August 9, 2024
Steuben County Lakes Council
317 South Wayne Street,
Suite 2A
Angola, IN 46703

STEUBEN COUNTY ENVIRONMENTAL DNA REPORT

INTRODUCTION

Twenty-nine wet weather samples were collected on July 10, 2024 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 fifth such sampling event, previous samples were collected in October 2020, June 2021, July 2022, and July 2023. At the request of the Steuben County Lakes Council some changes were made to the sampling locations for this year's 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	-84.824110	Clear Lake	2020, 2022, 2023
7	41.655504	-85.019579	Carpenter Creek	2020, 2021, 2022, 2023
8	41.656863	-85.023053	Carpenter Creek	2020, 2021, 2022, 2023
9	41.660794	-85.028259	Carpenter Creek	2020, 2021, 2022, 2023
10	41.667092	-85.036215	Carpenter Creek	2020, 2021, 2022, 2023
11	41.668156	-85.036002	Crooked Lake	2020, 2021, 2022, 2023
12	41.671376	-85.026319	Palfreyman Ditch	2020, 2021, 2022, 2023
13	41.664479	-85.018234	Palfreyman Ditch	2020, 2021, 2022, 2023
14	41.664136	-85.018111	Palfreyman Ditch	2020, 2021, 2022, 2023
15	41.664331	-85.017397	Palfreyman Ditch	2020, 2021, 2022, 2023
16	41.658881	-85.004190	Palfreyman Ditch	2020, 2021, 2022, 2023
17	41.719156	-85.033741	Snow Lake	2021, 2022, 2023
18	41.542063	-85.194894	McClish Lake	2021, 2022, 2023
19	41.546779	-85.191998	McClish Lake	2021, 2022, 2023
21	41.618222	-85.156835	Lake Arrowhead	2021, 2022, 2023
25	41.729612	-84.847775	Clear Lake	2022, 2023
26	41.651418	-85.174436	Pigeon River	2022, 2023
27	41.741473	-85.171456	Fawn River	2022, 2023
28	41.558906	-84.835616	Fish Creek	2022, 2023
29	41.631997	-84.937648	Pigeon River	2022, 2023
33	41.624472	-85.026793	Fox Lake	2022, 2023
35	41.625948	-85.031951	Fox Lake	2023

Site Number	Latitude	Longitude	Water Body	Years Sampled
36	41.696849	-85.088635	Lake Gage	2023
37	41.699593	-85.103777	Lake Gage	2023
38	41.713098	-85.119539	Lake Gage	2023
42	41.558207	-84.909597	Black Creek	2023
43	41.724940	-85.021640	Snow Lake	New
44	41.736380	-85.027050	Snow Lake	New
45	41.623720	-85.015570	Fox Lake	New

Table 1: July 10, 2024 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	Flow	E Coli
3	Clear Lake	Harry Teeter's Ditch	Medium/High	>2420
7	Carpenter Creek	CR 155 W	Medium	>2420
8	Carpenter Creek	CR 100 N	Medium/High	>2420
9	Carpenter Creek	CR 200 W	Medium/High	>2420
10	Carpenter Creek	Beach Road	High	>2420
11	Crooked Lake	Beach	None	794
12	Palfreyman Ditch	CR 200 W	Medium	>2420
13	Palfreyman Ditch	Downstream Northwood	Medium/High	1344
14	Palfreyman Ditch	Northwood	Medium	>2420
15	Palfreyman Ditch	Upstream Northwood	Medium/High	1680
16	Palfreyman Ditch	Wohlert	Medium/High	1344
17	Snow Lake	Discharge from Pokagon treatment plant	None	1
18	McClish Lake	Discharge on west end	Medium	176
19	McClish Lake	Inlet on east end	Low/Medium	>2420
21	Lake Arrowhead	Ditch entering	Medium	>2420

Site Number	Water Body	Description	Flow	E Coli
25	Clear Lake	Tributary to Clear Lake	High	>2420
26	Pigeon River	At IND 327 public access site	Medium	>2420
27	Fawn River	At IND 327 at the fish hatchery	Medium	927
28	Fish Creek	At IND 427	High	>2420
29	Pigeon River	At US 20 east of Angola	High	316
33	Fox Lake	At swimming beach	None	32
35	Fox Lake	Discharge of Fox Lake	Low	1684
36	Lake Gage	Outlet from Crooked Lake	Medium	275
37	Lake Gage	Inlet to Lake Gage	Medium/High	>2420
38	Lake Gage	Outlet from Lime Lake	High	45
42	Black Creek	Where Black Creek meets Hamilton Lake	High	>2420
43	Snow Lake	Lake Inlet from Big Otter	Medium/High	683
44	Snow Lake	Inlet for George	Medium/High	945
45	Fox Lake	Outlet	Medium	110

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*

			eDNA (Copies/100 mL)					
Site Number	Water Body	Volume Filtered (mL)	Human	Swine	Bovine	Poultry	Sheep	Dog
3	Clear Lake	60	2596	0	0	0	0	6
7	Carpenter Creek	37	30	0	0	0	0	0
8	Carpenter Creek	50	159	0	0	0	0	13
9	Carpenter Creek	50	12	0	68	8	0	5
10	Carpenter Creek	50	36	0	0	4	0	11
11	Crooked Lake	60	49	0	0	0	0	0
12	Palfreyman Ditch	40	53	0	0	0	0	0
13	Palfreyman Ditch	60	839	0	0	0	0	12
14	Palfreyman Ditch	60	0	0	0	20	0	26
15	Palfreyman Ditch	60	80	0	0	0	0	12
16	Palfreyman Ditch	95	75	0	0	8	0	57
17	Snow Lake	180	1062	49	62	23	0	31
18	McClish Lake	120	9	0	0	18	0	6
19	McClish Lake	25	86	0	0	0	0	0
21	Lake Arrowhead	60	114	0	40	0	0	0
25	Clear Lake	20	86	0	0	0	0	0

			eDNA (Copies/100 mL)					
Site Number	Water Body	Volume Filtered (mL)	Human	Swine	Bovine	Poultry	Sheep	Dog
26	Pigeon River	120	165	0	120	0	0	12
27	Fawn River	120	67	0	0	0	0	0
28	Fish Creek	15	137	0	47530	0	668	0
29	Pigeon River	70	286	0	41	0	0	0
33	Fox Lake	120	51	0	0	0	0	0
35	Fox Lake	60	74	0	0	0	0	0
36	Lake Gage	120	27	0	0	0	0	12
37	Lake Gage	60	1218	0	0	0	0	0
38	Lake Gage	180	17	0	0	0	0	0
42	Black Creek	11	124	0	1999	9	63	6
43	Snow Lake	120	24	0	0	19	0	29
44	Snow Lake	180	41	0	0	21	0	0
45	Fox Lake	120	61	0	0	0	0	2

