



## Results Information

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Customer ID: City of Plattsburgh NY  
Project #: P-300-A  
Sample Date Received: 7/14/17  
Date of Analysis: 7/17/17 – 7/21/17  
Sample Type: 14 Membrane Filters  
Analysis Type: MST

## Procedure:

Submitted membrane filter samples were tested for the presence of host source specific bacteria using target DNA markers. Samples received were from collections made on 6/23/17, 6/30/17 and 7/1/17.

Submitted samples for MST testing had the following sample designations and were assayed for the host source markers indicated:

<u>Sample</u>	<u>Host Source Markers Surveyed</u>
RW0 - 6/23/17	human, bovine, canine, geese, gull
RW1- 6/23/17	human, bovine, canine, geese, gull
RW2- 6/23/17	human, bovine, canine, geese, gull
RW3- 6/23/17	human, bovine, canine, geese, gull
RW4- 6/23/17	human, bovine, canine, geese, gull
RW17- 6/23/17	human, bovine, canine, geese, gull
RW6 - 6/23/17	human, bovine, canine, geese, gull
RW0 - 6/30/17	human, bovine, canine, geese, gull
RW 1- 6/30/17	human, bovine, canine, geese, gull
RW 2- 6/30/17	human, bovine, canine, geese, gull
RW3- 6/30/17	human, bovine, canine, geese, gull
RW4- 6/30/17	human, bovine, canine, geese, gull
RW17- 6/30/17	human, bovine, canine, geese, gull
RW5- 7/1/17	human, bovine, canine, geese, gull

## **Procedure:**

500 ml of each water sample analyzed were previously suctioned filtered through a 0.45 micron filter membrane that proceeded to capture any bacteria present in the water sample. Each individual filter membrane containing captured bacteria were placed in sterile tubes and submitted to Helix Biological Laboratory. DNA extractions were performed using the Gene Rite EZ ST-1 kit according the manufacturer's instructions.

Bacterial DNA extractions were used as DNA template for quantitative Real Time (RT) PCR analysis using a Stratagene Mx 3005p qPCR/Real Time instrument. qPCR analysis incorporated oligonucleotide primers that target specific marker DNA sequences unique to host source specific Bacteroides bacteria. Appropriate positive and negative control samples were included in qPCR experimentation. Following the qPCR procedure, data was analyzed and relative proportions of DNA markers testing positive were calculated.

Replicates of each sample were run for quality assurance purposes and to confirm the observed results.

## **Results:**

Results of the quantitative PCR testing indicate the presence (positive) or absence (negative) of host source specific DNA markers through PCR amplification of host source specific DNA marker sequences, as well as the proportional quantities of each host source DNA marker in instances where multiple host source DNA markers are detected.

A sample testing positive for a host source specific DNA marker indicates that the host source specific DNA marker was detected, thus confirming the presence of that host source specific DNA marker in the sample. A sample testing negative for a host source specific DNA marker indicates that the host source specific DNA marker was not detected, thus confirming the absence of that host source specific DNA marker in the sample.

<b><u>Sample</u></b>	<b><u>Collection Date</u></b>	<b><u>Source DNA Marker</u></b>	<b><u>Result</u></b>
RW0	6/23/17	Human	+
RW0	6/23/17	Bovine	+
RW0	6/23/17	Canine	+
RW0	6/23/17	Geese	+
RW0	6/23/17	Gull	+
RW1	6/23/17	Human	+
RW1	6/23/17	Bovine	+
RW1	6/23/17	Canine	+
RW1	6/23/17	Geese	+
RW1	6/23/17	Gull	+
RW2	6/23/17	Human	+
RW2	6/23/17	Bovine	+
RW2	6/23/17	Canine	+
RW2	6/23/17	Geese	+
RW2	6/23/17	Gull	+
RW3	6/23/17	Human	+
RW3	6/23/17	Bovine	+
RW3	6/23/17	Canine	+
RW3	6/23/17	Geese	+
RW3	6/23/17	Gull	+
RW4	6/23/17	Human	+
RW4	6/23/17	Bovine	+
RW4	6/23/17	Canine	+
RW4	6/23/17	Geese	+
RW4	6/23/17	Gull	+

RW17	6/23/17	Human	+
RW17	6/23/17	Bovine	+
RW17	6/23/17	Canine	+
RW17	6/23/17	Geese	+
RW17	6/23/17	Gull	+

