Biomonitor

August 31, 2023
Steuben County Lakes Council
317 South Wayne Street,
Suite 2A
Angola, IN 46703

STEUBEN COUNTY ENVIRONMENTAL DNA REPORT

INTRODUCTION

Thirty-three wet weather samples were collected on July 12, 2023 in and around Steuben County to determine the source of elevated *Escherichia coli (E. coli)* concentrations at several locations throughout the county. This was the fourth such sampling event, previous samples were collected in October 2020, June 2021, and July 2022. At the request of the Steuben County Lakes Council, 8 new sampling locations were added for the 2023 sampling event. The monitoring sites are summarized in Table 1 and maps are included in Appendix A. These samples were analyzed for *E. coli* and the presence of human, swine, bovine, poultry, sheep, and dog DNA.

Site Number	Latitude	Longitude	Water Body	Years Sampled
3	41.729226	-84.82411	Clear Lake	2020, 2022
4	41.7685738	-85.0201903	Lake George Watershed	2020, 2021, 2022
6	41.7713339	-85.004431	Lake George Watershed	2020, 2021, 2022
7	41.6555037	-85.0195794	Carpenter Creek	2020, 2021, 2022
8	41.6568634	-85.0230525	Carpenter Creek	2020, 2021, 2022
9	41.6607941	-85.0282594	Carpenter Creek	2020, 2021, 2022
10	41.6670917	-85.0362151	Carpenter Creek	2020, 2021, 2022
11	41.6681564	-85.0360016	Carpenter Creek/Crooked Lake	2020, 2021, 2022
12	41.6713764	-85.0263185	Palfreyman Ditch	2020, 2021, 2022
13	41.6644794	-85.0182336	Palfreyman Ditch	2020, 2021, 2022
14	41.6641362	-85.0181106	Palfreyman Ditch	2020, 2021, 2022
15	41.6643306	-85.0173968	Palfreyman Ditch	2020, 2021, 2022
16	41.6588809	-85.0041896	Palfreyman Ditch	2020, 2021, 2022
17	41.719156	-85.033741	Snow Lake	2021, 2022
18	41.542063	-85.194894	McClish Lake	2021, 2022
19	41.546779	-85.191998	McClish Lake	2021, 2022
21	41.618222	-85.156835	Lake Arrowhead	2021, 2022
25	41.729612	-84.847775	Clear Lake	2022

Site Number	Latitude	Longitude	Water Body	Years Sampled
26	41.651418	-85.174436	Pigeon River	2022
27	41.741473	-85.171456	Fawn River	2022
28	41.558906	-84.835616	Fish Creek	2022
29	41.631997	-84.937648	Pigeon River	2022
31	41.757408	-85.078012	Lake Pleasant	2022
33	41.624472	-85.026793	Fox Lake	2022
34	41.680974	-85.019476	Lake James	2022
35	41.63079	-85.02547	Fox Lake	New
36	41.696849	-85.088635	Lake Gage	New
37	41.699593	-85.103777	Lake Gage	New
38	41.713098	-85.119539	Lake Gage	New
39	41.746742	-84.799206	Little Long Lake	New
40	41.750647	-84.80119	Little Long Lake	New
41	41.627423	-85.165008	West Otter Lake	New
42	41.558207	-84.909597	Black Creek	New

Table 1: July 12, 2023 Sampling Locations

RESULTS

E. coli samples were filtered and incubated in the field by Biomonitor to stay within the short holding time for bacterial samples. A field sheet showing sample collection data is in Appendix B. The complete laboratory report can be found in Appendix C. Elevated *E. coli* levels were detected at many of the sampling locations. A summary of the *E. coli* results is displayed in Table 2 below.

A filtered sample was also collected, and the filter sent to be analyzed for human, dog, bovine, swine, poultry, and sheep DNA. The complete lab report can be found in Appendix D. A summary of the eDNA results is shown in Table 3 below. The filtered volume of sample is included in Table 3. Although this value has been normalized in the eDNA results, it is useful to consider the overall turbidity of the water body, since samples are filtered until the membrane fouls. A smaller volume of filtered water indicates that there were more particulates in the water. Figure 1 shows a graph with eDNA results plotted alongside *E. coli* results.

Site Number	Water Body	Description	Estimated Flow	E. Coli (CFU/100 mL)
3	Clear Lake	Harry Teeter's Ditch	Low	384
4	Lake George Watershed	Flint Road Boat Ramp	Low	112
6	Lake George Watershed	Kope Kon Road	Low	396

Site Number	Water Body	Description	Estimated Flow	E. Coli (CFU/100 mL)
7	Carpenter Creek	CR 155 W	Low	>2420
8	Carpenter Creek	CR 100 N	Med	1456
9	Carpenter Creek	CR 200 W	Low	1164
10	Carpenter Creek	Beach Road	Med	1254
11	Crooked Lake	Beach	None	694
12	Palfreyman Ditch	CR 200 W	Low	122
13	Palfreyman Ditch	Downstream Northwood	Low	1523
14	Palfreyman Ditch	Northwood	None	>2420
15	Palfreyman Ditch	Upstream Northwood	Low	2038
16	Palfreyman Ditch	Wohlert	Med	984
17	Snow Lake	Pokagon Treatment Plant	High	4
18	McClish Lake	Discharge on West End	Low	64
19	McClish Lake	Inlet on East End	Low	520
21	Lake Arrowhead	Ditch Entering	Low	750
25	Clear Lake	Tributary to Clear Lake	Low	>2420
26	Pigeon River	At IND 327	Med	200
27	Fawn River	IND 327 at the Fish Hatchery	Med	152
28	Fish Creek	At IND 427	Low	>2420
29	Pigeon River	At US 20 east of Angola	Low	828
31	Lake Pleasant	Tributary to Lake Pleasant	Low	>2420
33	Fox Lake	At Swimming Beach	None	10
34	Lake James	Discharge of Croxton ditch	Low	500
35	Fox Lake	Discharge of Fox Lake	Low	504
36	Lake Gage	Outlet from Crooked Lake	Low	164
37	Lake Gage	Inlet to Lake Gage	Low	>2420
38	Lake Gage	Outlet from Lime Lake	Med	12
39	Little Long Lake	East Tributary to Little Long Lake	Low	*
40	Little Long Lake	Derr Drain	Low	196
41	West Otter Lake	Tributary to West Otter	Low	582
42	Black Creek	Black Creek meets Hamilton Lake	Low	8

Table 2: *E. Coli* Results Summary

**Conditional formatting shows green highlights for E. coli below the Indiana Recreational Limit of 235 MPN/100 mL and red highlights above 1000 MPN/100 mL*

***2420 CFU/100 mL is the upper detection limit of the test*

Site Number	Water Body	Volume Filtered (mL)	eDNA (Copies/100 mL)					
			Bovine	Dog	Human	Poultry	Sheep	Swine
3	Clear Lake	30	0	0	0	0	0	50
4	Lake George Watershed	100	0	7	7	0	0	0
6	Lake George Watershed	100	0	14	157	7	0	0
7	Carpenter Creek	90	0	0	22	21	0	0
8	Carpenter Creek	100	0	0	36	0	0	0
9	Carpenter Creek	140	0	0	27	14	0	0
10	Carpenter Creek	150	0	0	1715	0	0	0
11	Carpenter Creek	150	0	0	53	0	0	0
12	Palfreyman Ditch	70	0	0	39	21	0	0
13	Palfreyman Ditch	150	0	10	32	0	0	0
14	Palfreyman Ditch	8	0	0	80	667	0	0
15	Palfreyman Ditch	150	0	87	86	0	0	20
16	Palfreyman Ditch	120	0	0	16	22	0	0
17	Snow Lake	60	20	6	327	64	0	86
18	McClish Lake	200	0	0	15	15	0	0
19	McClish Lake	300	0	0	24	0	0	0
21	Lake Arrowhead	150	0	0	59	1	0	0
25	Clear Lake	80	0	7	22	68	0	0
26	Pigeon River	150	0	0	26	15	0	32
27	Fawn River	180	0	0	79	9	0	0
28	Fish Creek	120	10	0	101	7	0	0
29	Pigeon River	120	0	5	35	0	0	0
31	Lake Pleasant	150	0	17	16	25	0	0
33	Fox Lake	150	0	0	26	0	0	0
34	Lake James	100	0	0	44	0	0	0
35	Fox Lake	25	0	0	323	0	0	9
36	Lake Gage	100	0	0	24	0	0	0
37	Lake Gage	150	0	5	116	14	0	0
38	Lake Gage	200	0	0	9	0	0	0
39	Little Long Lake	7	0	0	199	0	6	0
40	Little Long Lake	100	0	0	178	0	0	0
41	West Otter Lake	150	0	0	20	7	0	0
42	Black Creek	120	0	0	22	27	0	31

