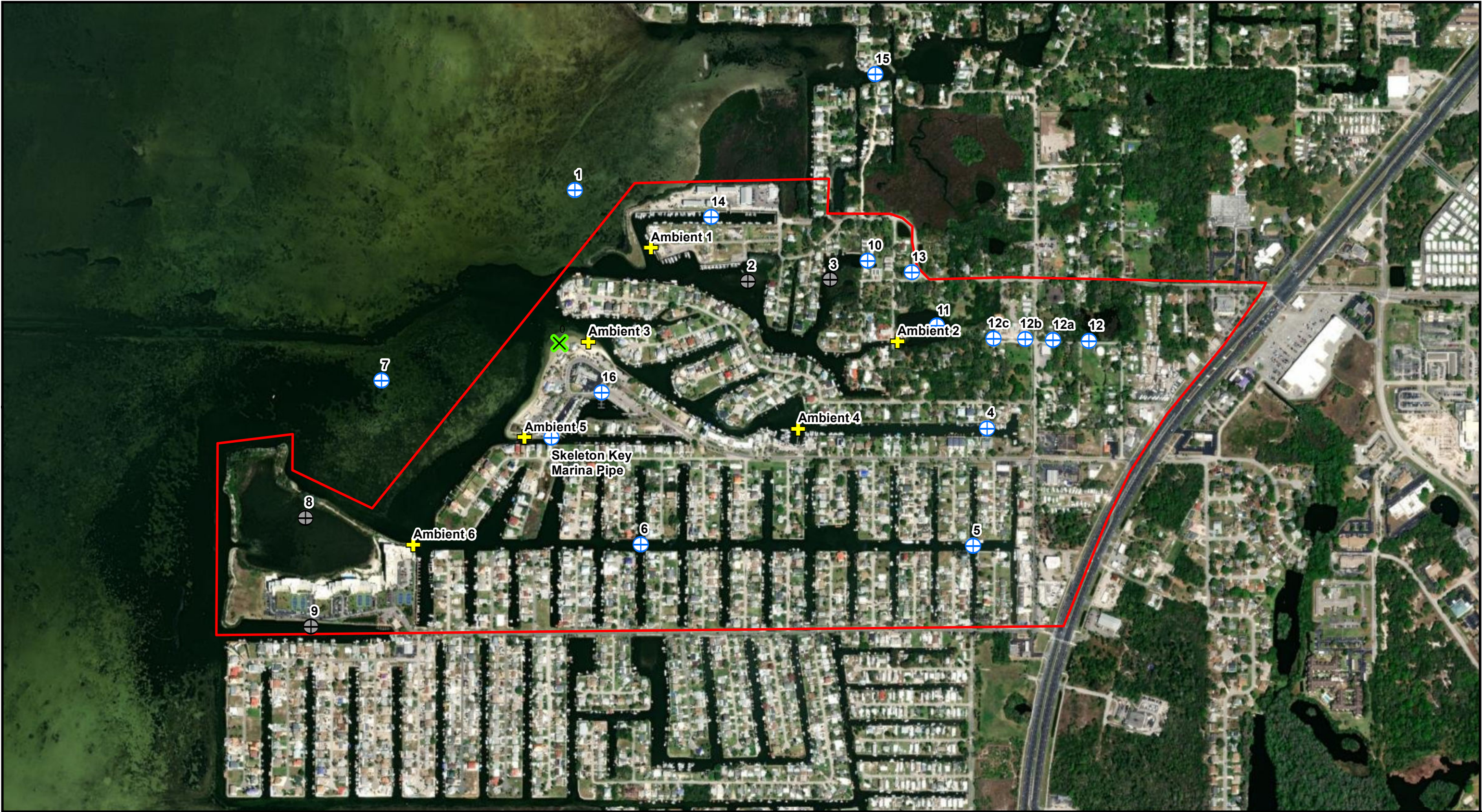




Feet 0 700 1 in = 700 feet	HUDSON BEACH POLLUTION SOURCE TRACKING FLORIDA DESIGN CONSULTANTS PASCO COUNTY, FLORIDA	Ambient Monitoring Site						SAMPLING LOCATION & AMBIENT MONITORING SITE MAP	
--	---	---	--	--	--	--	--	---	---