RW6	6/23/17	Human	+
RW6	6/23/17	Bovine	+
RW6	6/23/17	Canine	+
RW6	6/23/17	Geese	+
RW6	6/23/17	Gull	+

RW0	6/30/17	Human	+
RW0	6/30/17	Bovine	+
RW0	6/30/17	Canine	+
RW0	6/30/17	Geese	+
RW0	6/30/17	Gull	+

RW1	6/30/17	Human	+
RW1	6/30/17	Bovine	+
RW1	6/30/17	Canine	+
RW1	6/30/17	Geese	+
RW1	6/30/17	Gull	+

RW2	6/30/17	Human	+
RW2	6/30/17	Bovine	+
RW2	6/30/17	Canine	+
RW2	6/30/17	Geese	+
RW2	6/30/17	Gull	+

RW3	6/30/17	Human	+
RW3	6/30/17	Bovine	+
RW3	6/30/17	Canine	+
RW3	6/30/17	Geese	+
RW3	6/30/17	Gull	+
RW4	6/30/17	Human	+
RW4	6/30/17	Bovine	+
RW4	6/30/17	Canine	+
RW4	6/30/17	Geese	+
RW4	6/30/17	Gull	+
RW17	6/30/17	Human	+
RW17	6/30/17	Bovine	+
RW17	6/30/17	Canine	+
RW17	6/30/17	Geese	+
RW17	6/30/17	Gull	+
RW5	7/1/17	Human	+
RW5	7/1/17	Bovine	+
RW5	7/1/17	Canine	+
RW5	7/1/17	Geese	+
RW5	7/1/17	Gull	+

A positive result for a host source specific DNA marker at a collection station implies that host source is contributing to fecal contamination that may have been determined at the collection station during the collection period. A negative result for a source specific DNA marker at a collection station implies that host source is not contributing to fecal contamination that may have been determined at the collection station during the collection period.

### **Proportional Fold Differences of Source DNA Markers Testing Positive**

Quantitative MST analysis determines the proportional amounts of each host source specific DNA marker where multiple host source specific DNA markers are detected at a collection site.

Proportional amounts of host source specific DNA markers are expressed as a fold difference between the host source specific DNA marker detected in greater quantity versus the host source specific DNA marker detected in lesser quantity.

For example, if host source specific DNA marker "A" has a 2 fold greater proportional fold difference than host source specific DNA marker "B", this means that the quantity of host source specific DNA marker "A" detected was 2 times greater than the quantity of host source specific DNA marker "B" detected at that collection site.

**Asterisk (\*) denotes the host source detected in the lowest concentration**

**Sample ID**                      **Collection Date**  
**Sample RW0**                      **6/23/17**

<b><u>Host Sources Detected</u></b>	<b><u>Proportional Fold Difference</u></b>	
<b>Human</b>	<b>5.62</b>	<b>Human/Canine</b>
<b>Bovine</b>	<b>12.04</b>	<b>Bovine /Canine</b>
<b>*Canine</b>		
<b>Geese</b>	<b>4.32</b>	<b>Geese Canine</b>
<b>Gull</b>	<b>266.87</b>	<b>Gull Canine</b>

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<b><u>Sample ID</u></b>	<b><u>Collection Date</u></b>
Sample RW1	6/23/17

<b><u>Host Sources Detected</u></b>	<b><u>Proportional Fold Difference</u></b>
Human	68.59      Human/Geese
Bovine	30.27      Bovine /Geese
Canine	163.14      Canine/ Geese
*Geese	
Gull	1,296.13      Gull/Geese

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<b><u>Sample ID</u></b>	<b><u>Collection Date</u></b>
Sample RW2	6/23/17

<b><u>Host Sources Detected</u></b>	<b><u>Proportional Fold Difference</u></b>
Human	15.45      Human/Geese
Bovine	1.24      Bovine /Geese
Canine	13.45      Canine/ Geese
*Geese	
Gull	60.13      Gull/Geese

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<b><u>Sample ID</u></b>	<b><u>Collection Date</u></b>
Sample RW3	6/23/17

<b><u>Host Sources Detected</u></b>	<b><u>Proportional Fold Difference</u></b>
Human	9.51      Human/Geese
Bovine	10.78      Bovine /Geese
Canine	17.88      Canine/ Geese
*Geese	
Gull	124.50      Gull/Geese