Table 3: eDNA Results Summary

**Conditional formatting shows green highlights for no eDNA detected and red highlights above 1000 copies/100 mL*

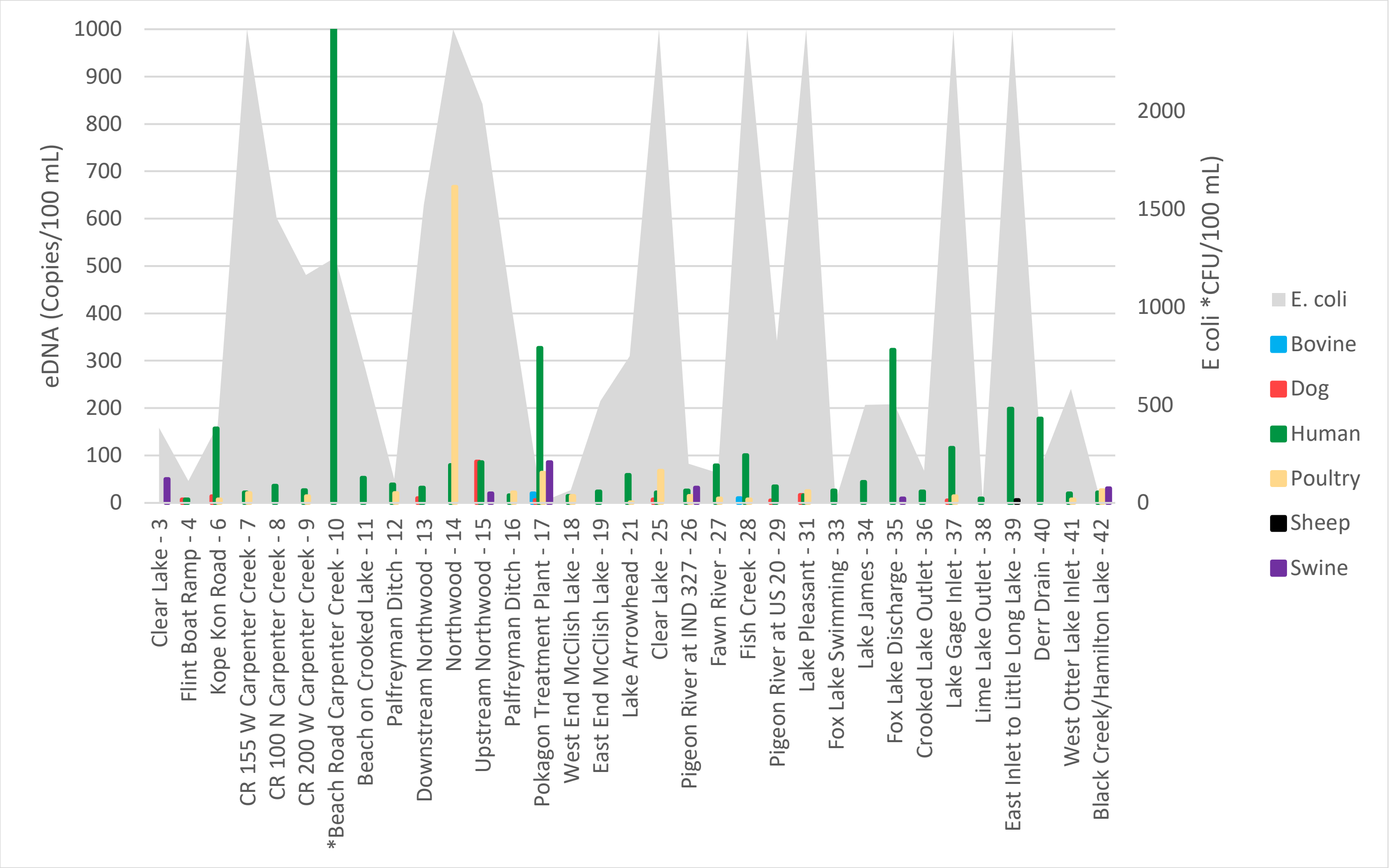


Figure 1: E. Coli and eDNA results

**The human eDNA value for site 10 is 1715 Copies per 100 mL of water*

CONCLUSIONS

As in previous years, *E. coli* was detected at high concentrations at many locations throughout the sampling area. The fact that the detection level was above the safe recreation level at the swimming beach on Crooked Lake is particularly concerning. Human DNA was found to be the main source of eDNA detected at the beach and throughout Steuben County. Tables 4 and 5 compare *E. coli* and Human eDNA levels from the past three sampling events.

During this sampling event, detections of farm animal DNA were higher relative to human DNA than seen in previous years. Most of these detections were still at low levels and should not be concerning. Particularly of note was the high level of poultry DNA detected in the unnamed ditch exiting the Northwood subdivision. This ditch contained water but was not flowing freely into Palfreyman Ditch at the time of sampling. It is possible that the poultry DNA is the result of a backyard chicken coop or some other hobby poultry farm. Additional investigation is recommended to confirm this hypothesis. It is worth comparing these results to other eDNA data throughout the country to provide context for which values are considered indicatory. Figure 2 shows a compilation of all the source tracking data analyzed by JonahVentures in 2021. Density refers to the relative likelihood to encounter host DNA at a given level across samples. Values a little over 1000 copies/100 mL are the most common for Human DNA.

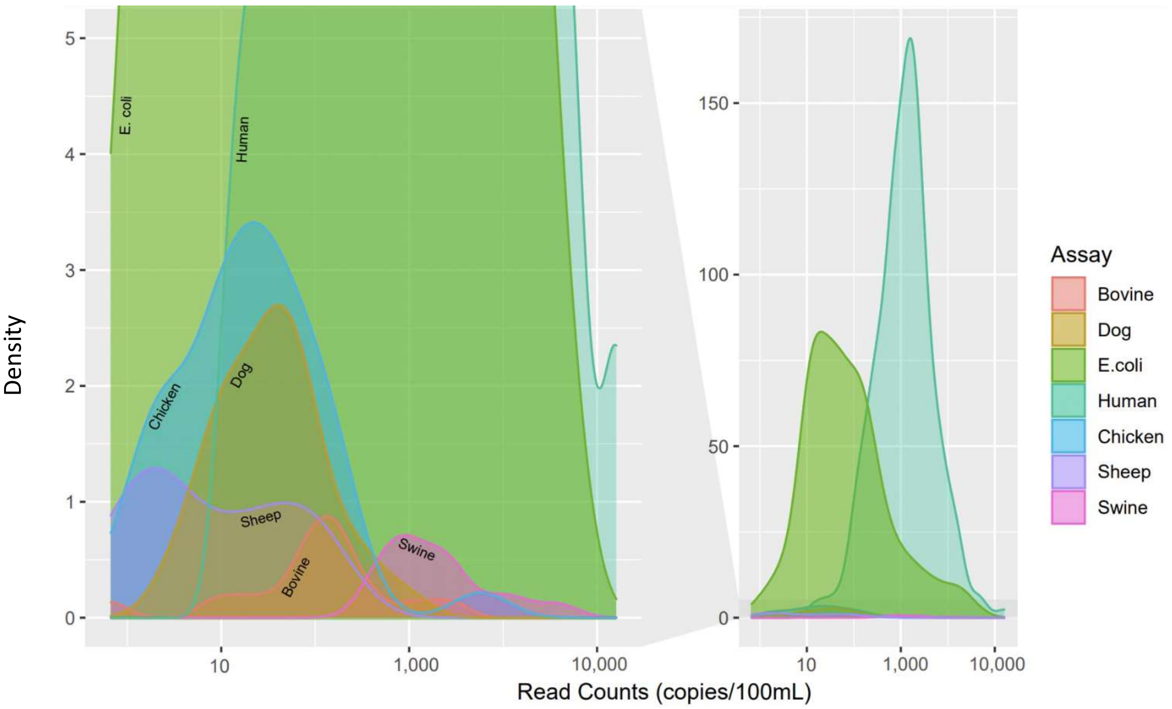


Figure 2: Graph generated by JonahVentures displaying all E. coli source tracking data analyzed by their laboratory in 2021

		E Coli (CFU/100 mL)			
Site Number	Water Body	Oct-20	Jun-21	Jul-22	Jul-23
3	Clear Lake	>2420	NT	>2420	384
4	Lake George Watershed	>2420	185	664	112
6	Lake George Watershed	178	82	524	396
7	Carpenter Creek	184	1046	420	>2420
8	Carpenter Creek	727	687	>2420	1456
9	Carpenter Creek	579	770	672	1164
10	Carpenter Creek	1300	249	>2420	1254
11	Carpenter Creek/Crooked Lake	4	25	2	694
12	Palfreyman Ditch	>2420	64	100	122
13	Palfreyman Ditch	>2420	>2420	816	1523
14	Palfreyman Ditch	NT	>2420	214	>2420
15	Palfreyman Ditch	>2420	1553	1538	2038
16	Palfreyman Ditch	>2420	1553	86	984
17	Snow Lake	NT	2	0	4

Site Number	Water Body	E Coli (CFU/100 mL)			
		Oct-20	Jun-21	Jul-22	Jul-23
18	McClish Lake	NT	308	276	64
19	McClish Lake	NT	64	>2420	520
21	Lake Arrowhead	NT	866	>2420	750
25	Clear Lake	NT	NT	280	>2420
26	Pigeon River	NT	NT	470	200
27	Fawn River	NT	NT	896	152
28	Fish Creek	NT	NT	>2420	>2420
29	Pigeon River	NT	NT	1210	828
31	Lake Pleasant	NT	NT	546	>2420
33	Fox Lake	NT	NT	54	10
34	Lake James	NT	NT	890	500
35	Fox Lake	NT	NT	NT	504
36	Lake Gage	NT	NT	NT	164
37	Lake Gage	NT	NT	NT	>2420
38	Lake Gage	NT	NT	NT	12
39	Little Long Lake	NT	NT	NT	*
40	Little Long Lake	NT	NT	NT	196
41	West Otter Lake	NT	NT	NT	582
42	Black Creek	NT	NT	NT	8

Table 4: E. Coli results from October 2020, June 2021, July 2022, and July 2023

**The upper detection limit of the m-coli blue E. coli test is 2420 CFU/100 mL*

***Conditional formatting shows green highlights for E. coli below the Indiana Recreational Limit of 235 MPN/100 mL and red highlights above 1000 MPN/100 mL*

****NT=Not Tested*

Site Number	Water Body	Human eDNA (Copies/100 mL)			
		Oct-20	Jun-21	Jul-22	Jul-23
3	Clear Lake	0	NT	1246	0
4	Lake George Watershed	1	71	1034	7
6	Lake George Watershed	31	44	702	157
7	Carpenter Creek	0	233	655	22
8	Carpenter Creek	5	89	759	36
9	Carpenter Creek	0	258	692	27
10	Carpenter Creek	18	110	868	1715
11	Carpenter Creek/Crooked Lake	33	36	78	53
12	Palfreyman Ditch	0	149	1360	39

Site Number	Water Body	Human eDNA (Copies/100 mL)			
		Oct-20	Jun-21	Jul-22	Jul-23
13	Palfreyman Ditch	60	264	854	32
14	Palfreyman Ditch	NT	90	364	80
15	Palfreyman Ditch	144	117	607	86
16	Palfreyman Ditch	40	193	338	16
17	Pokagon Treatment Plant	NT	1631	7367	327
18	McClish Lake	NT	78	182	15
19	McClish Lake	NT	230	242	24
21	Lake Arrowhead	NT	28	0	59
25	Clear Lake	NT	NT	1303	22
26	Pigeon River	NT	NT	358	26
27	Fawn River	NT	NT	469	79
28	Fish Creek	NT	NT	955	101
29	Pigeon River	NT	NT	1139	35
31	Lake Pleasant	NT	NT	511	16
33	Fox Lake	NT	NT	2126	26
34	Lake James	NT	NT	NT	44
35	Fox Lake	NT	NT	NT	323
36	Lake Gage	NT	NT	NT	24
37	Lake Gage	NT	NT	NT	116
38	Lake Gage	NT	NT	NT	9
39	Little Long Lake	NT	NT	NT	199
40	Little Long Lake	NT	NT	NT	178
41	West Otter Lake	NT	NT	NT	20
42	Black Creek	NT	NT	NT	22

Table 5: eDNA results from October 2020, June 2021, July 2022, and July 2023

**Conditional formatting shows green highlights for no eDNA detected and red highlights above 1000 copies/100 mL*

***NT = Not Tested*

Human DNA was detected throughout the sampling area but not at the high levels seen in 2022. This can largely be attributed to the smaller rain event preceding the 2023 sampling event. It is useful to keep in mind that humans and other animals are leaving behind DNA in the form of dead skin cells even without defecating and humans are the most common large animal in Steuben County. That may explain many of the lower human eDNA hits seen during this sampling event.