		Results (MPN/100 mL)		
		Team Field Recon Event	Event 1	Event 2
		10/23/2023	11/6/2023	11/20/2023
Previous 7 day cumulative rainfall (inches)		0	0	1.13
		High Tide: 7:21 Low Tide: 15:14	High Tide: 5:50 Low Tide: 13:45	High Tide: 4:47 Low Tide: 12:25
Sample ID	Location Description	Sample start: 10:05 Sample end: 10:05	Sample start: 11:35 Sample end: 14:40	Sample start: 9:46 Sample end: 13:00
Ambient 1	Canal near Shrimp Dock Dr.	-	ND	
Ambient 2	Hudson Creek at Pine St.	-	ND	
Ambient 3	RJS Beach Park (Spong Crawl Creek)	-	ND	
Ambient 3 (sediment)	Sample collected w/ sediment from beach	-	-	
Ambient 4	Sponge Crawl Creek at Yacht Club	-	10	
Ambient 5	RJS Beach Park S - Near Skeleton Key Marina	-	ND	
Ambient 6	NE Corner of Gulf Island	-	ND	
1	Northern off-shore	-	ND	
2 (removed location)	Canal b/t Shrimp Dock Dr & Hendry Ct (spring location)	-	-	
3 (removed location)	Location removed due to proximity to added locations	-	-	
4	End of Sponge Crawl Creek canal	-	NDA	
5	End of canal b/t Clark St & Skippers Sanctuary	-	ND	
6	Central canal location b/t Clark St & Skippers Sanctuary	-	10	
7	Southern off-shore	-	ND	
8 (removed location)	Within condo complex lagoon	-	-	
9 (removed location)	Canal behind condo complex	-	-	
10	Canal adj. to MHP w/ previous sewage release	-	ND	
11	Hudson Creek/tidally influenced pond	-	10	
12	Ditch on Apple Ave adj. to Hudson Water Works	>2400Δ	61Δ	
12a	West of Location 12, in ditch at intersection of Apple Ave & Old Dixie Hwy	-	-	
12b	West of 12a in ditch adj. to Apple St.	-	-	
12c	West of 12b at end of ditch on Apple St.	-	-	
13	SW pond E of MHP and adj. to Lift Station "Port Hudson PS32"	-	ND	
Pipe at Skeleton Key Marina	Pipe discharging gray-ish water from seawall at Skeleton Key Marina	-	10	
14	Canal directly adjacent to Port Hudson Marina	-	ND	
15	Mouth of Cow Creek canal, just north of project area	-	ND	
16	Stormwater pond at RJS Boat Ramp	-	31	

Bold values indicate detections of human waste tracers

Δ = HF-183 detected

□ = Acetaminophen detected

Bold borders indicate location was sampled for enterococci, HF-183, and acetaminophen

ND/1-70 MPN/100mL
71-409 MPN/100mL
409-1399 MPN/100mL
>1400 MPN/100mL/human tracer detection



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FINAL

Workorder: Hudson Pollution (T2321065)

October 31, 2023

RE: Workorder: T2321065 Hudson Pollution

Enclosed are the analytical results for sample(s) received by the laboratory on Monday October 23, 2023. Results reported herein conform to the most current NELAC standards, where applicable, unless otherwise narrated in the body of the report. The analytical results for the samples contained in this report were submitted for analysis as outlined by the Chain of Custody and results pertain only to these samples.

If you have any questions concerning this report, please feel free to contact me.