Table 3: eDNA Results Summary

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

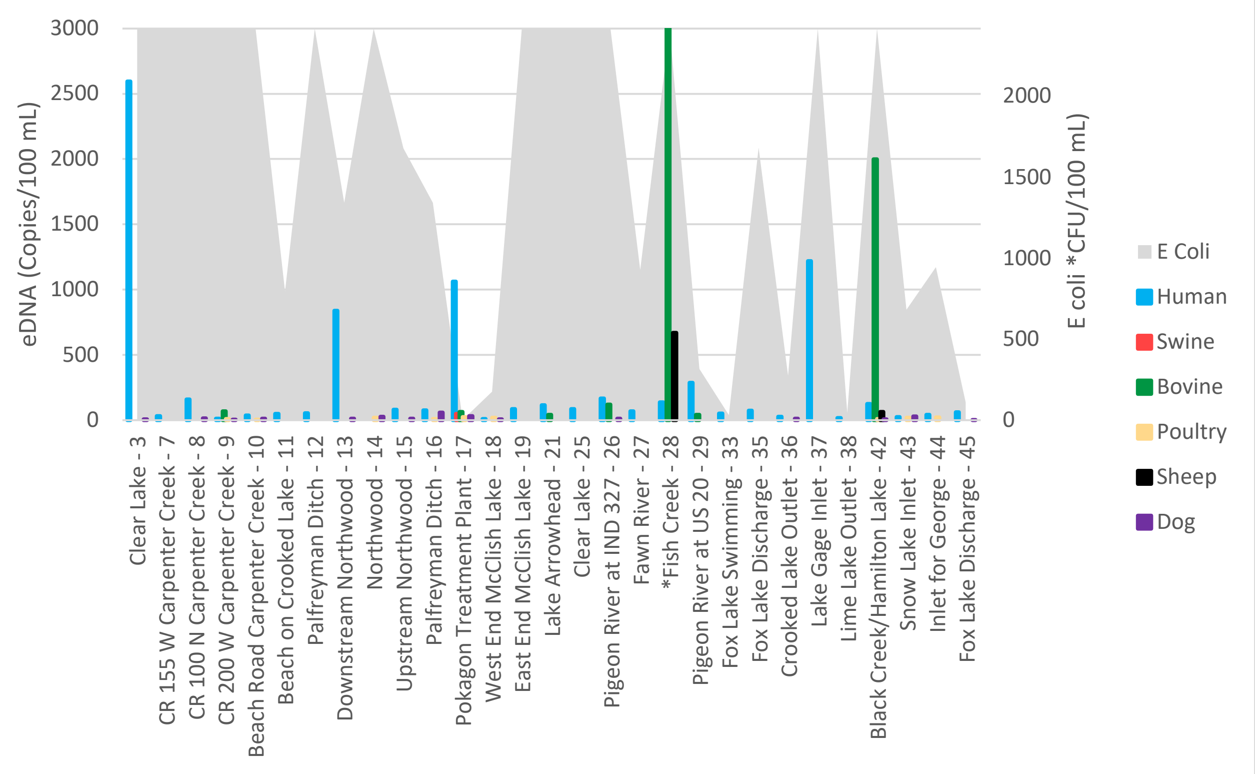


Figure 1: E. Coli and eDNA results

**The bovine eDNA value for site 28 is 47530 copies per 100 mL of water*

CONCLUSIONS

This year's sampling event is particularly interesting since the samples were collected during tropical storm Beryl. The USGS recorded 2.88 inches of rain at their stream gauge on Pigeon Creek in Angola [1]. This is the largest rainfall event captured in this study since the start of the project. Table 4 shows rainfall data from the National Weather Service for all five sampling events. Figure 2 shows the USGS stream gauge discharge data for Pigeon Creek in Angola.

Date	Precipitation (in.)
Oct. 22, 2020	0.83
Jun. 10, 2021	0.15
Jul. 6, 2022	2.06
Jul. 12, 2023	0.36
Jul.10, 2024	2.88

Table 4: Rainfall data for each sampling event [2]

This year, Site 28 had a large amount of bovine DNA detected. This site has had high *E. coli* detected at every sampling event so far, as seen in Table 5. This is the first year that bovine DNA has been detected at such high values. During the 2023 sampling event, bovine DNA was detected at 10 copies/100 mL [3]. One possible explanation for the sudden increase is that the large rain event caused a containment measure for an upstream cattle farm to fail.

Table 6 shows the amount of Human DNA detected during each sampling event. Once again, Human DNA was detected at low levels throughout the sampling area. 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 the influence of septic systems or other sewage sources and humans are the most common large animal in Steuben County. That may be the explanation for many of the human eDNA hits seen during this sampling event. The rainfall data in Table 4 helps to confirm this. During years where the rainfall was greater, a larger amount of human DNA was detected.

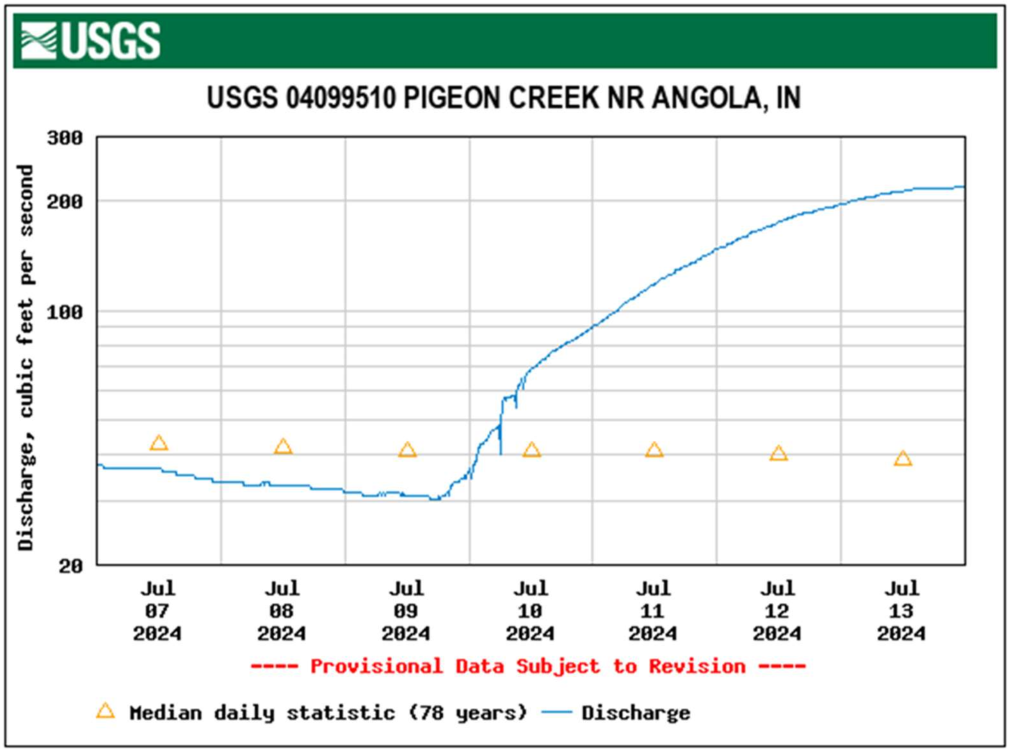


Figure 2: Pigeon Creek Discharge Data from USGS [1]

		E. Coli (CFU/100 mL)				
Site Number	**Water Body**	**Oct-20**	**Jun-21**	**Jul-22**	**Jul-23**	**Jul-24**
3	Clear Lake	>2420	NT	>2420	384	>2420
7	Carpenter Creek	184	1046	420	>2420	>2420
8	Carpenter Creek	727	687	>2420	1456	>2420
9	Carpenter Creek	579	770	672	1164	>2420
10	Carpenter Creek	1300	249	>2420	1254	>2420
11	Crooked Lake	4	25	2	694	794
12	Palfreyman Ditch	>2420	64	100	122	>2420
13	Palfreyman Ditch	>2420	>2420	816	1523	1344
14	Palfreyman Ditch	NT	>2420	214	>2420	>2420
15	Palfreyman Ditch	>2420	1553	1538	2038	1680
16	Palfreyman Ditch	>2420	1553	86	984	1344
17	Snow Lake	NT	2	0	4	1
18	McClish Lake	NT	308	276	64	176
19	McClish Lake	NT	64	>2420	520	>2420
21	Lake Arrowhead	NT	866	>2420	750	>2420
25	Clear Lake	NT	NT	280	>2420	>2420
26	Pigeon River	NT	NT	470	200	>2420

		E. Coli (CFU/100 mL)				
Site Number	Water Body	Oct-20	Jun-21	Jul-22	Jul-23	Jul-24
27	Fawn River	NT	NT	896	152	927
28	Fish Creek	NT	NT	>2420	>2420	>2420
29	Pigeon River	NT	NT	1210	828	316
33	Fox Lake	NT	NT	54	10	32
35	Fox Lake	NT	NT	NT	504	1684
36	Lake Gage	NT	NT	NT	164	275
37	Lake Gage	NT	NT	NT	>2420	>2420
38	Lake Gage	NT	NT	NT	12	45
42	Black Creek	NT	NT	NT	8	>2420
43	Snow Lake	NT	NT	NT	NT	683
44	Snow Lake	NT	NT	NT	NT	945
45	Fox Lake	NT	NT	NT	NT	110

Table 5: E. Coli results from 2020 to 2024 [3,4,5,6]

**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*

		Human eDNA (Copies/100 mL)				
Sample ID	Water Body	Oct-20	Jun-21	Jul-22	Jul-23	Jul-24
3	Clear Lake	0	NT	1246	0	2596
7	Carpenter Creek	0	233	655	22	30
8	Carpenter Creek	5	89	759	36	159
9	Carpenter Creek	0	258	692	27	12
10	Carpenter Creek	18	110	868	1715	36
11	Crooked Lake	33	36	78	53	49
12	Palfreyman Ditch	0	149	1360	39	53
13	Palfreyman Ditch	60	264	854	32	839
14	Palfreyman Ditch	NT	90	364	80	0
15	Palfreyman Ditch	144	117	607	86	80
16	Palfreyman Ditch	40	193	338	16	75
17	Snow Lake	NT	1631	7367	327	1062
18	McClish Lake	NT	78	182	15	9
19	McClish Lake	NT	230	242	24	86
21	Lake Arrowhead	NT	28	0	59	114