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<u><b>Sample ID</b></u>	<u><b>Collection Date</b></u>
Sample RW4	6/23/17

<u><b>Host Sources Detected</b></u>	<u><b>Proportional Fold Difference</b></u>
Human	4.26 Human/Canine
Bovine	2.41 Bovine /Canine
*Canine	
Geese	1.91 Geese/ Canine
Gull	47.84 Gull/Canine

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<u><b>Sample ID</b></u>	<u><b>Collection Date</b></u>
Sample RW17	6/23/17

<u><b>Host Sources Detected</b></u>	<u><b>Proportional Fold Difference</b></u>
Human	4.63 Human/Geese
Bovine	203.66 Bovine /Geese
Canine	202.25 Canine/Geese
*Geese	
Gull	666.29 Gull/Geese

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<u><b>Sample ID</b></u>	<u><b>Collection Date</b></u>
Sample RW6	6/23/17

<u><b>Host Sources Detected</b></u>	<u><b>Proportional Fold Difference</b></u>
*Human	
Bovine	1.99 Bovine /Human
Canine	4.41 Canine/Human
Geese	6.11 Geese/Human
Gull	55.72 Gull/Human

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<u>Sample ID</u>	<u>Collection Date</u>
Sample RW0	6/30/17

<u>Host Sources Detected</u>	<u>Proportional Fold Difference</u>
Human	576.03 Human/Geese
Bovine	340.14 Bovine /Geese
Canine	803.41 Canine/Geese
*Geese	
Gull	286.03 Gull/Geese

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<u>Sample ID</u>	<u>Collection Date</u>
Sample RW1	6/30/17

<u>Host Sources Detected</u>	<u>Proportional Fold Difference</u>
Human	146.02 Human/Gull
Bovine	494.56 Bovine /Gull
Canine	699.41 Canine/Gull
Geese	5.35 Geese/Gull
*Gull	

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<u>Sample ID</u>	<u>Collection Date</u>
Sample RW2	6/30/17

<u>Host Sources Detected</u>	<u>Proportional Fold Difference</u>
Human	139.10 Human/Geese
Bovine	99.04 Bovine /Geese
Canine	227.54 Canine/Geese
*Geese	
Gull	30.48 Gull/Geese

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<u><b>Sample ID</b></u>	<u><b>Collection Date</b></u>
Sample RW3	6/30/17

<u><b>Host Sources Detected</b></u>	<u><b>Proportional Fold Difference</b></u>
Human	157.59      Human/Geese
Bovine	600.49      Bovine /Geese
Canine	685.02      Canine/Geese
*Geese	
Gull	458.25      Gull/Geese

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<u><b>Sample ID</b></u>	<u><b>Collection Date</b></u>
Sample RW4	6/30/17

<u><b>Host Sources Detected</b></u>	<u><b>Proportional Fold Difference</b></u>
Human	27.47      Human/Gull
Bovine	4,299.64      Bovine /Gull
Canine	347.29      Canine/Gull
Geese	4.03      Geese/Gull
*Gull	

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<u><b>Sample ID</b></u>	<u><b>Collection Date</b></u>
Sample RW17	6/30/17

<u><b>Host Sources Detected</b></u>	<u><b>Proportional Fold Difference</b></u>
Human	445.72      Human/Geese
Bovine	254.23      Bovine /Geese
Canine	17.75      Canine/Geese
*Geese	
Gull	26,615.89      Gull/Geese

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**Sample ID**  
Sample RW5

**Collection Date**  
7/1/17

**Host Sources Detected**

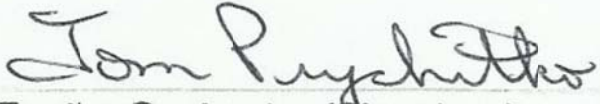
Human  
Bovine  
Canine  
\*Geese  
Gull

**Proportional Fold Difference**

106.89      Human/Geese  
65.80      Bovine /Geese  
142.02      Canine/Geese  
  
308.69      Gull/Geese

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These results were verified by Tom Prychitko, Laboratory Director for Helix Biological Laboratory.

  
For the Contractor (Signature)

All sample analysis performed has met with the requirements of the Helix Biological Laboratory Quality Control / Quality Assurance plan for Microbial Source Tracking analysis.