Sincerely,

Sheila Wilcox, Assistant Lab Manager
SWilcox@aellab.com

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FINAL

Workorder: Hudson Pollution (T2321065)

Sample Summary

Lab ID	Sample ID	Matrix	Method	Date Collected	Date Received	Analytes Reported	Basis
T2321065001	12	WA	ENTEROLERT/ QUANTI-TRAY	10/23/2023 10:05	10/23/2023 15:31	1	NA

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FINAL

Workorder: Hudson Pollution (T2321065)

Workorder Summary

Batch Comments

MICt/7060 - Enterococci,EPA 1600, Water

The aliquots analyzed for Enterococci sample T2321065001 and its dilution resulted in a tabulated value that is off scale, over the high range. The actual concentration for Enterococci is estimated to be higher than the value reported. The sample is qualified with a J to indicate the estimation.

Task Comments

T2321065001 (12) - MICt/7060 - Enterococci,EPA 1600, Water

J4|Estimated Result

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FINAL

Workorder: Hudson Pollution (T2321065)

Analytical Results Qualifiers

Parameter Qualifiers

- U The compound was analyzed for but not detected.
- I The reported value is between the laboratory method detection limit and the laboratory practical quantitation limit.

Lab Qualifiers

- T DOH Certification #E84589 (FL NELAC) AEL-Tampa

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FINAL

Workorder: Hudson Pollution (T2321065)

Analytical Results

Lab ID: T2321065001
Sample ID: 12

Date Collected: 10/23/2023 10:05
Date Received: 10/23/2023 15:31

Matrix: Water

Parameter	Results	Units	PQL	MDL	DF	Prepared	Analyzed	Lab
Microbiology (ENTEROLERT/ QUANTI-TRAY)								
Enterococcus	2400	MPN/100 mL	1.0	1.0	1	10/23/2023 16:15	10/23/2023 16:15	T

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FINAL

Workorder: Hudson Pollution (T2321065)

QC Results

QC Batch: MICT/7060
Preparation Method: ENTEROLERT/ QUANTI-TRAY
Associated Lab IDs: T2321065001

Analysis Method: ENTEROLERT/ QUANTI-TRAY

Method Blank(5012583)

Parameter	Results	Units	PQL	MDL	Lab
Enterococcus	1.0 U	MPN/100 mL	1.0	1.0	T

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FINAL

Workorder: Hudson Pollution (T2321065)

QC Cross Reference

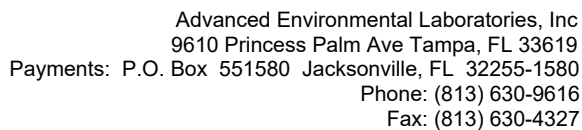
Lab ID	Sample ID	Prep Batch	Prep Method
MICt/7060 - ENTEROLERT/ QUANTI-TRAY			
T2321065001	12		

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Workorder: Hudson Pollution (T2321065)

Tuesday, October 31, 2023 8:45:03 AM
Dates and times are displayed using (-04:00)
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DRAFT

Workorder: Hudson Pollution (T2322172)

November 16, 2023

Michael Grzywacz
Flatwoods
8306 ILaurel Fair Circle Ste12
Tampa,

RE: Workorder: T2322172 Hudson Pollution

Dear Michael Grzywacz:

Enclosed are the analytical results for sample(s) received by the laboratory on Monday November 6, 2023. Results reported herein conform to the most current NELAC standards, where applicable, unless otherwise narrated in the body of the report. The analytical results for the samples contained in this report were submitted for analysis as outlined by the Chain of Custody and results pertain only to these samples.

If you have any questions concerning this report, please feel free to contact me.

Sincerely,

Sheila Wilcox, Assistant Lab Manager
SWilcox@aellab.com

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DRAFT

Workorder: Hudson Pollution (T2322172)