Sample ID	Water Body	Human eDNA (Copies/100 mL)				
		Oct-20	Jun-21	Jul-22	Jul-23	Jul-24
25	Clear Lake	NT	NT	1303	22	86
26	Pigeon River	NT	NT	358	26	165
27	Fawn River	NT	NT	469	79	67
28	Fish Creek	NT	NT	955	101	137
29	Pigeon River	NT	NT	1139	35	286
33	Fox Lake	NT	NT	2126	26	51
35	Fox Lake	NT	NT	NT	323	74
36	Lake Gage	NT	NT	NT	24	27
37	Lake Gage	NT	NT	NT	116	1218
38	Lake Gage	NT	NT	NT	9	17
42	Black Creek	NT	NT	NT	22	124
43	Snow Lake	NT	NT	NT	NT	24
44	Snow Lake	NT	NT	NT	NT	41
45	Fox Lake	NT	NT	NT	NT	61

Table 6: Human eDNA results from 2020 to 2024 [3,4,5,6]

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

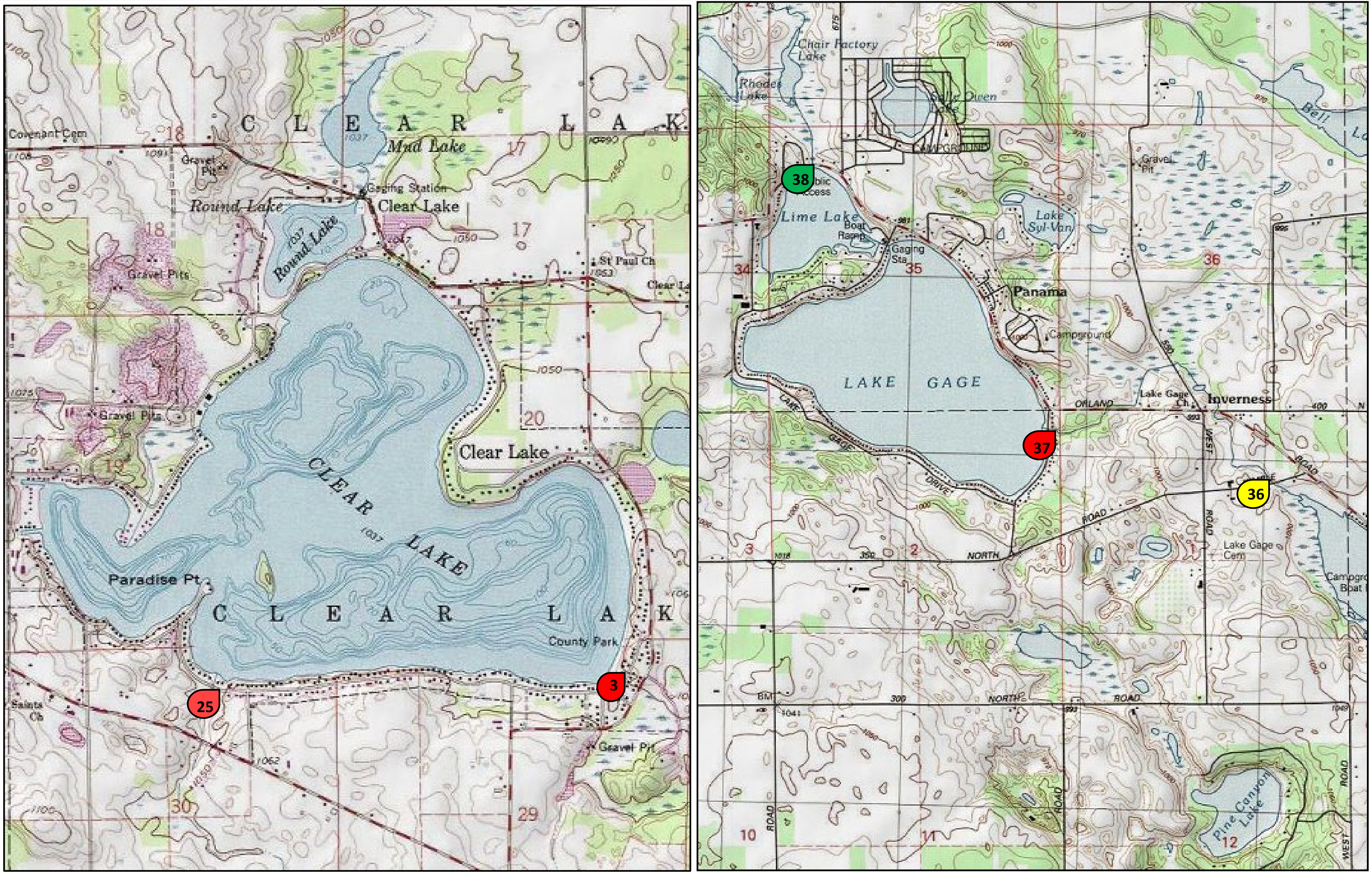
***NT = Not Tested*

REFERENCES

1. U.S. Geological Survey. (2021, August 6). *Pigeon Creek NR Angola, IN – 04099510*. USGS. https://waterdata.usgs.gov/monitoringlocation/04099510
2. National Weather Service. (2024, August 7). *Climatological Data for Angola, IN.* NOWData. https://www.weather.gov/wrh/Climate?wfo=iwx
3. Biomonitor. (2023). *Steuben County Environmental DNA Report.* July 2023. Submitted to the Steuben County Lakes Council August 2023.
4. Biomonitor. (2022). *Steuben County Environmental DNA Report.* July 2022. Submitted to the Steuben County Lakes Council September 2022.
5. Biomonitor. (2021). *Steuben County Environmental DNA Report.* June 2021. Submitted to the Steuben County Lakes Council July 2021.
6. Biomonitor. (2020). *Steuben County Environmental DNA Report.* October 2020. Submitted to the Steuben County Lakes Council November 2020.

APPENDIX A – July 2024 Sampling Maps

Steuben County eDNA Sampling Locations with *Escherichia coli* Results



Legend:

⬠ 17 Site ID

July 2024 *E. Coli* results:

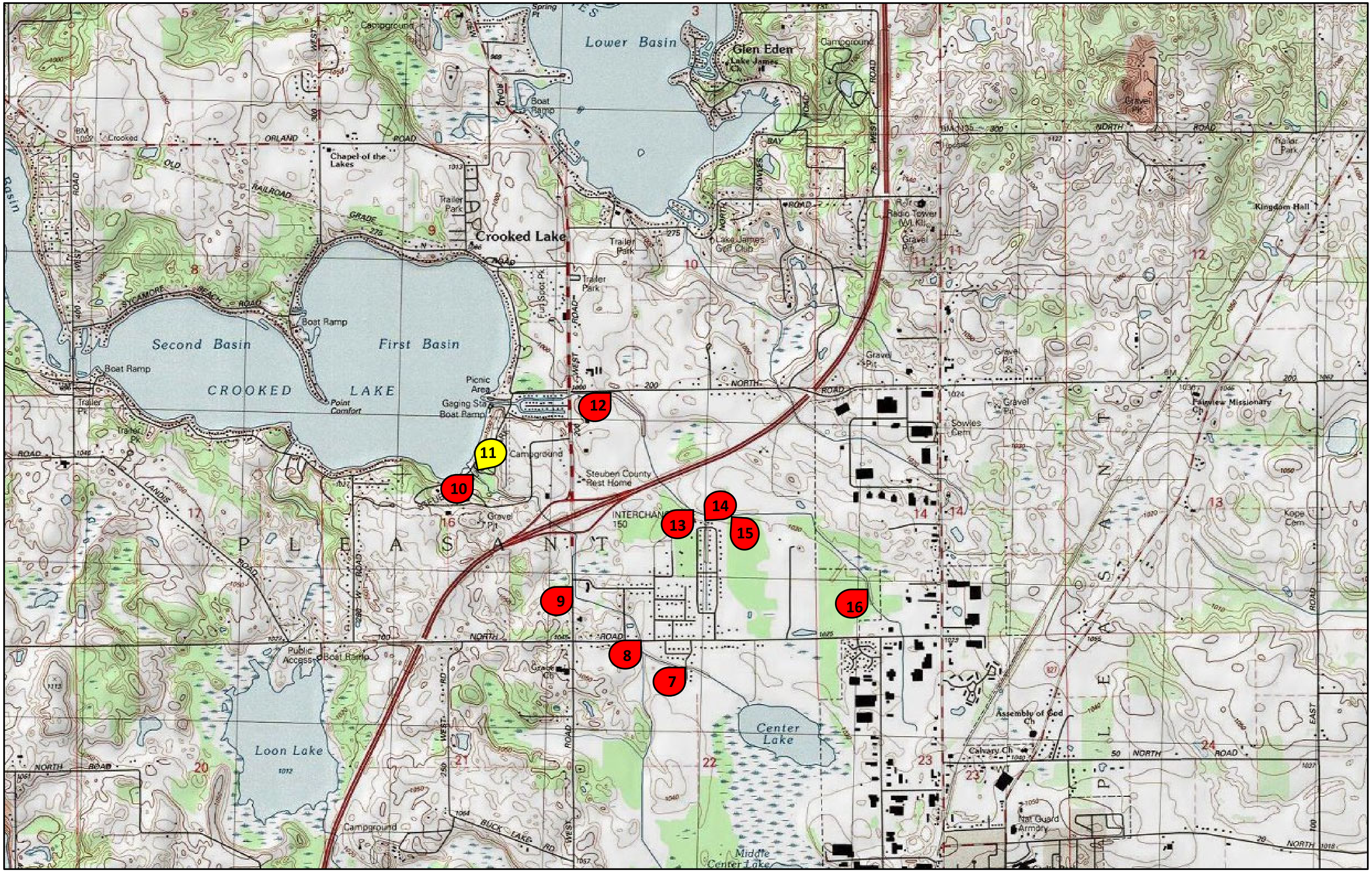
🟢 0 - 234 MPN/100 mL

🟡 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:

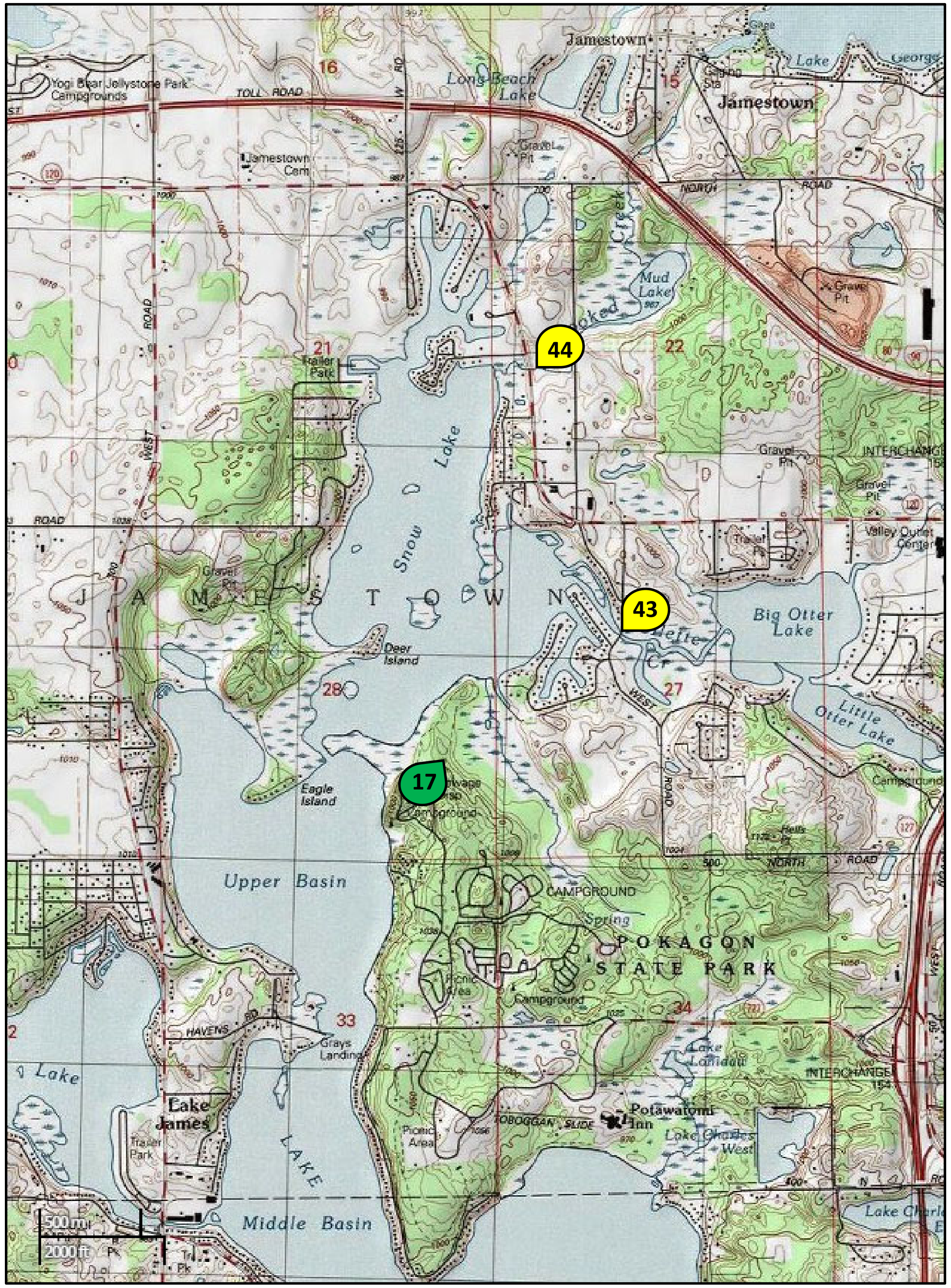
17 Site ID

July 2024 *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:

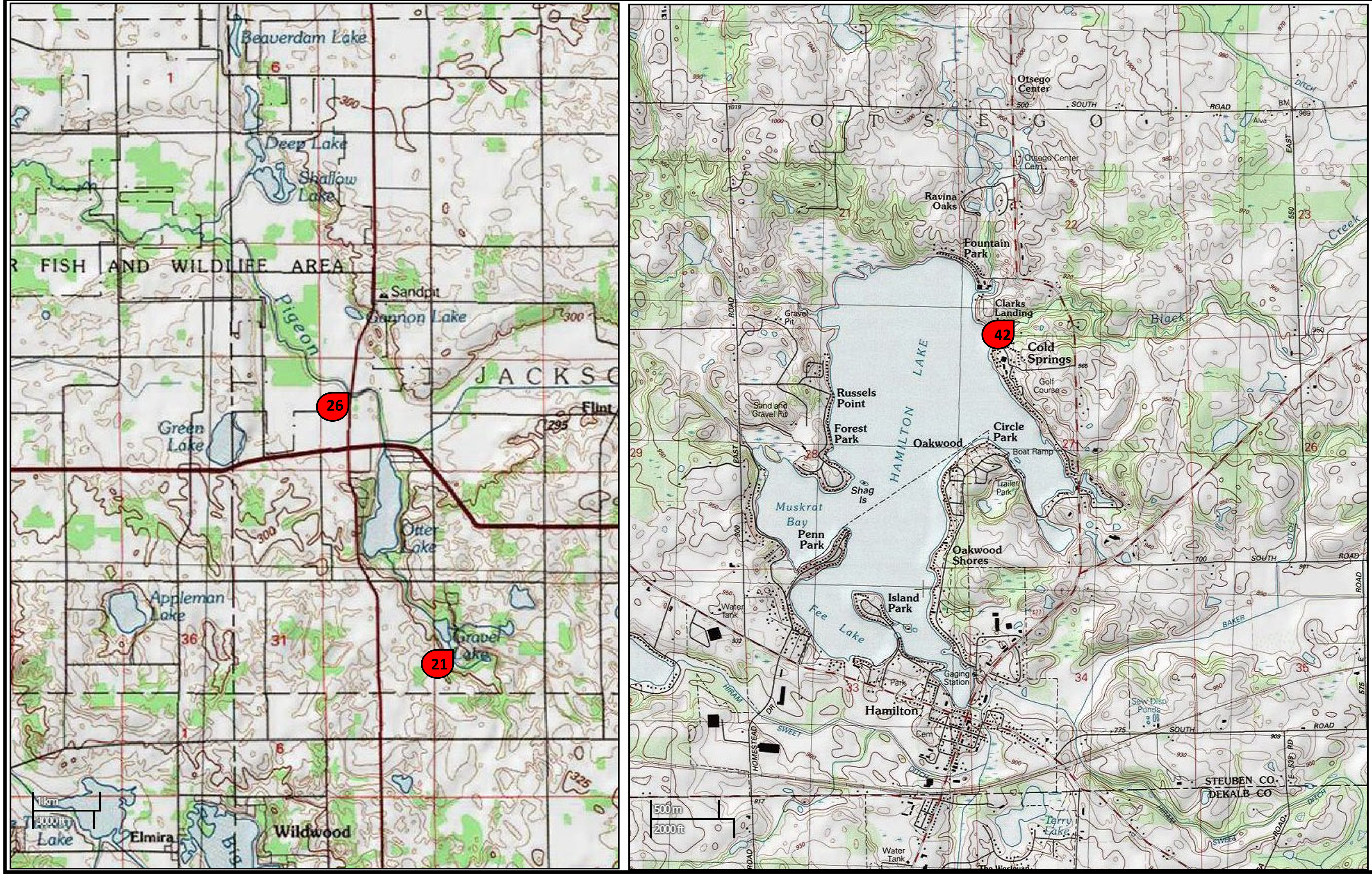
17 Site ID

July 2024 *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:

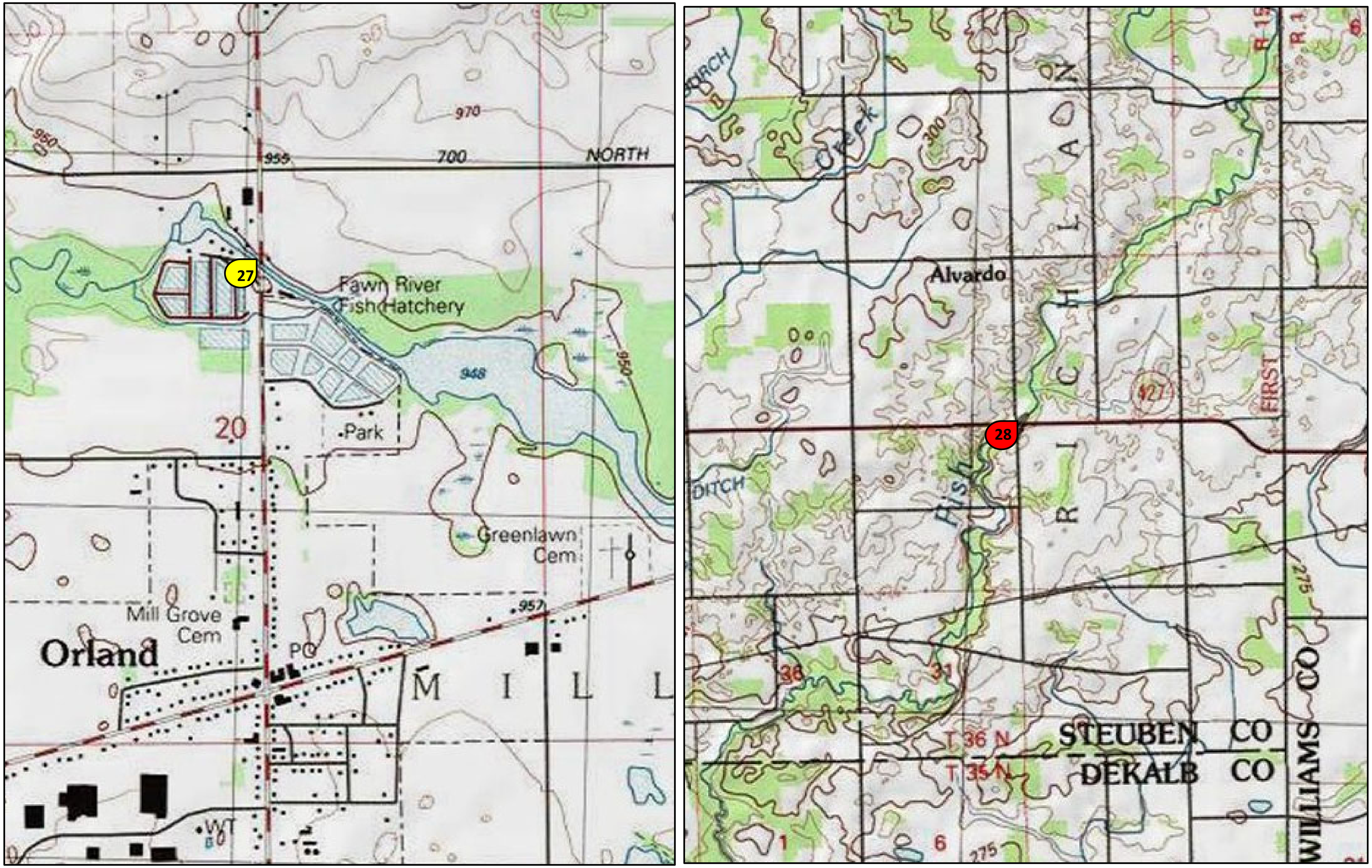
17 Site ID

July 2024 *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 2024 *E. Coli* results:

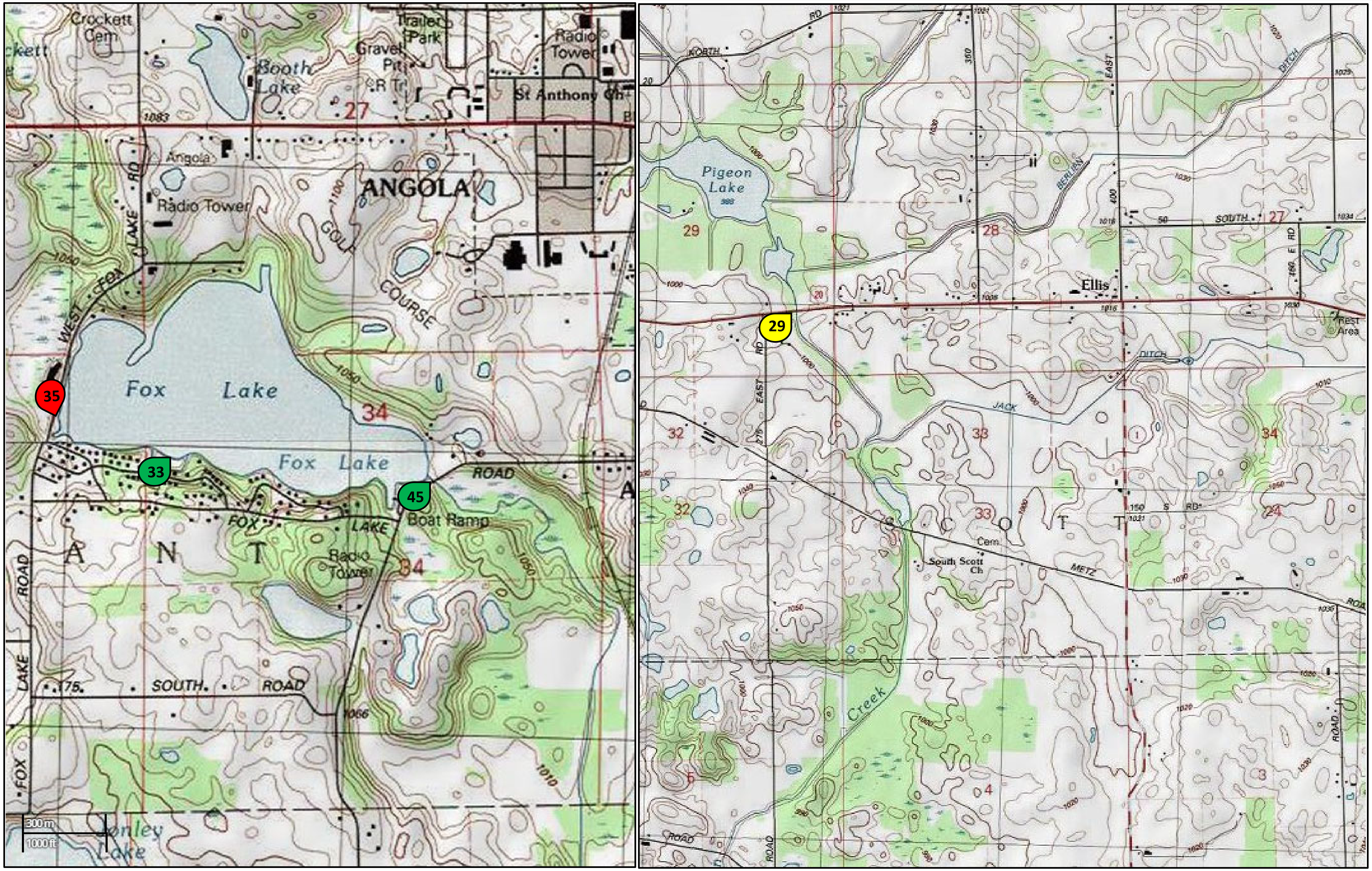
🟢 0 - 234 MPN/100 mL

🟡 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 2024 *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 2024 Field Sheet