APPENDIX A – July 2023 Sampling Maps
APPENDIX B – July 2023 Field Sheet
APPENDIX C – July 2023 E. coli Lab Report
APPENDIX D – July 2023 eDNA Lab Report

APPENDIX A – July 2023 Sampling Maps

Steuben County eDNA Sampling Locations with *Escherichia coli* Results

Legend:

17 Site ID

July 2022 *E. Coli* results:

🟢 0 - 234 MPN/100 mL

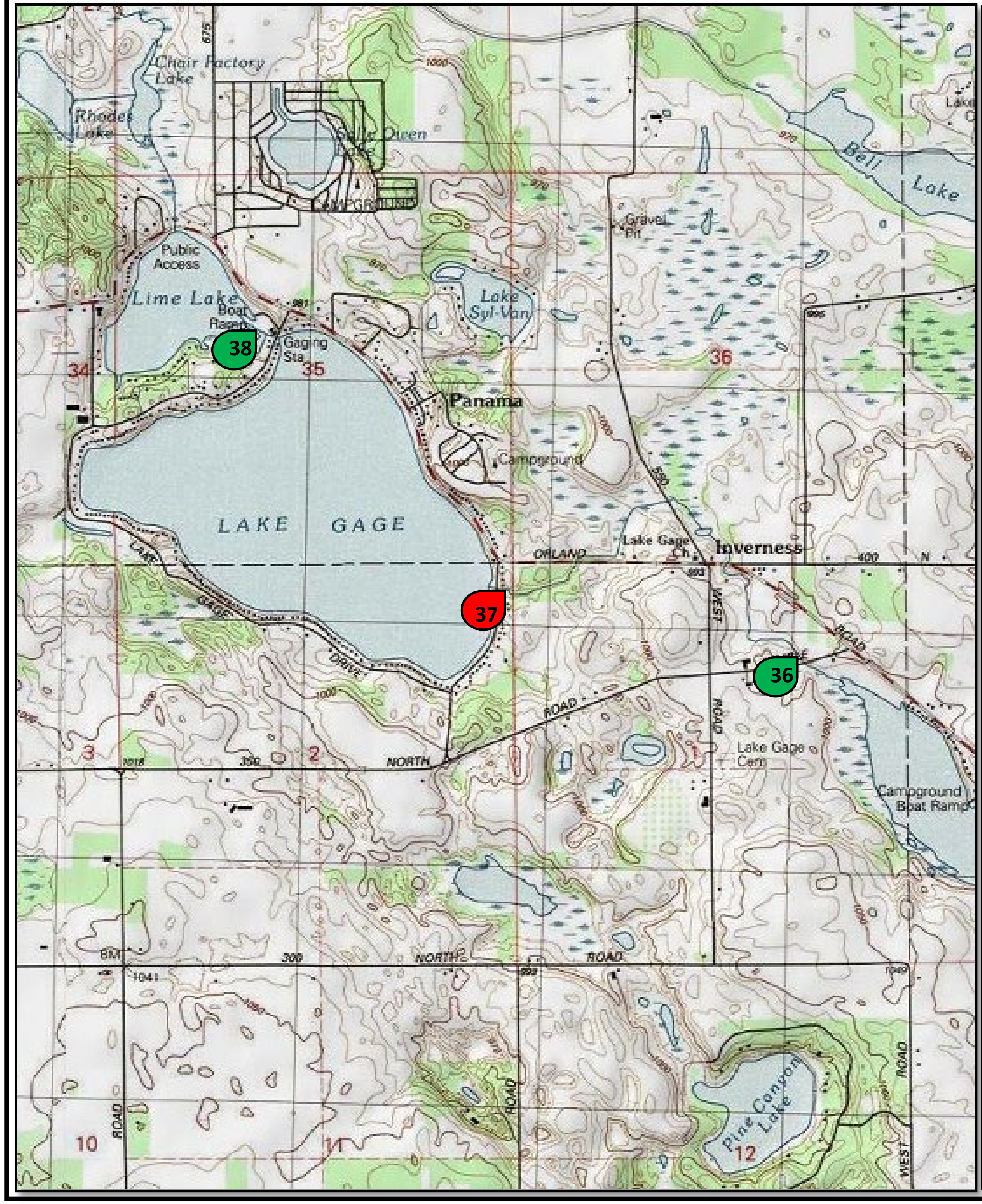
🟡 235 - 999 MPN/100 mL

🔴 1000 – 2420 MPN/100 mL

BASEMAP:
USGS Topographic Maps 2000-2023

Biomonitor

Steuben County eDNA Sampling Locations with *Escherichia coli* Results



Legend:

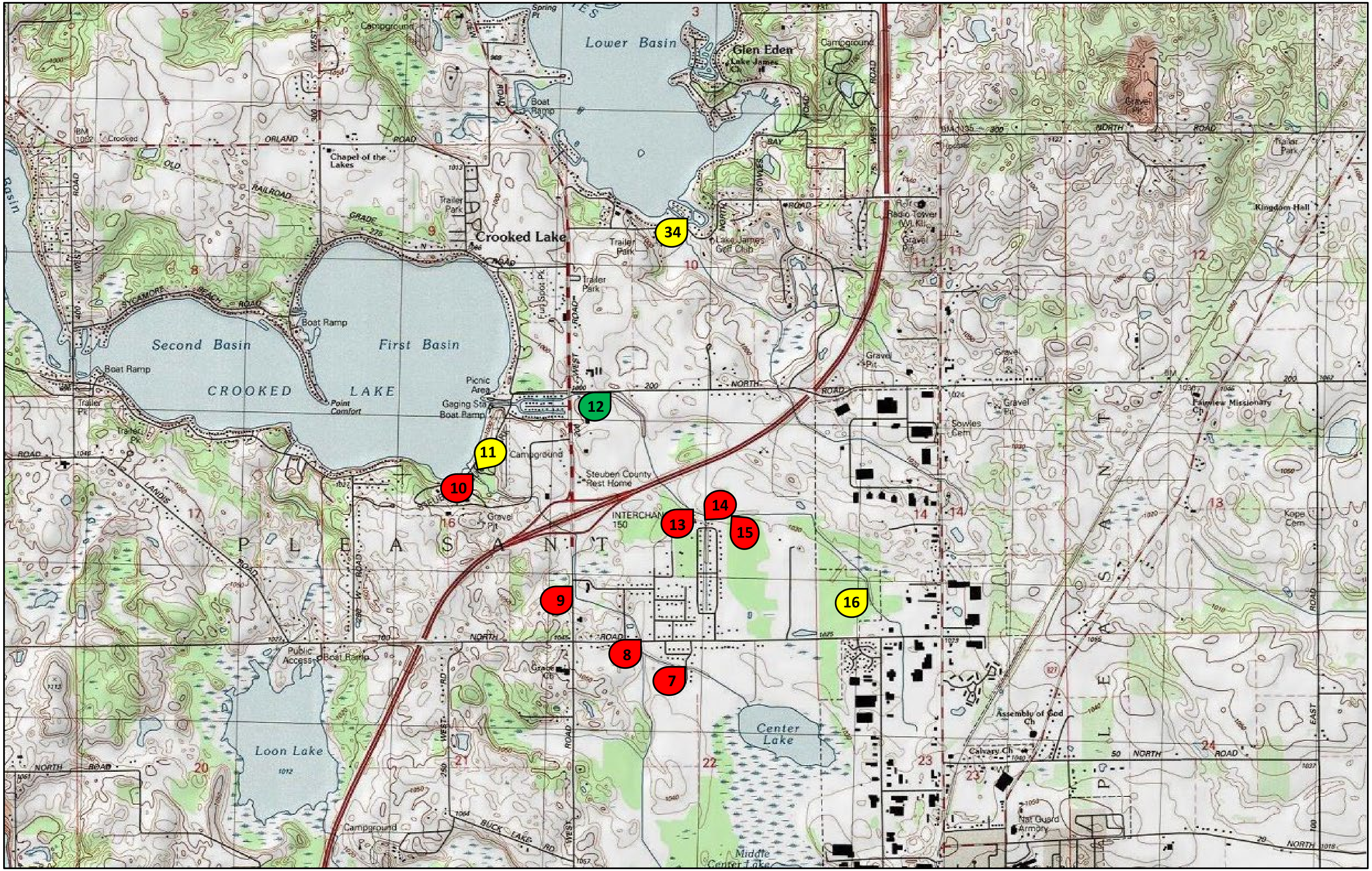
17 Site ID

July 2022 *E. Coli* results:

- 🟩 0 - 234 MPN/100 mL
- 🟨 235 - 999 MPN/100 mL
- 🟥 1000 – 2420 MPN/100 mL

BASEMAP:
USGS Topographic Maps 2000-2023

Biomonitor



Steuben County eDNA Sampling Locations with *Escherichia coli* Results

Legend:

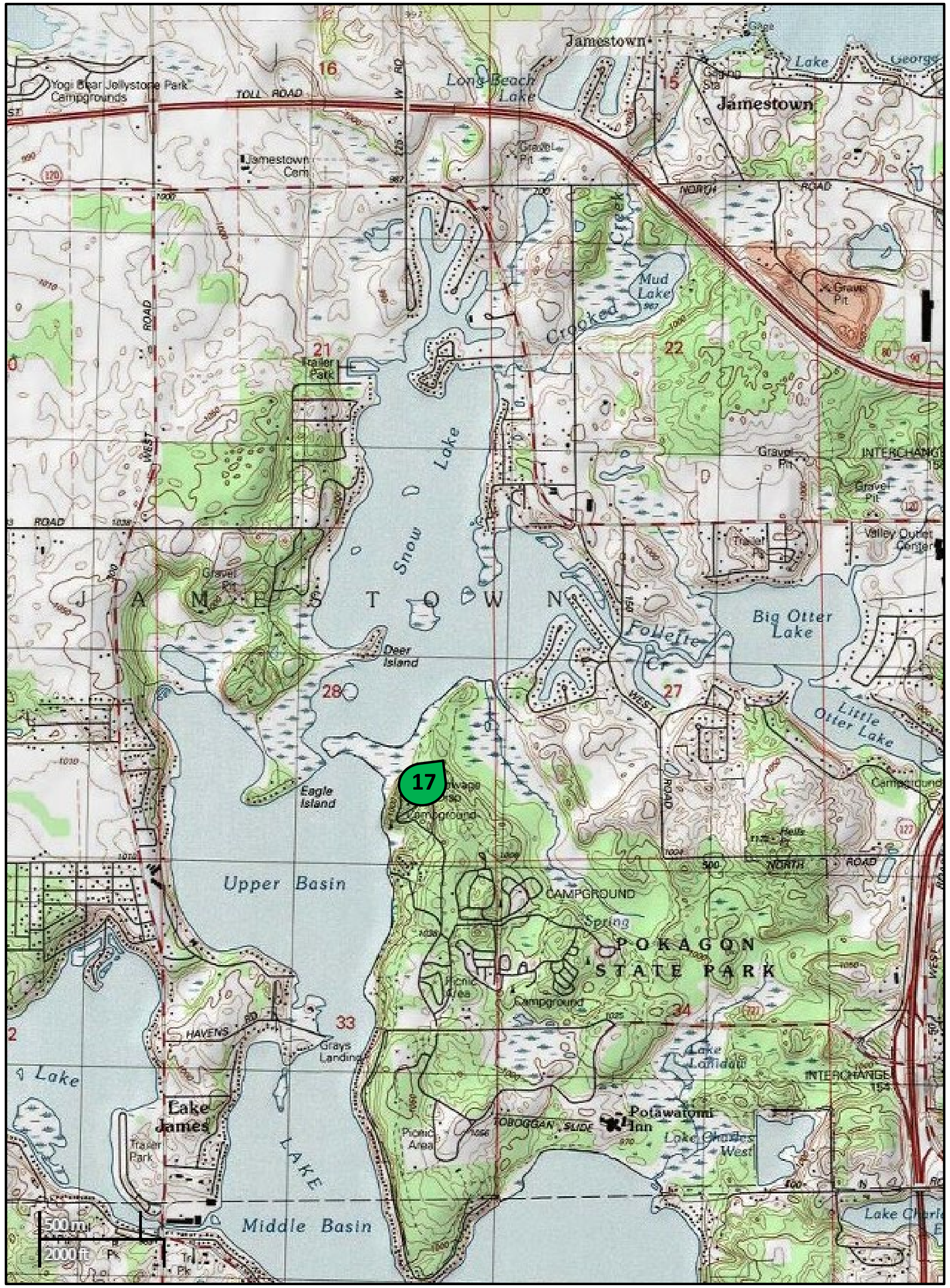
17 Site ID

July 2022 *E. Coli* results:

🟩	0 - 234 MPN/100 mL
🟨	235 - 999 MPN/100 mL
🟥	1000 – 2420 MPN/100 mL

BASEMAP:
USGS Topographic Maps 2000-2022

Biomonitor



Steuben County eDNA Sampling Locations with *Escherichia coli* Results

Legend:

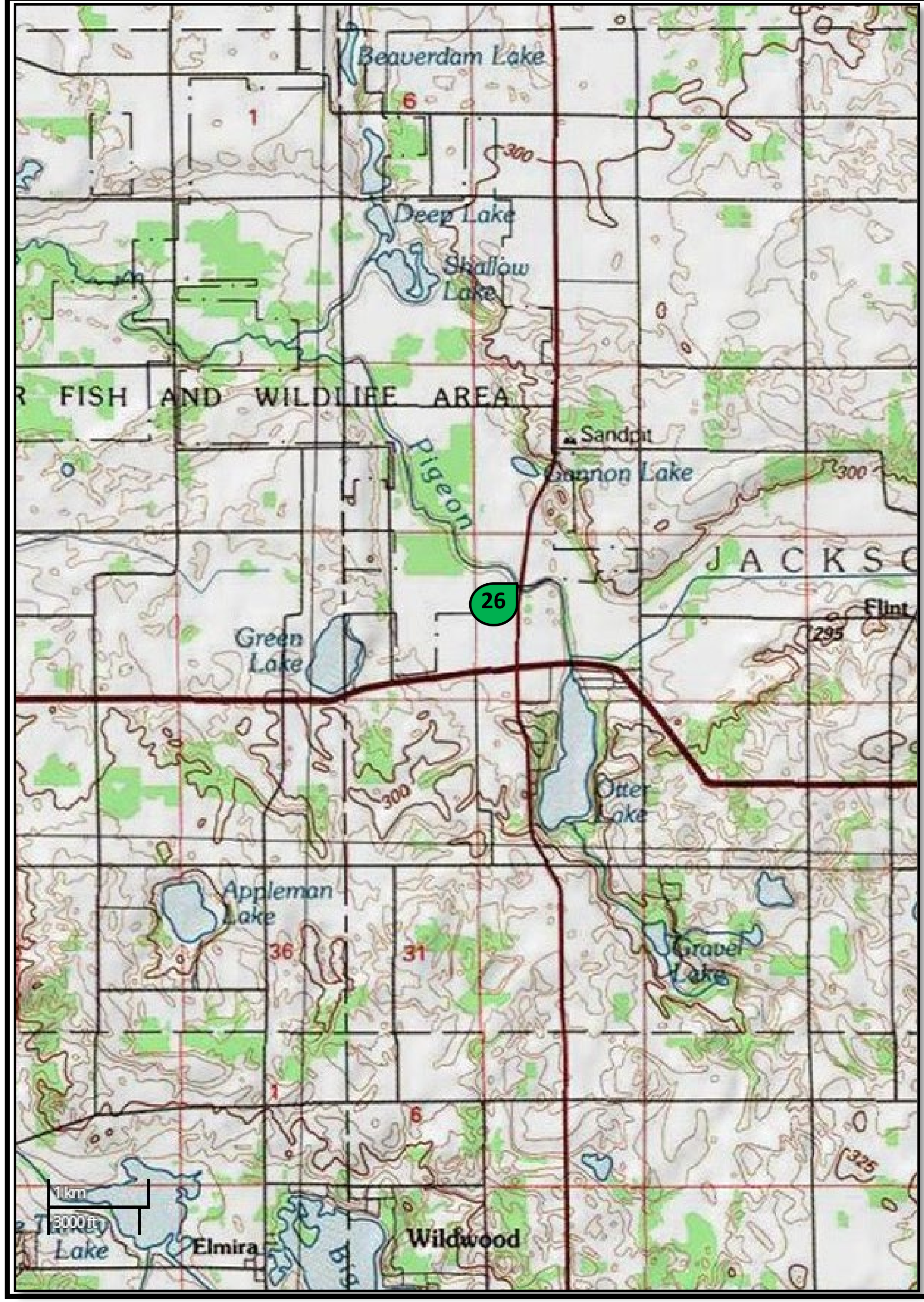
17	Site ID

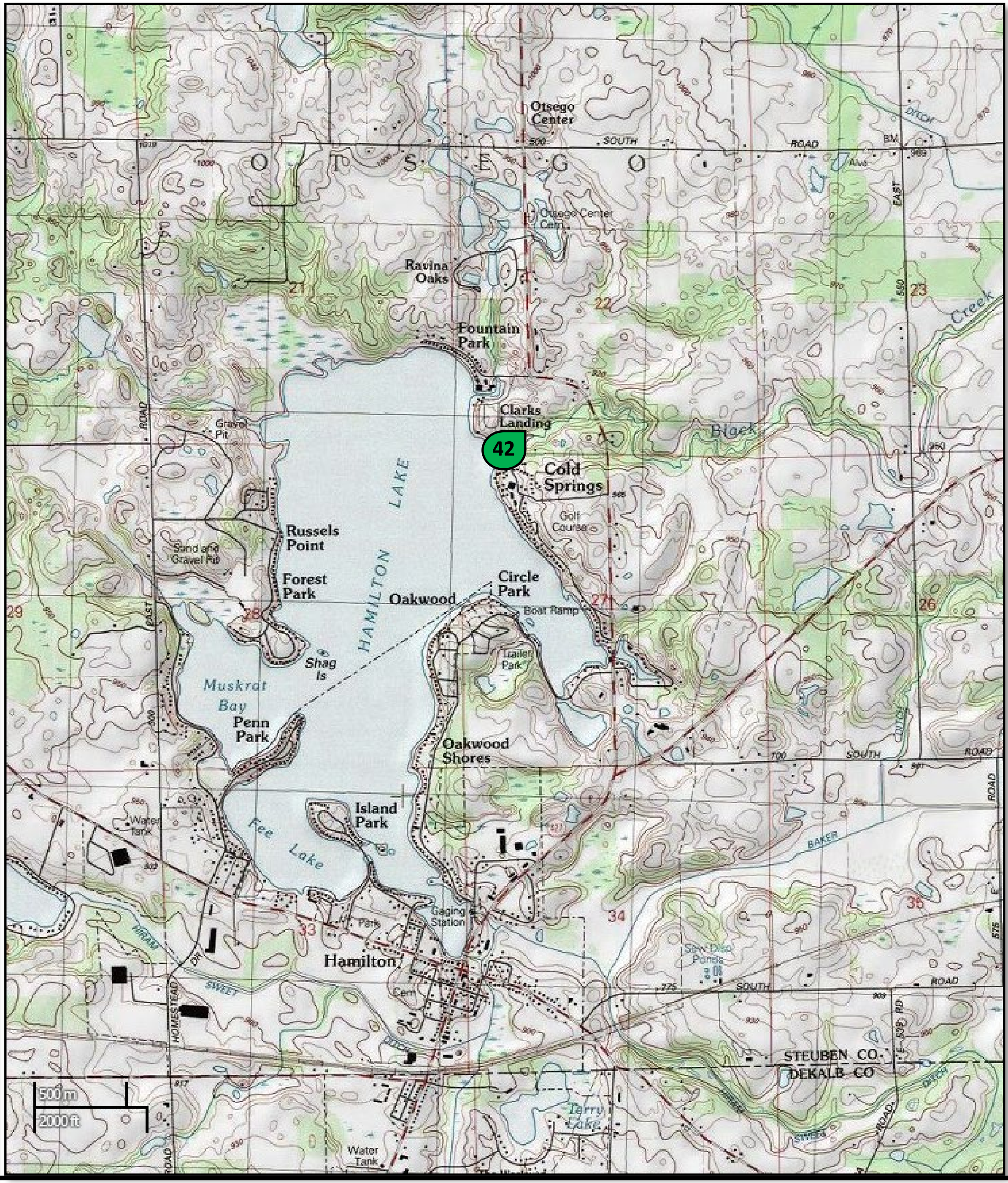
July 2022 *E. Coli* results:

🟩	0 - 234 MPN/100 mL
🟨	235 - 999 MPN/100 mL
🟥	1000 – 2420 MPN/100 mL

BASEMAP:
USGS Topographic Maps 2000-2022

Biomonitor





Steuben County eDNA Sampling Locations with *Escherichia coli* Results

Legend:

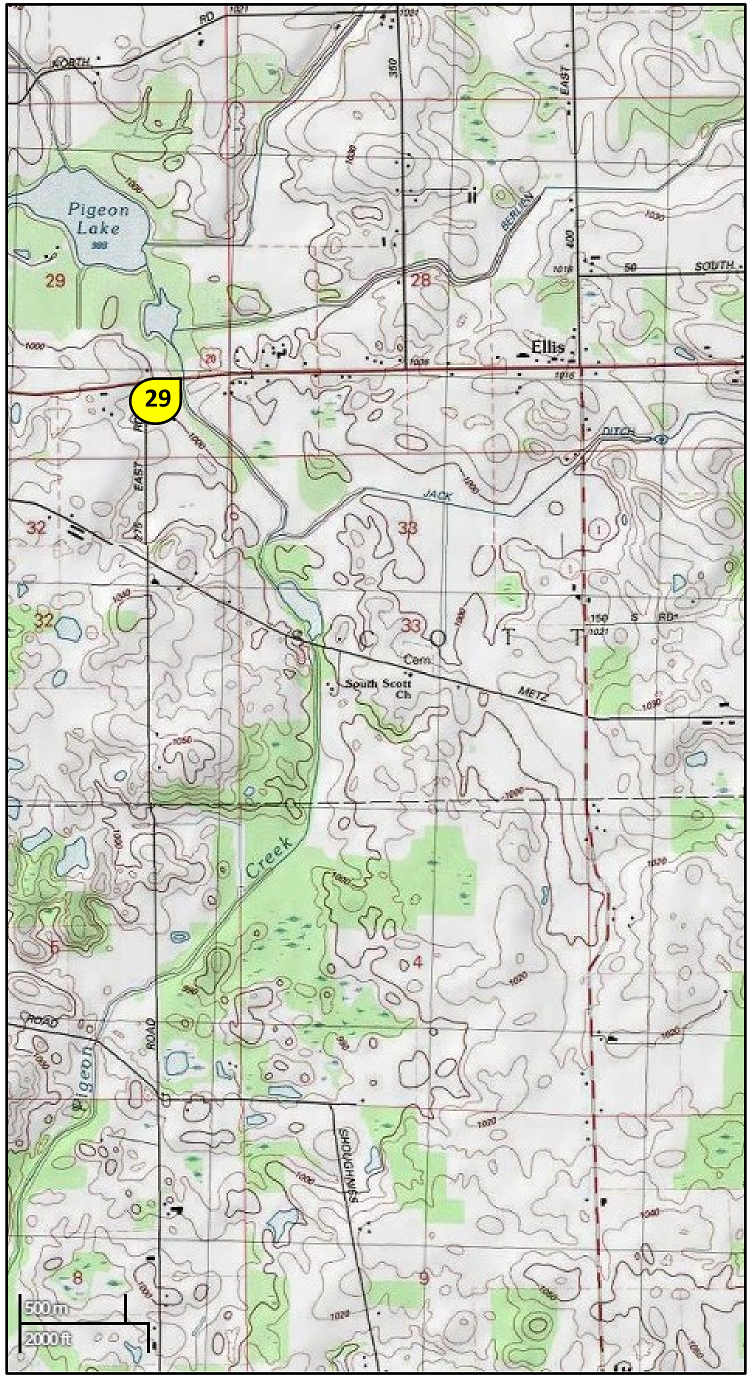
17 Site ID

July 2022 *E. Coli* results:

🟩	0 - 234 MPN/100 mL
🟨	235 - 999 MPN/100 mL
🟥	1000 – 2420 MPN/100 mL

BASEMAP:
USGS Topographic Maps 2000-2023

Biomonitor



Steuben County eDNA Sampling Locations with *Escherichia coli* Results

Legend:

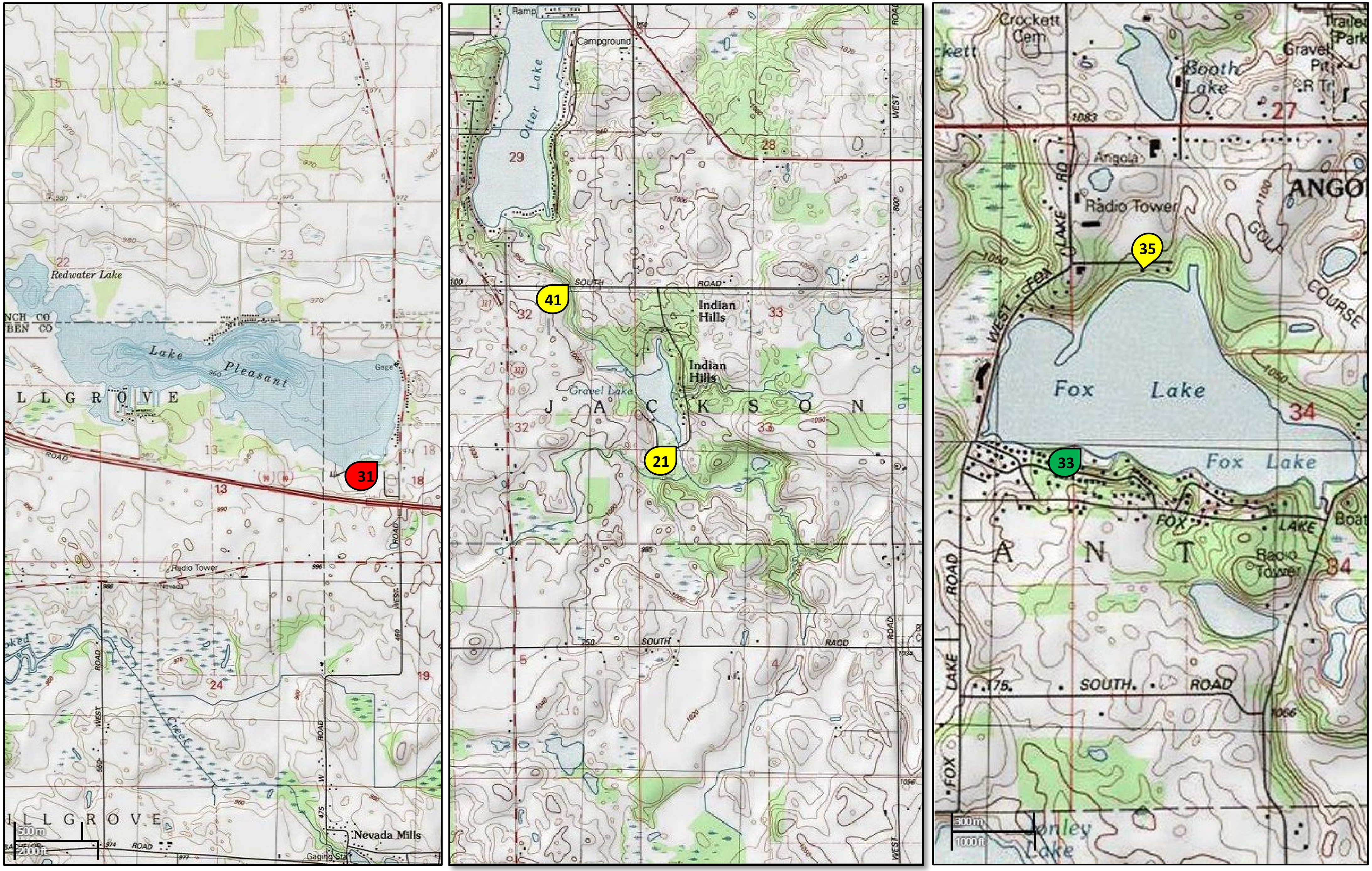
17	Site ID

July 2022 *E. Coli* results:

🟩	0 - 234 MPN/100 mL
🟨	235 - 999 MPN/100 mL
🟥	1000 – 2420 MPN/100 mL

BASEMAP:
USGS Topographic Maps 2000-2022

Biomonitor



Steuben County eDNA Sampling Locations with *Escherichia coli* Results

Legend:

17 Site ID

July 2022 *E. Coli* results:

- 🟩 0 - 234 MPN/100 mL
- 🟨 235 - 999 MPN/100 mL
- 🟥 1000 – 2420 MPN/100 mL

BASEMAP:
USGS Topographic Maps 2000-2023

Biomonitor

APPENDIX B – July 2023 Field Sheet

Date: 7/12/2023

Weather: Sunny, 70s
↳ to Rain 80s

Personel: Arizona Fox
Michael Britton
Sophie Gilliland

Northwood P

Dock site?