Sample Summary

Lab ID	Sample ID	Matrix	Method	Date Collected	Date Received	Analytes Reported	Basis
T2322172001	Skeleton Key Marina	WA	ENTEROLERT/ QUANTI-TRAY	11/06/2023 11:35	11/06/2023 15:52	1	NA
T2322172002	15	WA	ENTEROLERT/ QUANTI-TRAY	11/06/2023 11:53	11/06/2023 15:52	1	NA
T2322172003	1	WA	ENTEROLERT/ QUANTI-TRAY	11/06/2023 12:03	11/06/2023 15:52	1	NA
T2322172004	Ambient 2	WA	ENTEROLERT/ QUANTI-TRAY	11/06/2023 12:15	11/06/2023 15:52	1	NA
T2322172005	10	WA	ENTEROLERT/ QUANTI-TRAY	11/06/2023 12:24	11/06/2023 15:52	1	NA
T2322172006	Ambient 1	WA	ENTEROLERT/ QUANTI-TRAY	11/06/2023 12:32	11/06/2023 15:52	1	NA
T2322172007	14	WA	ENTEROLERT/ QUANTI-TRAY	11/06/2023 12:36	11/06/2023 15:52	1	NA
T2322172008	4	WA	ENTEROLERT/ QUANTI-TRAY	11/06/2023 12:55	11/06/2023 15:52	1	NA
T2322172009	Ambient 4	WA	ENTEROLERT/ QUANTI-TRAY	11/06/2023 13:01	11/06/2023 15:52	1	NA
T2322172010	Ambient 3	WA	ENTEROLERT/ QUANTI-TRAY	11/06/2023 13:08	11/06/2023 15:52	1	NA
T2322172011	7	WA	ENTEROLERT/ QUANTI-TRAY	11/06/2023 13:14	11/06/2023 15:52	1	NA
T2322172012	5	WA	ENTEROLERT/ QUANTI-TRAY	11/06/2023 13:33	11/06/2023 15:52	1	NA
T2322172013	6	WA	ENTEROLERT/ QUANTI-TRAY	11/06/2023 13:41	11/06/2023 15:52	1	NA
T2322172014	Ambient 6	WA	ENTEROLERT/ QUANTI-TRAY	11/06/2023 13:48	11/06/2023 15:52	1	NA
T2322172015	Ambient 5	WA	ENTEROLERT/ QUANTI-TRAY	11/06/2023 13:54	11/06/2023 15:52	1	NA
T2322172016	16	WA	ENTEROLERT/ QUANTI-TRAY	11/06/2023 14:11	11/06/2023 15:52	1	NA
T2322172017	11	WA	ENTEROLERT/ QUANTI-TRAY	11/06/2023 14:20	11/06/2023 15:52	1	NA
T2322172018	12	WA	ENTEROLERT/ QUANTI-TRAY	11/06/2023 14:40	11/06/2023 15:52	1	NA
T2322172019	13	WA	ENTEROLERT/ QUANTI-TRAY	11/06/2023 14:26	11/06/2023 15:52	1	NA





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DRAFT

Workorder: Hudson Pollution (T2322172)

Analytical Results Qualifiers

Parameter Qualifiers

- U The compound was analyzed for but not detected.
- I The reported value is between the laboratory method detection limit and the laboratory practical quantitation limit.

Lab Qualifiers

- T DOH Certification #E84589 (FL NELAC) AEL-Tampa

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Workorder: Hudson Pollution (T2322172)

Analytical Results

Lab ID: T2322172001
Sample ID: Skeleton Key Marina

Date Collected: 11/06/2023 11:35
Date Received: 11/06/2023 15:52

Matrix: Water

Parameter	Results	Units	PQL	MDL	DF	Prepared	Analyzed	Lab
Microbiology (ENTEROLERT/ QUANTI-TRAY)								
Enterococcus	10	MPN/100 mL	10	10	10	11/06/2023 16:39	11/06/2023 16:39	T

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Workorder: Hudson Pollution (T2322172)

Analytical Results

Lab ID: T2322172002
Sample ID: 15

Date Collected: 11/06/2023 11:53
Date Received: 11/06/2023 15:52

Matrix: Water

Parameter	Results	Units	PQL	MDL	DF	Prepared	Analyzed	Lab
Microbiology (ENTEROLERT/ QUANTI-TRAY)								
Enterococcus	10 U	MPN/100 mL	10	10	10	11/06/2023 16:39	11/06/2023 16:39	T

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DRAFT

Workorder: Hudson Pollution (T2322172)