[redacted]

Date: 7/10/24

Weather: overcast rain 70's

Personnel: AF, MB

Sample ID	Sample Code	Time Sampled	eDNA Vol Filtered	Flow	Notes	e.coli Vol Filtred
28	18582FWD	1003	15 mL	High		50 mL
42	YCTPWJ0V	1032	11 ml	High very	Picture taken	20 ml
29	JCB7VUAZ	1050	70 mL	High		50 mL
3	0BOJX9IT	1117	60 mL	med high		50 mL
25	8HGDBWU7	1129	20 mL	high		20 mL
44	RH99KI1R	1130	180 mL	med high	not new?	50 mL
43	Q0GH3ET1	1200	120 mL	med high		50 ml
17	VD5XRWRB	1216	180 mL	NA	campground 1A group camp >	50 mL
13	WRCEF70B	1255	60 mL	Med/high		50 mL
14	C4PH8L52	1310	60 mL	Med	See picture	50 mL
15	71KA0YGD	1325	60 mL	Med/high		50 mL
16	TFNAL8A3	1336	95 mL	Med/high		50 mL
45	6N3JN5L2	1349	120 mL	med		50 mL
33	KC7TH8GI	1358	120 mL	NA		50 mL
35	OGX8DUBD	1418	60 mL	Low	not marked correctly Freola Stock	850 mL
7	NONADUE6	1432	37 mL	Med	overgrown	50 mL
8	PDO2O0BD	1439	50 mL	Med/High	Big Fish	50 mL
9	UEZMHS9U	1447	50 mL	Med/High		50 mL
12	ZEYK0X4O	1455	40 mL	Med		50 mL
11	DNIK90FV	1500	60 mL	NA	Beach	50 mL
10	SLGKRGZ3	1505	50 mL	high		50 mL
36	D9HIAJDE	1526	120 mL	med.		50 mL
37	TMCXZG40	1534	60 mL	med/High		50 mL
38	XVKTEDG7	1540	180 mL	high		50 mL
27	MZ8Q3RI0	1554	120 mL	med		50 mL
26	H571IUSC	1615	120 mL	med		50 mL
21	MNX5VG63	1626	60 mL	med		50 mL
19	1UXEVLFI	1641	25 mL	low/med		50 mL
18	N5AK4PMH	1653	120 mL	med		50 mL

APPENDIX C – July 2024 E. coli Lab Report

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

July 13, 2024

The following table includes results from 30 total samples. This includes 29 site samples and a lab blank (LB). Samples collected and cultures inoculated by Biomonitor laboratory personnel on July 10, 2024.

SAMPLE ID	*E. coli* CFU/100 mL
Site - 3	>2420
Site - 7	>2420
Site - 8	>2420
Site - 9	>2420
Site - 10	>2420
Site - 11	794
Site - 12	>2420
Site - 13	1344
Site - 14	>2420
Site - 15	1680
Site - 16	1344
Site - 17	1
Site - 18	176
Site - 19	>2420
Site - 21	>2420
Site - 25	>2420
Site - 26	>2420
Site - 27	927
Site - 28	>2420
Site - 29	316
Site - 33	32
Site - 35	1684
Site - 36	275
Site - 37	>2420
Site - 38	45
Site - 42	>2420
Site - 43	683
Site - 44	945
Site - 45	110
Lab Blank	0

APPENDIX D – July 2024 eDNA Lab Report

JONAH VENTURES
KNOWLEDGE IN SEQUENCE

July 22 2024

For more information go to www.jonahventures.com

info@jonahventures.com

Report prepared for arizona@biomonitor.com

BatchId = JVB3658

Number of samples analyzed = 29

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	E.Coli01	Human01	Swine01	Bovine01
0BOJX9IT	2,907	2,596	0	0
18582FWD	177,048	137	0	47,530
1UXEVLFI	2,471	86	0	0
6N3JN5L2	14	61	0	0
71KA0YGD	2,269	80	0	0
8HGDBWU7	2,091	86	0	0
C4PH8LS2	2,579	0	0	0
D9HIAJDE	250	27	0	0
DNIK90FV	518	49	0	0
H571IUSC	2,632	165	0	120
JCB7VUAZ	911	286	0	41
KC7TH8GI	53	51	0	0
MNX5VG63	3,477	114	0	40
MZ8Q3RI0	1,551	67	0	0
N5AK4PMH	90	9	0	0
NONADUE6	2,228	30	0	0
OGX8DUBD	2,606	74	0	0
PDO2O0BD	1,912	159	0	0
Q0GH3ET1	1,167	24	0	0
RH99KI1R	1,287	41	0	0
SLGKRGZ3	2,045	36	0	0
TFNAL8A3	5,182	75	0	0
TMCXZG40	3,668	1,218	0	0

SampleId	E.Coli01	Human01	Swine01	Bovine01
UEZMHS9U	2,218	12	0	68
VD5XRWRB	2,004	1,062	49	62
WRCEF70B	3,218	839	0	0
XVKTEDG7	27	17	0	0
YCTPWJ0V	24,965	124	0	1,999
ZEYK0X4O	1,728	53	0	0

SampleId	Poultry01	Sheep01	Dog01
0BOJX9IT	0	0	6
18582FWD	0	668	0
1UXEVLFI	0	0	0
6N3JN5L2	0	0	2
71KA0YGD	0	0	12
8HGDBWU7	0	0	0
C4PH8LS2	20	0	26
D9HIAJDE	0	0	12
DNIK90FV	0	0	0
H571IUSC	0	0	12
JCB7VUAZ	0	0	0
KC7TH8GI	0	0	0
MNX5VG63	0	0	0
MZ8Q3RI0	0	0	0
N5AK4PMH	18	0	6
NONADUE6	0	0	0
OGX8DUBD	0	0	0
PDO2O0BD	0	0	13
Q0GH3ET1	19	0	29
RH99KI1R	21	0	0
SLGKRGZ3	4	0	11

SampleId	Poultry01	Sheep01	Dog01
TFNAL8A3	8	0	57
TMCXZG40	0	0	0
UEZMHS9U	8	0	5
VD5XRWRB	23	0	31
WRCEF70B	0	0	12
XVKTEDG7	0	0	0
YCTPWJ0V	9	63	6
ZEYK0X4O	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	E.Coli01	Human01	Swine01	Bovine01
0BOJX9IT	100.0	100.0	0.0	0.0
18582FWD	100.0	100.0	0.0	100.0
1UXEVLFI	100.0	100.0	0.0	0.0
6N3JN5L2	33.3	100.0	0.0	0.0
71KA0YGD	100.0	66.7	0.0	0.0
8HGDBWU7	100.0	100.0	0.0	0.0
C4PH8LS2	100.0	0.0	0.0	0.0
D9HIAJDE	100.0	66.7	0.0	0.0
DNIK90FV	100.0	100.0	0.0	0.0
H571IUSC	100.0	100.0	0.0	33.3
JCB7VUAZ	100.0	100.0	0.0	66.7
KC7TH8GI	66.7	66.7	0.0	0.0
MNX5VG63	100.0	66.7	0.0	33.3
MZ8Q3RI0	100.0	100.0	0.0	0.0
N5AK4PMH	100.0	33.3	0.0	0.0
NONADUE6	100.0	33.3	0.0	0.0
OGX8DUBD	100.0	66.7	0.0	0.0
PDO2O0BD	100.0	100.0	0.0	0.0
Q0GH3ET1	100.0	100.0	0.0	0.0
RH99KI1R	100.0	100.0	0.0	0.0
SLGKRGZ3	100.0	100.0	0.0	0.0
TFNAL8A3	100.0	100.0	0.0	0.0