Site Number	Time Sampled	Sample Code	Filtered Volume	Estimated Flow	Notes
19	9:40	Y8VQVD0R	300 ml	low –	
18	9:52	WXZV1I1V	200 ml	low	paddle boarders observed while sampling
21	10:12	WWAD5H7B	150 ml	low +	
41	10:21	NFV1IMV1	150 ml	low	
26	10:34	7RUN50AQ	150 ml	med +	
33	11:04	TLWF1GRX	150 ml	NA	no swimmers
35	11:26	8VAL8072 ~~[illegible]~~	25 ml	low –	(41.6257899, -85.0319722) difficult to sample (mud, algae)
8	11:38	GFY014EY	100 ml	med	
7	11:47	6BUJWD78	90 ml	low	Visible dog poop, very pungent
16	11:54	54990008	120 ml	med	white cat in culvert
13	12:16	F8ZBOLU0	150 ml	low +	
14	12:23	P0ESICEZ	8 ml	low –	Sheen on water, stagnant
15	12:32	M16VLDQN	150 ml	low +	Racoon Prints
9	12.53	D71CUW6D	140 ml	low +	
10	1305	43E19YTX	150 ml	med –	
11	1310	KEGNNTKU	150 ml	NA	Swimmers observed , goose poop
12	1322	G36FIUMP	70 ml	low –	
34	1333	E714PIN9	100 ml	low +	Started raining 13:39*
17	14:03	Z19FVVQ4	60 ml	high	(still lightly raining) membrane didn't fill ↓ go thru campground *2 gates
36	14:31	Y8F5JJ6S	100 ml	low	↓ 14:35 loaded incubator!!
37	14:39	3HFMW31D	150 ml	low +	
38	14:49	APC3KP10	200 ml	med +	
27	15:06	PNILOCF1	180 ml	med +	Dead baby deer
31	15:25	35TQH4FI	150 ml	low	
4	15:41	S4CC7WJH	100 ml	low	Moved across street (not hole in yard)
6	15:53	70927D7J	100 ml	low –	~~[illegible]~~
40	16:18	TKMCRFVG	100 ml	low +	
39	16:32	FDVV09X8 ~~[illegible]~~	7 ml	low –	~~[illegible]~~ ecoli 20 ml stagnant "Bourbon"
3	17:08	4ZCV07K9	30 ml	low	[redacted]
25	17:18	6P4G354F	80 ml	low –	
29	17:38	D49Z66WR	120 ml	low +	Used bucket sampler
42	17:56 [redacted]	YB2CUW5F	120 ml	low –	
28	18:17	H3UXIH	120 ml	low +	construction – npp[redacted], up road, raccoon prints

*Final batch in Incubator 7:01 pm

APPENDIX C – July 2023 E. coli Lab Report

Simultaneous Detection and Enumeration of Total Coliforms and *Escherichia coli* using m-ColiBlue24 Membrane Filtration

July 13, 2023

The following table includes results from 34 total samples. This includes 33 site samples and a lab blank (LB). Samples collected and cultures inoculated by Biomonitor laboratory personnel on July 12, 2023.

SAMPLE ID	*E. coli* CFU/100 mL
Site - 3	384
Site - 4	112
Site - 6	396
Site - 7	>2420
Site - 8	1456
Site - 9	1164
Site - 10	1254
Site - 11	694
Site - 12	122
Site - 13	1523
Site - 14	>2420
Site - 15	2038
Site - 16	984
Site - 17	4
Site - 18	64
Site - 19	520
Site - 21	750
Site - 25	>2420
Site - 26	200
Site - 27	152
Site - 28	>2420
Site - 29	828
Site - 31	>2420
Site - 33	10
Site - 34	500
Site - 35	504
Site - 36	164
Site - 37	>2420
Site - 38	12
Site - 39	*
Site - 40	196
Site - 41	582
Site - 42	8
Lab Blank	0

APPENDIX D – July 2023 eDNA Lab Report

JONAH VENTURES

KNOWLEDGE IN SEQUENCE

July 25 2023

For more information go to www.jonahventures.com

info@jonahventures.com

Report prepared for arizona@biomonitor.com

BatchId = JVB2498

Number of samples analyzed = 33

Average number of copies detected

This table shows the average number of copies detected for each target organism in each sample. Values represent the average number of copies / 100 mL when sample volume was provided. When sample volumes are not known, the values indicate the estimated number of copies in the sample.

SampleId	Bovine01	Dog01	E.coli01	Human01	Poultry01	Sheep01	Swine01
2I9FVVQ4	20	6	55	327	64	0	86
35TQH4FI	0	17	2	16	25	0	0
3HFMW3ID	0	5	1930	116	14	0	0
43EI9YTX	0	0	72	1715	0	0	0
4ZCVO7K9	0	0	917	0	0	0	50
54990OO8	0	0	4	16	22	0	0
6BUJWD78	0	0	6	22	21	0	0
6P4G354F	0	7	12	22	68	0	0
70927D7J	0	14	5	157	7	0	0
7RUN5OAQ	0	0	0	26	15	0	32
APC3KPI0	0	0	65	9	0	0	0
BVALB072	0	0	403	323	0	0	0
D49Z66WR	0	5	1	35	0	0	0
D71CUW6D	0	0	154	27	14	0	0
E7I4PIN9	0	0	26	44	0	0	0
F8ZBOLU0	0	10	0	32	0	0	0
FDVV09X0	0	0	31	199	0	6	0
G36FIUMP	0	0	11	39	21	0	0
GFYOI4EY	0	0	545	36	0	0	0
KEGNNTKU	0	0	0	53	0	0	0
M16VLDQN	0	87	0	86	0	0	20
NFV1IMV1	0	0	27	20	7	0	0
P0ESICEZ	0	0	42085	80	667	0	0
PNILOCF1	0	0	1	79	9	0	0
S4CC7WJH	0	7	42	7	0	0	0
SIH3UXIH	10	0	454	101	7	0	0
TKMCRFVG	0	0	8	178	0	0	0
TLWF1GRX	0	0	0	26	0	0	0
WWAD5H7B	0	0	95	59	1	0	0
WX2V1IIV	0	0	0	15	15	0	0
XB2CUW5F	0	0	0	22	27	0	31
Y8F5JJ6S	0	0	0	21	0	0	9
Y8VQVD0R	0	0	427	24	0	0	0

Percent of replicates above detection limit

This table provides data on what percentage of the replicates that were run were above the detection limit. The detection limit is as high as the lowest positive on the calibration curve, but can be up to an order of magnitude lower. For example, a calibration curve might generate a positive at 100 copies and no positive for 10 copies, but the actual detection limit would be 11 copies. See the next section for the range of copy numbers estimated for each assay.

SampleId	Bovine01	Dog01	E.coli01	Human01	Poultry01	Sheep01	Swine01
2I9FVVQ4	66.7	33.3	100.0	100.0	66.7	0.0	33.3
35TQH4FI	0.0	66.7	33.3	100.0	33.3	0.0	0.0
3HFMW3ID	0.0	33.3	100.0	100.0	66.7	0.0	0.0
43EI9YTX	0.0	0.0	100.0	100.0	0.0	0.0	0.0
4ZCVO7K9	0.0	0.0	100.0	0.0	0.0	0.0	33.3
54990OO8	0.0	0.0	66.7	100.0	33.3	0.0	0.0
6BUJWD78	0.0	0.0	33.3	66.7	33.3	0.0	0.0
6P4G354F	0.0	33.3	66.7	66.7	33.3	0.0	0.0
70927D7J	0.0	33.3	100.0	100.0	33.3	0.0	0.0
7RUN5OAQ	0.0	0.0	0.0	100.0	66.7	0.0	33.3
APC3KPI0	0.0	0.0	100.0	66.7	0.0	0.0	0.0
BVALB072	0.0	0.0	100.0	100.0	0.0	0.0	0.0
D49Z66WR	0.0	33.3	33.3	100.0	0.0	0.0	0.0
D71CUW6D	0.0	0.0	100.0	66.7	33.3	0.0	0.0
E7I4PIN9	0.0	0.0	100.0	66.7	33.3	0.0	0.0
F8ZBOLU0	0.0	33.3	0.0	66.7	0.0	0.0	0.0
FDVV09X0	0.0	0.0	33.3	33.3	0.0	33.3	0.0
G36FIUMP	0.0	0.0	33.3	100.0	33.3	0.0	0.0
GFYOI4EY	0.0	0.0	100.0	100.0	0.0	0.0	0.0
KEGNNTKU	0.0	0.0	0.0	100.0	0.0	0.0	0.0
M16VLDQN	0.0	100.0	0.0	100.0	0.0	0.0	33.3
NFV1IMV1	0.0	0.0	100.0	66.7	100.0	0.0	0.0
P0ESICEZ	0.0	0.0	100.0	66.7	100.0	0.0	0.0
PNILOCF1	0.0	0.0	33.3	100.0	33.3	0.0	0.0
S4CC7WJH	0.0	33.3	100.0	66.7	0.0	0.0	0.0
SIH3UXIH	33.3	0.0	100.0	100.0	33.3	0.0	0.0
TKMCRFVG	0.0	0.0	33.3	100.0	0.0	0.0	0.0
TLWF1GRX	0.0	0.0	0.0	100.0	0.0	0.0	0.0
WWAD5H7B	0.0	0.0	100.0	100.0	33.3	0.0	0.0
WX2V1IIV	0.0	0.0	0.0	100.0	33.3	0.0	0.0
XB2CUW5F	0.0	0.0	0.0	66.7	33.3	0.0	33.3
Y8F5JJ6S	0.0	0.0	0.0	100.0	0.0	0.0	33.3
Y8VQVD0R	0.0	0.0	100.0	100.0	0.0	0.0	0.0

Detailed results

The following table provides the estimated copy number for individual technical replicates for each qPCR assay. Missing values indicate failed reactions or outliers that were removed from the analysis.