Analytical Results

Lab ID: T2322172003		Date Collected: 11/06/2023 12:03		Matrix: Water				
Sample ID: 1		Date Received: 11/06/2023 15:52						
Parameter	Results	Units	PQL	MDL	DF	Prepared	Analyzed	Lab
Microbiology (ENTEROLERT/ QUANTI-TRAY)								
Enterococcus	10 U	MPN/100 mL	10	10	10	11/06/2023 16:39	11/06/2023 16:39	T

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Workorder: Hudson Pollution (T2322172)

Analytical Results

Lab ID: T2322172004		Date Collected: 11/06/2023 12:15		Matrix: Water				
Sample ID: Ambient 2		Date Received: 11/06/2023 15:52						
Parameter	Results	Units	PQL	MDL	DF	Prepared	Analyzed	Lab
Microbiology (ENTEROLERT/ QUANTI-TRAY)								
Enterococcus	10	MPN/100 mL	10	10	10	11/06/2023 16:39	11/06/2023 16:39	T

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Workorder: Hudson Pollution (T2322172)

Analytical Results

Lab ID: T2322172005
Sample ID: 10

Date Collected: 11/06/2023 12:24
Date Received: 11/06/2023 15:52

Matrix: Water

Parameter	Results	Units	PQL	MDL	DF	Prepared	Analyzed	Lab
Microbiology (ENTEROLERT/ QUANTI-TRAY)								
Enterococcus	10 U	MPN/100 mL	10	10	10	11/06/2023 16:39	11/06/2023 16:39	T

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Workorder: Hudson Pollution (T2322172)

Analytical Results

Lab ID: T2322172006
Sample ID: Ambient 1

Date Collected: 11/06/2023 12:32
Date Received: 11/06/2023 15:52

Matrix: Water

Parameter	Results	Units	PQL	MDL	DF	Prepared	Analyzed	Lab
Microbiology (ENTEROLERT/ QUANTI-TRAY)								
Enterococcus	10 U	MPN/100 mL	10	10	10	11/06/2023 16:39	11/06/2023 16:39	T

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DRAFT

Workorder: Hudson Pollution (T2322172)

Analytical Results

Lab ID: T2322172007		Date Collected: 11/06/2023 12:36		Matrix: Water				
Sample ID: 14		Date Received: 11/06/2023 15:52						
Parameter	Results	Units	PQL	MDL	DF	Prepared	Analyzed	Lab
Microbiology (ENTEROLERT/ QUANTI-TRAY)								
Enterococcus	10 U	MPN/100 mL	10	10	10	11/06/2023 16:39	11/06/2023 16:39	T

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DRAFT

Workorder: Hudson Pollution (T2322172)

Analytical Results

Lab ID: T2322172008
Sample ID: 4

Date Collected: 11/06/2023 12:55
Date Received: 11/06/2023 15:52

Matrix: Water

Parameter	Results	Units	PQL	MDL	DF	Prepared	Analyzed	Lab
Microbiology (ENTEROLERT/ QUANTI-TRAY)								
Enterococcus	10 U	MPN/100 mL	10	10	10	11/06/2023 16:39	11/06/2023 16:39	T

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DRAFT

Workorder: Hudson Pollution (T2322172)

Analytical Results

Lab ID: T2322172009
Sample ID: Ambient 4

Date Collected: 11/06/2023 13:01
Date Received: 11/06/2023 15:52

Matrix: Water

Parameter	Results	Units	PQL	MDL	DF	Prepared	Analyzed	Lab
Microbiology (ENTEROLERT/ QUANTI-TRAY)								
Enterococcus	10	MPN/100 mL	10	10	10	11/06/2023 16:39	11/06/2023 16:39	T

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Workorder: Hudson Pollution (T2322172)

Analytical Results

Lab ID: T2322172010
Sample ID: Ambient 3

Date Collected: 11/06/2023 13:08
Date Received: 11/06/2023 15:52

Matrix: Water

Parameter	Results	Units	PQL	MDL	DF	Prepared	Analyzed	Lab
Microbiology (ENTEROLERT/ QUANTI-TRAY)								
Enterococcus	10 U	MPN/100 mL	10	10	10	11/06/2023 16:39	11/06/2023 16:39	T

