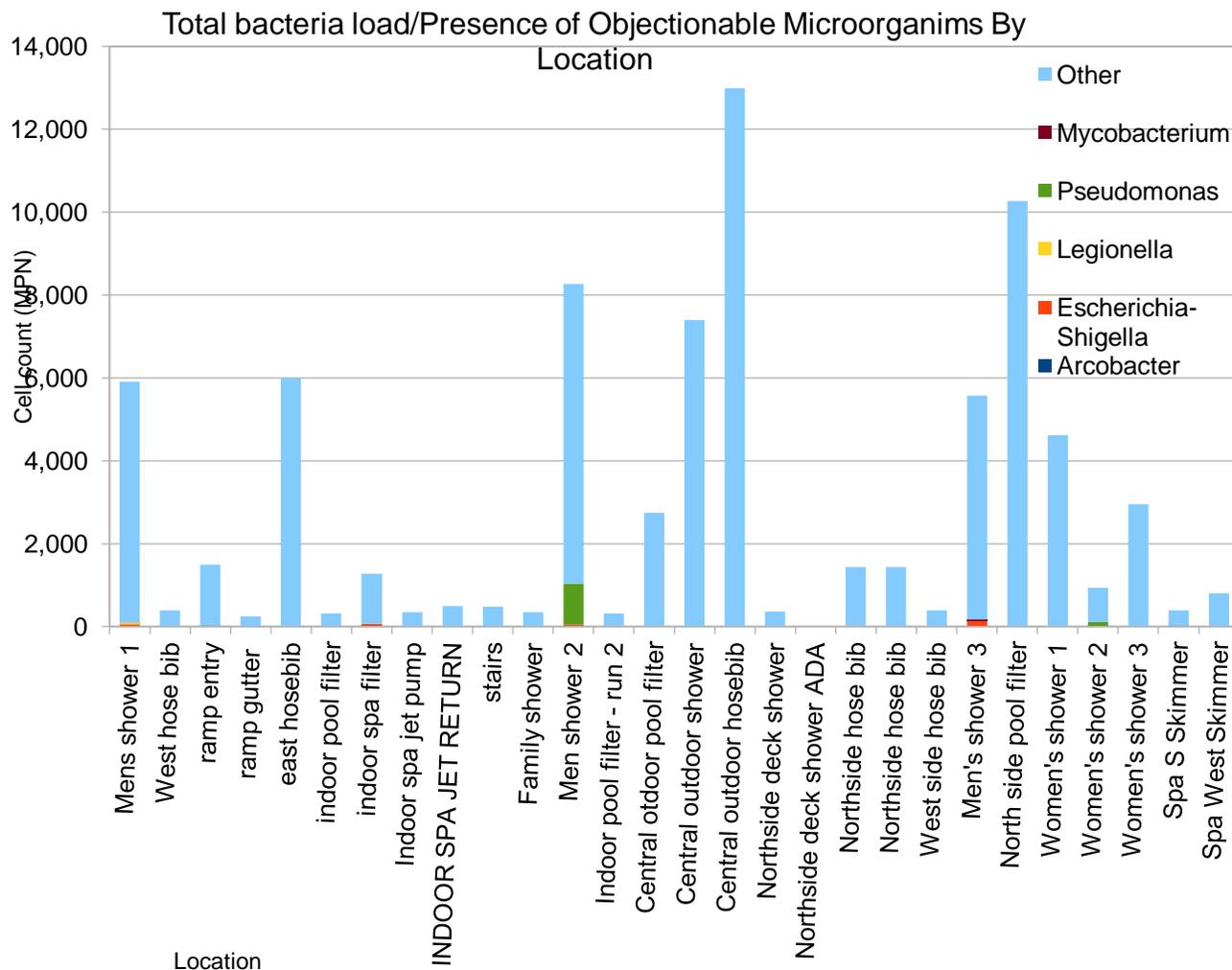
Figure 4: Summary Data for Total Microbial and Free Chlorine test results

Overall the results showed a strong correlation between HPC and ExactBlue results. ExactBlue results show no false negative results (all test that were below 500 MPN on ExactBlue had HPC counts below 550 CFU/ml). Based on HPC results, there were 9 “perceived false positive results” (HPC count was below 500CFU/ml but MPN value for ExactBlue was over 500). These results are a result of the fact

that ExactBlue also detects biofilm particles and viable but non-culturable cells (bacteria that do not form colonies on R2A plate, such as Mycobacteria, Legionella, and many other).

Free Chlorine results did not show the expected correlation with total bacteria counts for some of the samples, in some with no chlorine the total bacteria count was very low, in other, even at the recommended Free Chlorine levels, there were significant cell counts both on HPC and ExactBlue (Men’s showers for example)

The 16s rRNA test results also showed that samples with high total cell count had higher risk of pathogenic or opportunistic bacteria



Traces of Legionella spp was only found in one sample (Men Showers 1), however the % of Legionella in the sample was very low (less than 1%, or less than 20 MPN), and the specific type could not be identified (there is no indication that the strain found was Legionella Pneumophila sp1)

*Pseudomonas* spp. (opportunistic bacteria that can lead to pneumonia in immune-compromised patients) was also identified, in higher percentages in samples Men Showers 2, Men Showers 3, Women Showers 2)

Coliform bacteria was also detected, at low levels, in Men Shower 2, Men Shower 3 and in traces in Indoor spa filter, skimmers and ramp gutter. These results are expected in a shower.

The outdoor samples and hose-bibs mostly showed presence of biofilm-forming environmental bacteria, which is expected.

## Conclusion

Overall the test showed good correlation between HPC and ExactBlue results, confirmed also by the 16s rRNA test results.

Except the samples from shower (men's and women's) all other samples showed presence of biofilm, but no pathogenic or opportunistic bacteria.

Testing showed relatively high levels of biofilm, biofilm-forming bacteria and opportunistic bacteria, which suggest uneven usage. Attention should be paid by either disinfecting shower-heads or flushing the lines on a daily basis. A more extreme option, if this does not resolve the situation, is installing point-of-use filtration shower-heads.