SampleId	Rep 1	Rep 2	Rep 3
Bovine01			
2I9FVVQ4	0	12	47
35TQH4FI	0	0	0
3HFMW3ID	0	0	0
43EI9YTX	0	0	0
4ZCVO7K9	0	0	0
54990OO8	0	0	0
6BUJWD78	0	0	0
6P4G354F	0	0	0
70927D7J	0	0	0
7RUN5OAQ	0	0	0
APC3KPI0	0	0	0
BVALB072	0	0	0
D49Z66WR	0	0	0
D71CUW6D	0	0	0
E7I4PIN9	0	0	0
F8ZBOLU0	0	0	0
FDVV09X0	0	0	0
G36FIUMP	0	0	0
GFYOI4EY	0	0	0
KEGNNTKU	0	0	0
M16VLDQN	0	0	0
NFV1IMV1	0	0	0
P0ESICEZ	0	0	0
PNILOCF1	0	0	0
S4CC7WJH	0	0	0
SIH3UXIH	0	0	29
TKMCRFVG	0	0	0
TLWF1GRX	0	0	0
WWAD5H7B	0	0	0
WX2V1IIV	0	0	0
XB2CUW5F	0	0	0
Y8F5JJ6S	0	0	0
Y8VQVD0R	0	0	0
Dog01			
2I9FVVQ4	0	0	18
35TQH4FI	0	14	38
3HFMW3ID	0	15	0
43EI9YTX	0	0	0
4ZCVO7K9	0	0	0
54990OO8	0	0	0
6BUJWD78	0	0	0
6P4G354F	21	0	0
70927D7J	41	0	0
7RUN5OAQ	0	0	0

APC3KPI0	0	0	0
BVALB072	0	0	0
D49Z66WR	16	0	0
D71CUW6D	0	0	0
E7I4PIN9	0	0	0
F8ZBOLU0	0	31	0
FDVV09X0	0	0	0
G36FIUMP	0	0	0
GFYOI4EY	0	0	0
KEGNNTKU	0	0	0
M16VLDQN	85	114	61
NFV1IMV1	0	0	0
P0ESICEZ	0	0	0
PNILOCF1	0	0	0
S4CC7WJH	22	0	0
SIH3UXIH	0	0	0
TKMCRFVG	0	0	0
TLWF1GRX	0	0	0
WWAD5H7B	0	0	0
WX2V1IIV	0	0	0
XB2CUW5F	0	0	0
Y8F5JJ6S	0	0	0
Y8VQVD0R	0	0	0
E.coli01			
2I9FVVQ4	88	51	26
35TQH4FI	0	5	0
3HFMW3ID	1982	1969	1840
43EI9YTX	115	47	54
4ZCVO7K9	1066	913	772
54990OO8	6	0	6
6BUJWD78	18	0	0
6P4G354F	25	0	12
70927D7J	8	2	5
7RUN5OAQ	0	0	0
APC3KPI0	68	94	32
BVALB072	407	565	237
D49Z66WR	0	0	3
D71CUW6D	148	147	168
E7I4PIN9	36	31	12
F8ZBOLU0	0	0	0
FDVV09X0	0	0	92
G36FIUMP	0	32	0
GFYOI4EY	602	512	521
KEGNNTKU	0	0	0
M16VLDQN	0	0	0
NFV1IMV1	23	46	11
P0ESICEZ	45036	37874	43346
PNILOCF1	0	0	2
S4CC7WJH	14	63	50
SIH3UXIH	377	434	551
TKMCRFVG	0	0	24
TLWF1GRX	0	0	0
WWAD5H7B	77	100	107

WX2V1IIV	0	0	0
XB2CUW5F	0	0	0
Y8F5JJ6S	0	0	0
Y8VQVD0R	422	436	422
Human01			
2I9FVVQ4	471	259	251
35TQH4FI	30	4	15
3HFMW3ID	85	66	198
43EI9YTX	2087	1500	1559
4ZCVO7K9	0	0	0
54990OO8	22	4	22
6BUJWD78	0	45	22
6P4G354F	38	29	0
70927D7J	181	115	175
7RUN5OAQ	26	40	12
APC3KPI0	8	20	0
BVALB072	339	186	445
D49Z66WR	42	26	37
D71CUW6D	33	0	47
E7I4PIN9	28	0	105
F8ZBOLU0	0	10	87
FDVV09X0	0	0	597
G36FIUMP	79	16	23
GFYOI4EY	44	1	64
KEGNNTKU	43	90	27
M16VLDQN	76	56	125
NFV1IMV1	40	0	19
P0ESICEZ	0	58	182
PNILOCF1	71	56	111
S4CC7WJH	6	16	0
SIH3UXIH	140	88	74
TKMCRFVG	116	94	325
TLWF1GRX	32	16	31
WWAD5H7B	76	48	52
WX2V1IIV	13	14	17
XB2CUW5F	23	42	0
Y8F5JJ6S	15	31	16
Y8VQVD0R	32	20	21
Poultry01			
2I9FVVQ4	42	0	149
35TQH4FI	0	76	0
3HFMW3ID	0	29	14
43EI9YTX	0	0	0
4ZCVO7K9	0	0	0
54990OO8	0	66	0
6BUJWD78	0	0	62
6P4G354F	0	205	0
70927D7J	0	0	21
7RUN5OAQ	28	0	18
APC3KPI0	0	0	0
BVALB072	0	0	0
D49Z66WR	0	0	0

D71CUW6D	43	0	0
E7I4PIN9	0	1	0
F8ZBOLU0	0	0	0
FDVV09X0	0	0	0
G36FIUMP	0	64	0
GFYOI4EY	0	0	0
KEGNNTKU	0	0	0
M16VLDQN	0	0	0
NFV1IMV1	7	6	8
P0ESICEZ	239	253	1508
PNILOCF1	27	0	0
S4CC7WJH	0	0	0
SIH3UXIH	0	0	22
TKMCRFVG	0	0	0
TLWF1GRX	0	0	0
WWAD5H7B	3	0	0
WX2V1IIV	0	44	0
XB2CUW5F	82	0	0
Y8F5JJ6S	0	0	0
Y8VQVD0R	0	0	0
Sheep01			
2I9FVVQ4	0	0	0
35TQH4FI	0	0	0
3HFMW3ID	0	0	0
43EI9YTX	0	0	0
4ZCVO7K9	0	0	0
54990OO8	0	0	0
6BUJWD78	0	0	0
6P4G354F	0	0	0
70927D7J	0	0	0
7RUN5OAQ	0	0	0
APC3KPI0	0	0	0
BVALB072	0	0	0
D49Z66WR	0	0	0
D71CUW6D	0	0	0
E7I4PIN9	0	0	0
F8ZBOLU0	0	0	0
FDVV09X0	19	0	0
G36FIUMP	0	0	0
GFYOI4EY	0	0	0
KEGNNTKU	0	0	0
M16VLDQN	0	0	0
NFV1IMV1	0	0	0
P0ESICEZ	0	0	0
PNILOCF1	0	0	0
S4CC7WJH	0	0	0
SIH3UXIH	0	0	0
TKMCRFVG	0	0	0
TLWF1GRX	0	0	0
WWAD5H7B	0	0	0
WX2V1IIV	0	0	0
XB2CUW5F	0	0	0
Y8F5JJ6S	0	0	0

Y8VQVD0R	0	0	0
Swine01			
2I9FVVQ4	259	0	0
35TQH4FI	0	0	0
3HFMW3ID	0	0	0
43EI9YTX	0	0	0
4ZCVO7K9	150	0	0
54990OO8	0	0	0
6BUJWD78	0	0	0
6P4G354F	0	0	0
70927D7J	0	0	0
7RUN5OAQ	0	0	95
APC3KPI0	0	0	0
BVALB072	0	0	0
D49Z66WR	0	0	0
D71CUW6D	0	0	0
E7I4PIN9	0	0	0
F8ZBOLU0	0	0	0
FDVV09X0	0	0	0
G36FIUMP	0	0	0
GFYOI4EY	0	0	0
KEGNNTKU	0	0	0
M16VLDQN	60	0	0
NFV1IMV1	0	0	0
P0ESICEZ	0	0	0
PNILOCF1	0	0	0
S4CC7WJH	0	0	0
SIH3UXIH	0	0	0
TKMCRFVG	0	0	0
TLWF1GRX	0	0	0
WWAD5H7B	0	0	0
WX2V1IIV	0	0	0
XB2CUW5F	0	0	92
Y8F5JJ6S	27	0	0
Y8VQVD0R	0	0	0

Sample metadata

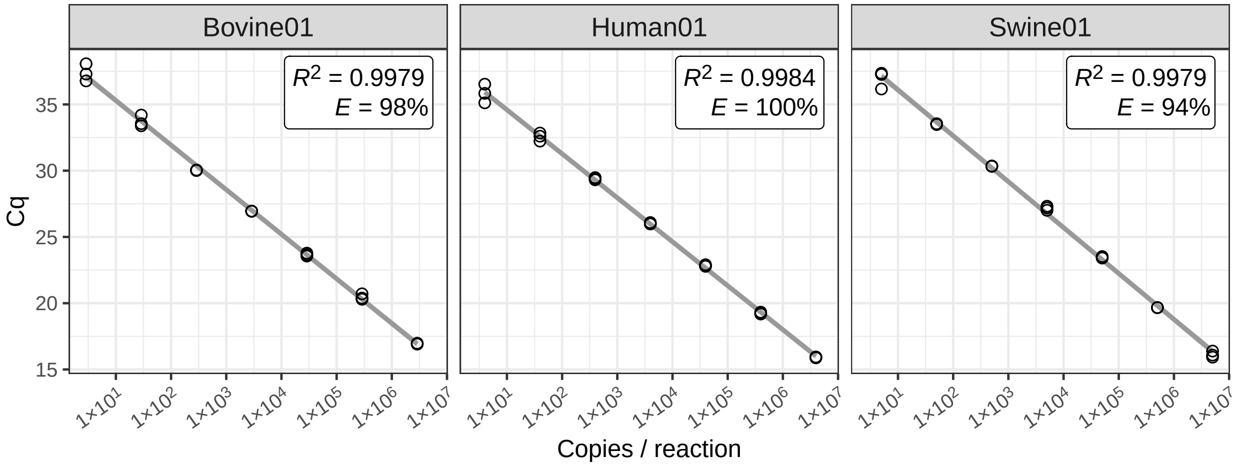
SampleId	Volume Water (ml)
2I9FVVQ4	60
35TQH4FI	150
3HFMW3ID	150
43EI9YTX	150
4ZCVO7K9	30
54990OO8	120
6BUJWD78	90
6P4G354F	80
70927D7J	100
7RUN5OAQ	150
APC3KPI0	200
BVALB072	25
D49Z66WR	120
D71CUW6D	140
E7I4PIN9	100
F8ZBOLU0	150
FDVV09X0	7
G36FIUMP	70
GFYOI4EY	100
KEGNNTKU	150
M16VLDQN	150
NFV1IMV1	150
P0ESICEZ	8
PNILOCF1	180
S4CC7WJH	100
SIH3UXIH	120
TKMCRFVG	100
TLWF1GRX	150
WWAD5H7B	150
WX2V1IIV	200
XB2CUW5F	120
Y8F5JJ6S	100
Y8VQVD0R	300