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DRAFT

Workorder: Hudson Pollution (T2322172)

Analytical Results

Lab ID: T2322172011		Date Collected: 11/06/2023 13:14		Matrix: Water				
Sample ID: 7		Date Received: 11/06/2023 15:52						
Parameter	Results	Units	PQL	MDL	DF	Prepared	Analyzed	Lab
Microbiology (ENTEROLERT/ QUANTI-TRAY)								
Enterococcus	10 U	MPN/100 mL	10	10	10	11/06/2023 16:39	11/06/2023 16:39	T

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DRAFT

Workorder: Hudson Pollution (T2322172)

Analytical Results

Lab ID: T2322172012
Sample ID: 5

Date Collected: 11/06/2023 13:33
Date Received: 11/06/2023 15:52

Matrix: Water

Parameter	Results	Units	PQL	MDL	DF	Prepared	Analyzed	Lab
Microbiology (ENTEROLERT/ QUANTI-TRAY)								
Enterococcus	10 U	MPN/100 mL	10	10	10	11/06/2023 16:39	11/06/2023 16:39	T

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DRAFT

Workorder: Hudson Pollution (T2322172)

Analytical Results

Lab ID: T2322172013		Date Collected: 11/06/2023 13:41		Matrix: Water				
Sample ID: 6		Date Received: 11/06/2023 15:52						
Parameter	Results	Units	PQL	MDL	DF	Prepared	Analyzed	Lab
Microbiology (ENTEROLERT/ QUANTI-TRAY)								
Enterococcus	10	MPN/100 mL	10	10	10	11/06/2023 16:39	11/06/2023 16:39	T

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DRAFT

Workorder: Hudson Pollution (T2322172)

Analytical Results

Lab ID: T2322172014
Sample ID: Ambient 6

Date Collected: 11/06/2023 13:48
Date Received: 11/06/2023 15:52

Matrix: Water

Parameter	Results	Units	PQL	MDL	DF	Prepared	Analyzed	Lab
Microbiology (ENTEROLERT/ QUANTI-TRAY)								
Enterococcus	10 U	MPN/100 mL	10	10	10	11/06/2023 16:39	11/06/2023 16:39	T

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DRAFT

Workorder: Hudson Pollution (T2322172)

Analytical Results

Lab ID: T2322172015
Sample ID: Ambient 5

Date Collected: 11/06/2023 13:54
Date Received: 11/06/2023 15:52

Matrix: Water

Parameter	Results	Units	PQL	MDL	DF	Prepared	Analyzed	Lab
Microbiology (ENTEROLERT/ QUANTI-TRAY)								
Enterococcus	10 U	MPN/100 mL	10	10	10	11/06/2023 16:39	11/06/2023 16:39	T

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DRAFT

Workorder: Hudson Pollution (T2322172)

Analytical Results

Lab ID: T2322172016		Date Collected: 11/06/2023 14:11		Matrix: Water				
Sample ID: 16		Date Received: 11/06/2023 15:52						
Parameter	Results	Units	PQL	MDL	DF	Prepared	Analyzed	Lab
Microbiology (ENTEROLERT/ QUANTI-TRAY)								
Enterococcus	31	MPN/100 mL	10	10	10	11/06/2023 16:39	11/06/2023 16:39	T

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Fax: (813) 630-4327

DRAFT

Workorder: Hudson Pollution (T2322172)

Analytical Results

Lab ID: T2322172017
Sample ID: 11

Date Collected: 11/06/2023 14:20
Date Received: 11/06/2023 15:52

Matrix: Water

Parameter	Results	Units	PQL	MDL	DF	Prepared	Analyzed	Lab
Microbiology (ENTEROLERT/ QUANTI-TRAY)								
Enterococcus	10	MPN/100 mL	10	10	10	11/06/2023 16:39	11/06/2023 16:39	T

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DRAFT

Workorder: Hudson Pollution (T2322172)