SampleId	E.Coli01	Human01	Swine01	Bovine01
TMCXZG40	100.0	100.0	0.0	0.0
UEZMHS9U	100.0	33.3	0.0	66.7
VD5XRWRB	100.0	100.0	33.3	33.3
WRCEF70B	100.0	100.0	0.0	0.0
XVKTEDG7	66.7	33.3	0.0	0.0
YCTPWJ0V	100.0	100.0	0.0	33.3
ZEYK0X4O	100.0	66.7	0.0	0.0

SampleId	Poultry01	Sheep01	Dog01
0BOJX9IT	0.0	0	33.3
18582FWD	0.0	100	0.0
1UXEVLFI	0.0	0	0.0
6N3JN5L2	0.0	0	33.3
71KA0YGD	0.0	0	33.3
8HGDBWU7	0.0	0	0.0
C4PH8LS2	66.7	0	100.0
D9HIAJDE	0.0	0	33.3
DNIK90FV	0.0	0	0.0
H571IUSC	0.0	0	33.3
JCB7VUAZ	0.0	0	0.0
KC7TH8GI	0.0	0	0.0
MNX5VG63	0.0	0	0.0
MZ8Q3RI0	0.0	0	0.0
N5AK4PMH	33.3	0	33.3
NONADUE6	0.0	0	0.0
OGX8DUBD	0.0	0	0.0
PDO2O0BD	0.0	0	33.3
Q0GH3ET1	33.3	0	66.7
RH99KI1R	66.7	0	0.0

SampleId	Poultry01	Sheep01	Dog01
SLGKRGZ3	33.3	0	66.7
TFNAL8A3	33.3	0	66.7
TMCXZG40	0.0	0	0.0
UEZMHS9U	33.3	0	33.3
VD5XRWRB	66.7	0	100.0
WRCEF70B	0.0	0	66.7
XVKTEDG7	0.0	0	0.0
YCTPWJ0V	33.3	100	33.3
ZEYK0X4O	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.

TestId	SampleId	Rep 1	Rep 2	Rep 3
Bovine01				
	0BOJX9IT	0	0	0
	18582FWD	46253	51097	45239
	1UXEVLFI	0	0	0
	6N3JN5L2	0	0	0
	71KA0YGD	0	0	0
	8HGDBWU7	0	0	0
	C4PH8LS2	0	0	0
	D9HIAJDE	0	0	0
	DNIK90FV	0	0	0
	H571IUSC	361	0	0
	JCB7VUAZ	54	69	0
	KC7TH8GI	0	0	0
	MNX5VG63	0	121	0
	MZ8Q3RI0	0	0	0
	N5AK4PMH	0	0	0
	NONADUE6	0	0	0
	OGX8DUBD	0	0	0
	PDO2O0BD	0	0	0
	Q0GH3ET1	0	0	0
	RH99KI1R	0	0	0
	SLGKRGZ3	0	0	0
	TFNAL8A3	0	0	0
	TMCXZG40	0	0	0

TestId	SampleId	Rep 1	Rep 2	Rep 3
	UEZMHS9U	0	92	112
	VD5XRWRB	0	186	0
	WRCEF70B	0	0	0
	XVKTEDG7	0	0	0
	YCTPWJ0V	5996	0	0
	ZEYK0X4O	0	0	0
Dog01				
	0BOJX9IT	0	17	0
	18582FWD	0	0	0
	1UXEVLFI	0	0	0
	6N3JN5L2	0	6	0
	71KA0YGD	0	0	36
	8HGDBWU7	0	0	0
	C4PH8LS2	34	19	26
	D9HIAJDE	0	0	35
	DNIK90FV	0	0	0
	H571IUSC	0	35	0
	JCB7VUAZ	0	0	0
	KC7TH8GI	0	0	0
	MNX5VG63	0	0	0
	MZ8Q3RI0	0	0	0
	N5AK4PMH	0	19	0
	NONADUE6	0	0	0
	OGX8DUBD	0	0	0
	PDO2O0BD	40	0	0
	Q0GH3ET1	0	72	16
	RH99KI1R	0	0	0
	SLGKRGZ3	17	15	0
	TFNAL8A3	104	67	0

TestId	SampleId	Rep 1	Rep 2	Rep 3
	TMCXZG40	0	0	0
	UEZMHS9U	15	0	0
	VD5XRWRB	31	16	47
	WRCEF70B	18	0	19
	XVKTEDG7	0	0	0
	YCTPWJ0V	0	0	18
	ZEYK0X4O	0	0	0
E.Coli01				
	0BOJX9IT	3057	2834	2830
	18582FWD	172407	159551	199185
	1UXEVLFI	3427	2026	1960
	6N3JN5L2	0	0	42
	71KA0YGD	2110	2241	2456
	8HGDBWU7	1762	2070	2442
	C4PH8LS2	1761	2451	3524
	D9HIAJDE	21	337	392
	DNIK90FV	322	634	599
	H571IUSC	2645	2415	2836
	JCB7VUAZ	1117	946	671
	KC7TH8GI	109	0	51
	MNX5VG63	2871	3592	3969
	MZ8Q3RI0	1730	1601	1322
	N5AK4PMH	107	45	117
	NONADUE6	2676	2610	1397
	OGX8DUBD	2473	2837	2509
	PDO2O0BD	2538	1256	1941
	Q0GH3ET1	1178	1031	1292
	RH99KI1R	1401	1212	1248
	SLGKRGZ3	2214	1597	2324

TestId	SampleId	Rep 1	Rep 2	Rep 3
	TFNAL8A3	4498	5561	5487
	TMCXZG40	4049	4028	2927
	UEZMHS9U	2256	2349	2049
	VD5XRWRB	1731	2106	2174
	WRCEF70B	3744	3085	2824
	XVKTEDG7	0	31	49
	YCTPWJ0V	24456	22701	27737
	ZEYK0X4O	1918	1417	1849
Human01				
	0BOJX9IT	3026	2527	2236
	18582FWD	181	89	140
	1UXEVLFI	119	59	79
	6N3JN5L2	72	73	37
	71KA0YGD	0	129	110
	8HGDBWU7	69	81	109
	C4PH8LS2	0	0	0
	D9HIAJDE	30	0	50
	DNIK90FV	52	74	22
	H571IUSC	186	173	136
	JCB7VUAZ	328	208	323
	KC7TH8GI	0	56	98
	MNX5VG63	0	141	202
	MZ8Q3RI0	86	52	64
	N5AK4PMH	0	26	0
	NONADUE6	91	0	0
	OGX8DUBD	108	115	0
	PDO2O0BD	433	25	20
	Q0GH3ET1	17	23	31
	RH99KI1R	81	23	19

TestId	SampleId	Rep 1	Rep 2	Rep 3
	SLGKRGZ3	39	22	47
	TFNAL8A3	81	41	104
	TMCXZG40	1342	888	1423
	UEZMHS9U	0	35	0
	VD5XRWRB	1181	1013	992
	WRCEF70B	985	1011	521
	XVKTEDG7	0	50	0
	YCTPWJ0V	224	101	47
	ZEYK0X4O	61	0	97
Poultry01				
	0BOJX9IT	0	0	0
	18582FWD	0	0	0
	1UXEVLFI	0	0	0
	6N3JN5L2	0	0	0
	71KA0YGD	0	0	0
	8HGDBWU7	0	0	0
	C4PH8LS2	0	22	38
	D9HIAJDE	0	0	0
	DNIK90FV	0	0	0
	H571IUSC	0	0	0
	JCB7VUAZ	0	0	0
	KC7TH8GI	0	0	0
	MNX5VG63	0	0	0
	MZ8Q3RI0	0	0	0
	N5AK4PMH	0	54	0
	NONADUE6	0	0	0
	OGX8DUBD	0	0	0
	PDO2O0BD	0	0	0
	Q0GH3ET1	0	0	56

TestId	SampleId	Rep 1	Rep 2	Rep 3
	RH99KI1R	27	36	0
	SLGKRGZ3	0	11	0
	TFNAL8A3	0	24	0
	TMCXZG40	0	0	0
	UEZMHS9U	0	23	0
	VD5XRWRB	0	49	21
	WRCEF70B	0	0	0
	XVKTEDG7	0	0	0
	YCTPWJ0V	0	0	28
	ZEYK0X4O	0	0	0
Sheep01				
	0BOJX9IT	0	0	0
	18582FWD	881	519	603
	1UXEVLFI	0	0	0
	6N3JN5L2	0	0	0
	71KA0YGD	0	0	0
	8HGDBWU7	0	0	0
	C4PH8LS2	0	0	0
	D9HIAJDE	0	0	0
	DNIK90FV	0	0	0
	H571IUSC	0	0	0
	JCB7VUAZ	0	0	0
	KC7TH8GI	0	0	0
	MNX5VG63	0	0	0
	MZ8Q3RI0	0	0	0
	N5AK4PMH	0	0	0
	NONADUE6	0	0	0
	OGX8DUBD	0	0	0
	PDO2O0BD	0	0	0