Methods and calibration curves

The following pages provide details of the methods used for each qPCR assay and the associated standard curves. Each assay in each run is associated with a calibration curve based typically on a series of 7, 10-fold dilutions of a standard with a known concentration. The calibration curves show the relationship between the $\log_{10}$-transformed standard concentration and the number of PCR cycles at which the detection threshold was reached (Cq). A linear regression is applied to this relationship and the r2 intercept and slope extracted for further analyses.

- RunId = An internal identifier for the standard curve(s) used to calculate copy numbers in the submitted samples. Assays that share a RunId are multiplexed (i.e., multiple targets amplified in a single reaction).
- R^2 = The coefficient of determination, or goodness of fit for the linear relationship (should be > 0.98).
- (E) = The reaction efficiency, or how close to a doubling of product was achieved with each PCR cycle. For a 10-fold dilution, 100% efficiency is for ~3.3 cycles per 10-fold dilution. A range of values is acceptable here, but we try to keep efficiency between 85% - 110%.

RunID: JVQ0369



Human Forward primer: 5’ CAGCAGCCATTCAAGCAATCC 3

Human Reverse primer: 5’ GGTGGAGACCTAATTGGGCTGATTAG 3

Human Probe: 5’ /5Cy5/TATCGGCGA/TAO/TATCGGTTTCATCCTCG/3IAbRQSp/ 3

Bovine Forward primer: 5’ CAGCAGCCCTACAAGACCTGT 3

Bovine Reverse primer: 5’ GAGGCCAAATTGGGCGGATTAT 3

Bovine Probe: 5’ /5HEX/CATCGGCGACATTGGTTTCATTTTAG/BHQ1/ 3

Swine Forward primer: 5’ ACAGCTGCACTACAAGCAATGC 3

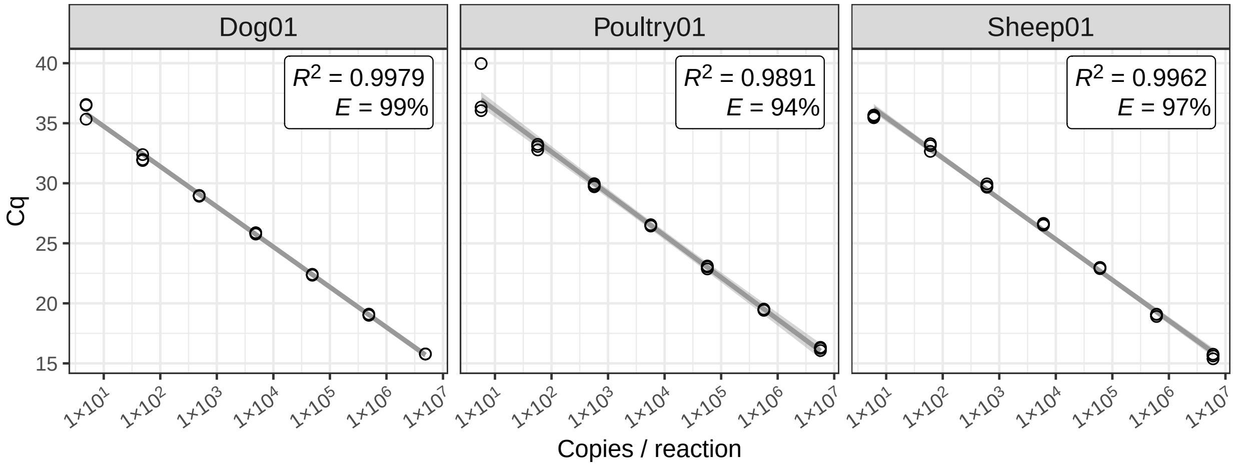
Swine Reverse primer: 5’ GGATGTAGTCCGAATTGAGCTGATTAT 3

Swine Probe: 5’ /56-FAM/CATCGGAGA/ZEN/CATTGGATTTGTCCTAT/3IABkFQ/ 3

Primer/probe reference: Cadwell et al., 200

Amplicons from the NADH dehydrogenase, subunit 5 (ND5) gene were amplified via qPCR from genomic DNA samples using the ND5 FWD and ND5 REV primers, and ND5 Probes corresponding to human, bovine, and swine. A standard curve was generated for each run to correspond to targeted regions of each of the ND5 genes. qPCR reactions contain 4.0 uL of QuantaBio PerfeCTa Multiplex qPCR ToughMix Low ROX (Catalog Number 89497-290), 500 nM of each primer, 300 nM of each probe, 4.0 uL of gDNA (or 1.33 uL of each gBlock for standard curve wells), and 2.4 uL of Nuclease-free H2O for a total reaction volume of 20 uL. qPCR amplification was carried out on the QuantStudio 5 qPCR instrument with the following thermal profile conditions: 1 cycle of initial denaturation for 5 minutes at 95 C; followed by 50 cycles of 15 seconds at 95 C and 1 minute at 60 C.

RunID: JVQ0370



Dog Forward primer: 5' CACATCTAAGCAACGCAGCATAA 3'

Dog Reverse primer: 5' AGATCGGCGACTAAAAGTCAGAA 3'

Dog Probe: 5' /5HEX/TCCGGCCCC/ZEN/TTAGCCAATGCC/3IABkFQ/ 3'

Poultry Forward primer: 5' CGTYATCACAAACCTATTCTCAGCAAT 3'

Poultry Reverse primer: 5' TTGGGTTGTCGACTGAAAATCC 3'

Poultry Probe: 5' /5Cy5/CCCTACATY/TAO/GGACAMACCCTAGTAGAGTGAGCC/3IAbRQSp/ 3'

Sheep Forward primer: 5' GCAATACACTATACACCTGACACAACAA 3'

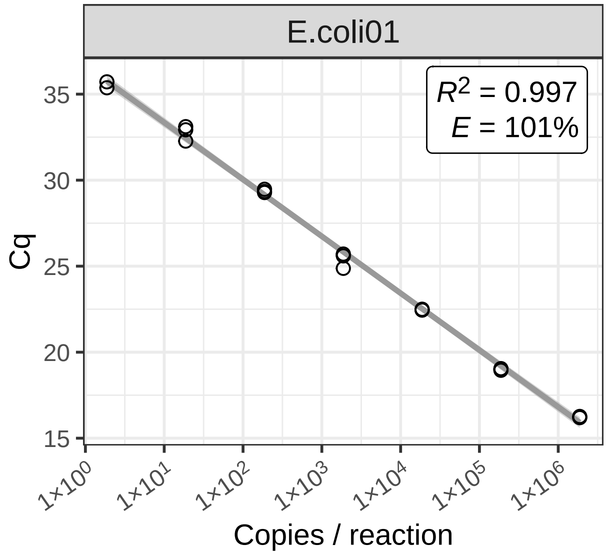
Sheep Reverse primer: 5' CAGATAAAAAATATTGATGCCCCGTTTG 3'

Sheep Probe: 5' /56-FAM/CTCCTCTGT/ZEN/AACCCACATTTGCCGAGA/3IABkFQ/ 3'

Primer/probe reference: Dancer et al., 2014

Amplicons from the Cytochrome b (Cytb) gene were amplified via qPCR from genomic DNA samples using the Cytb FWD and Cytb REV primers, and Cytb Probes corresponding to dog, poultry, and sheep. A standard curve was generated for each run to correspond to targeted regions of each of the Cytb genes. qPCR reactions contain 4.0 uL of QuantaBio PerfeCTa Multiplex qPCR ToughMix Low ROX (Catalog Number 89497-290), 500 nM of each primer, 300 nM of each probe, 4.0 uL of gDNA (or 1.33 uL of each gBlock for standard curve wells), and 2.4 uL of Nuclease-free H2O for a total reaction volume of 20 uL. qPCR amplification was carried out on the Agilent AriaMx qPCR instrument with the following thermal profile conditions: 1 cycle of initial denaturation for 5 minutes at 95 C; followed by 50 cycles of 15 seconds at 95 C and 1 minute at 60 C.

RunID: JVQ0371



Forward primer: 5' CAATGGTGATGTCAGCGTT 3

Reverse primer: 5' ACACTCTGTCCGGCTTTTG 3

Probe: 5' /56-FAM/TTGCAACTG/ZEN/GACAAGGCACCAGC/3IABkFQ/ 3

Primer/probe reference: Srinivasan et al., 201

An amplicon from the uidA gene was amplified via qPCR from genomic DNA samples using E. coli FWD and REV primers and probe. A standard curve was generated for each run to correspond to targeted region of the E. coli, uidA gene. Each qPCR reaction is run in triplicate and contains 8.0 uL of QuantaBio PerfeCTa qPCR ToughMix Low ROX (Catalog Number 97065-966), 500 nM of each primer, 300 nM of probe, 4.0 uL of gDNA, and 4.8 uL of Nuclease-free H2O for a total reaction volume of 20 uL. qPCR amplification was carried out on the QuantStudio 5 qPCR instrument with the following thermal profile conditions: 1 cycle of initial denaturation for 5 minutes at 95 C; followed by 50 cycles of 15 seconds at 95 C and 1 minute at 60 C.