Analytical Results

Lab ID: T2322172018
Sample ID: 12

Date Collected: 11/06/2023 14:40
Date Received: 11/06/2023 15:52

Matrix: Water

Parameter	Results	Units	PQL	MDL	DF	Prepared	Analyzed	Lab
Microbiology (ENTEROLERT/ QUANTI-TRAY)								
Enterococcus	61	MPN/100 mL	10	10	10	11/06/2023 16:39	11/06/2023 16:39	T

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DRAFT

Workorder: Hudson Pollution (T2322172)

Analytical Results

Lab ID: T2322172019
Sample ID: 13

Date Collected: 11/06/2023 14:26
Date Received: 11/06/2023 15:52

Matrix: Water

Parameter	Results	Units	PQL	MDL	DF	Prepared	Analyzed	Lab
Microbiology (ENTEROLERT/ QUANTI-TRAY)								
Enterococcus	10 U	MPN/100 mL	10	10	10	11/06/2023 16:39	11/06/2023 16:39	T

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DRAFT

Workorder: Hudson Pollution (T2322172)

QC Results

QC Batch: MICt/7158
Preparation Method: ENTEROLERT/ QUANTI-TRAY
Analysis Method: ENTEROLERT/ QUANTI-TRAY
Associated Lab IDs: T2322172001, T2322172002, T2322172003, T2322172004, T2322172005, T2322172006, T2322172007, T2322172008, T2322172009, T2322172010, T2322172011, T2322172012, T2322172013, T2322172014, T2322172015, T2322172016, T2322172017, T2322172018, T2322172019

Method Blank(5032965)

Parameter	Results	Units	PQL	MDL	Lab
Enterococcus	1.0 U	MPN/100 mL	1.0	1.0	T





DRAFT

Workorder: Hudson Pollution (T2322172)

QC Cross Reference

Lab ID	Sample ID	Prep Batch	Prep Method
MICt/7158 - ENTEROLERT/ QUANTI-TRAY			
T2322172001	Skeleton Key Marina		
T2322172002	15		
T2322172003	1		
T2322172004	Ambient 2		
T2322172005	10		
T2322172006	Ambient 1		
T2322172007	14		
T2322172008	4		
T2322172009	Ambient 4		
T2322172010	Ambient 3		
T2322172011	7		
T2322172012	5		
T2322172013	6		
T2322172014	Ambient 6		
T2322172015	Ambient 5		
T2322172016	16		
T2322172017	11		
T2322172018	12		
T2322172019	13		

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☐ Tallahassee: 2539 North Monroe St., Suite D, FL 32303 • 850.219.6274 • Lab ID: E611095

☐ Gainesville: 4965 SW 41st Blvd., FL 32608 • 352.377.2349 • Lab ID: E62001
☐ Miramar: 10200 USA Today Way, FL 33025 • 954.889.2288 • Lab ID: E62335
☒ Tampa: 9610 Princess Palm Ave., FL 33619 • 813.630.9616 • Lab ID: E64589

Client Name: Flatwoods Consulting

Project Name: Hudson Pollution

Address: 8306 Laurel Fair Circle

Project Number:

Phone: 610-739-4499

PO Number:

FAX:

FDEP Facility No:

Contact: Michael Graywell

FDEP Facility Addr:

Sampled By: Michael Graywell

Special Instructions:

Standard

Rush

ADAPT

EQUIS

Other

ANALYSIS REQUIRED

Enterococci



LABORATORY I.D. NUMBER

SAMPLE ID

SAMPLE DESCRIPTION

Grab Comp

DATE

TIME

MATRIX

NO. COUNT

Preservation

Fold-Filtered?