TestId	SampleId	Rep 1	Rep 2	Rep 3
	Q0GH3ET1	0	0	0
	RH99KI1R	0	0	0
	SLGKRGZ3	0	0	0
	TFNAL8A3	0	0	0
	TMCXZG40	0	0	0
	UEZMHS9U	0	0	0
	VD5XRWRB	0	0	0
	WRCEF70B	0	0	0
	XVKTEDG7	0	0	0
	YCTPWJ0V	79	54	56
	ZEYK0X4O	0	0	0
Swine01				
	0BOJX9IT	0	0	0
	18582FWD	0	0	0
	1UXEVLFI	0	0	0
	6N3JN5L2	0	0	0
	71KA0YGD	0	0	0
	8HGDBWU7	0	0	0
	C4PH8LS2	0	0	0
	D9HIAJDE	0	0	0
	DNIK90FV	0	0	0
	H571IUSC	0	0	0
	JCB7VUAZ	0	0	0
	KC7TH8GI	0	0	0
	MNX5VG63	0	0	0
	MZ8Q3RI0	0	0	0
	N5AK4PMH	0	0	0
	NONADUE6	0	0	0
	OGX8DUBD	0	0	0

TestId	SampleId	Rep 1	Rep 2	Rep 3
	PDO2O0BD	0	0	0
	Q0GH3ET1	0	0	0
	RH99KI1R	0	0	0
	SLGKRGZ3	0	0	0
	TFNAL8A3	0	0	0
	TMCXZG40	0	0	0
	UEZMHS9U	0	0	0
	VD5XRWRB	0	146	0
	WRCEF70B	0	0	0
	XVKTEDG7	0	0	0
	YCTPWJ0V	0	0	0
	ZEYK0X4O	0	0	0

Sample metadata

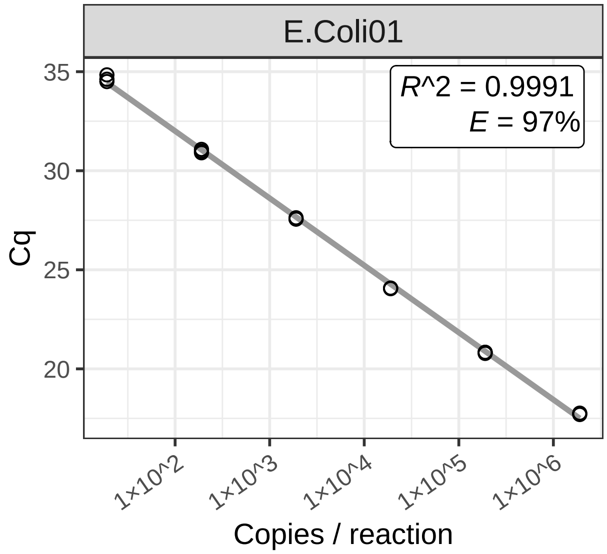
SampleId	Volume Water (ml)
0BOJX9IT	100
18582FWD	100
1UXEVLFI	100
6N3JN5L2	100
71KA0YGD	100
8HGDBWU7	100
C4PH8LS2	100
D9HIAJDE	100
DNIK90FV	100
H571IUSC	100
JCB7VUAZ	100
KC7TH8GI	100
MNX5VG63	100
MZ8Q3RI0	100
N5AK4PMH	100
NONADUE6	100
OGX8DUBD	100
PDO2O0BD	100
Q0GH3ET1	100
RH99KI1R	100
SLGKRGZ3	100
TFNAL8A3	100
TMCXZG40	100
UEZMHS9U	100
VD5XRWRB	100
WRCEF70B	100
XVKTEDG7	100
YCTPWJ0V	100
ZEYK0X4O	100

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: JVQ0560



Forward primer: 5’ CAATGGTGATGTCAGCGTT 3’

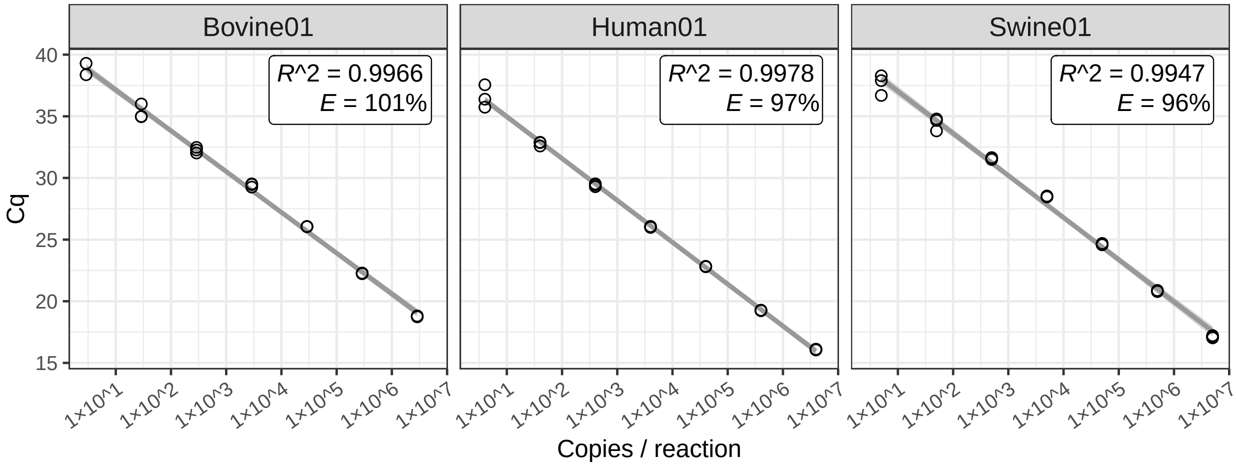
Reverse primer: 5’ ACACTCTGTCCGGCTTTTG 3’

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

Primer/probe reference: Srinivasan et al., 2011

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.

RunID: JVQ0584



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’

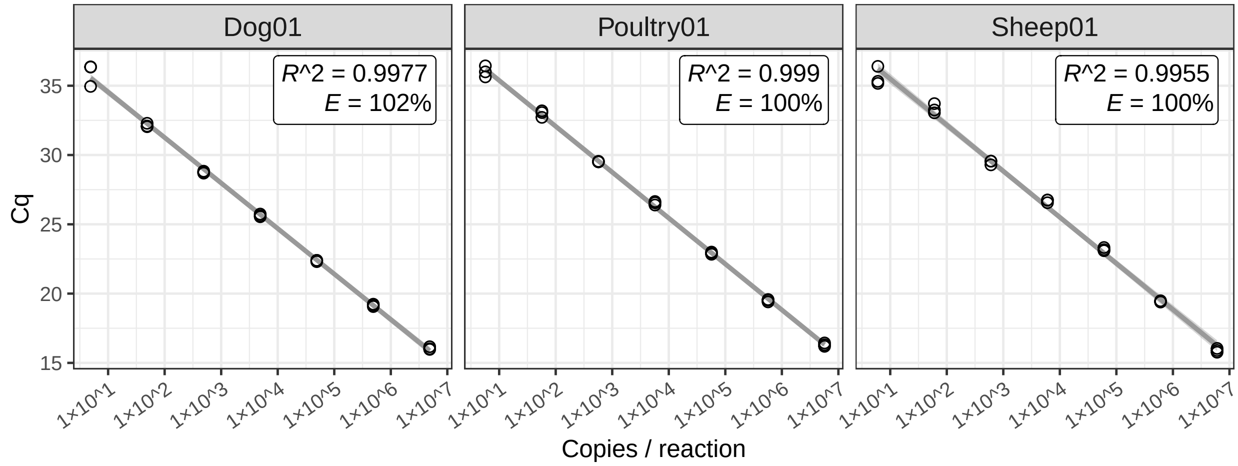
Swine Reverse primer: 5’ GGATGTAGTCCGAATTGAGCTGATTAT 3’

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

Primer/probe reference: Cadwell et al., 2007

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: JVQ0585



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.