No

Yes

Matrix

Count

Skeleton Key Marina Pipe (17)

Grab

11/6/13

11:35

Water

1

11:53

Water

1

15

12:03

Water

1

12:15

Water

1

12:24

Water

1

12:32

Water

1

12:36

Water

1

12:55

Water

1

13:01

Water

1

13:08

Water

1

13:08

Water

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13:08

Water

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13:08

Water

1

Ambient 1

Ambient 2

Ambient 3

Ambient 4

Ambient 5

Ambient 6

Ambient 7

Ambient 8

Ambient 9

Ambient 10

Ambient 11

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Ambient 236



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☒ Tampa: 9610 Princess Palm Ave., FL 33619 • 813.630.9616 • Lab ID: E84589

Page 2 of 2

Client Name: <u>Flatwoods Consulting</u>		Project Name: <u>Hudson Pollution</u>		BOTTLE SIZE & TYPE																							
Address: <u>8306 Laurel Fair Circle</u>		Project Number:																									
Site: <u>120 Tampa FL 33610</u>		PO Number:																									
Phone: <u>610-739-4444</u>		FDEP Facility No:																									
FAX:		FDEP Facility Addr:																									
Contact: <u>Michael Grylls</u>		Special Instructions:																									
Sampled By: <u>Standard</u>		Rush																									
AEL Profile #:		ADAPT		EQUIS		Other																					
SAMPLE ID		SAMPLE DESCRIPTION		Grab Comp		DATE		SAMPLING TIME		MATRIX		NO. COUNT		ANALYSIS REQUIRED		LABORATORY I.D. NUMBER											
7				Grab		11/6/23		13:14		MW		1		Enterococci		611											
5								13:33								012											
6								13:41								013											
Ambient								13:48								014											
Ambient								13:54								015											
16								14:11								016											
11								14:20								017											
12								14:40		SW						018											
13								14:26		SW						019											
Matrix Code: WW=wastewater, SW=surface water, GW=ground water, DW=drinking water, MW=marine water, O=oil, A=air, SO=soil, SL=sludge																Preservation Code: I=ice, H=HCl, S=H2SO4, N=HNO3, T=Sodium Thiosulfate, AA=Ascorbic/HCl, AB=Ascorbic/NaOH											
Received on ice ☒ Yes ☐ No ☐ Temp taken from sample ☐ Temp from blank ☒ Where required, pH checked																Temp. when received (observed) <u>12</u> °C Temp. when received (corrected) _____ °C											
DCN: AD-D051web Form last revised 07/26/2022																Device used for measuring Temp by unique identifier (circle IR temp gun used) J: 9A G: LT-1 LT-2 T: 10A A: 3A M: 3A S: 1V F: 1A											
Relinquished by: <u>[Signature]</u> Date: <u>11/6/23</u> Time: <u>15:52</u>																Received by: <u>[Signature]</u> Date: <u>11/6/23</u> Time: <u>15:52</u>											
1																FOR DRINKING WATER USE: (When PWS information not otherwise supplied) PWS ID: _____ Contact Person: _____ Supplier of Water: _____ Site Address: _____											
2																											
3																											
4																											



805 Pinnacle Drive, Suite M, Linthicum Heights, MD 21090

Fecal Host Quantification ID Test Results Report

Detection and quantification of the fecal host associated gene biomarker by quantitative Polymerase Chain Reaction (qPCR) DNA analytical technology

ND: Not Detected DNQ: Detected, Not Quantifiable

ROQ: Detected, Quantifiable

Submitter: Flatwoods Consulting Group
Report Generated: November 14, 2023

[illegible]

Reported Results Authorized By: Kaveh Emdad, Lab Services Scientist

Results reported herein apply only to the sample matrices as received.

Results reported herein relate to the genetic material extracted from the sample matrix processed and included in the analysis.

Revision 2.2

Effective Date: 11/11/2021



805 Pinnacle Drive, Suite M, Linthicum Heights, MD 21090

Fecal Host Quantification ID Test Results Report

Sample Processing and Analysis Information

Submitter: Flatwoods Consulting Group
Report Generated: November 14, 2023

[illegible]Reported Results Authorized By: Kaveh Emdad, Lab Services Scientist

Results reported herein apply only to the sample matrices as received.

Results reported herein relate to the genetic material extracted from the sample matrix processed and included in the analysis.

Revision 2.2
Effective Date: 11/11/2021



qPCR Analysis QAQC information

Submitter: Flatwoods Consulting Group
Report Generated: November 14, 2023

[illegible]

Reported Results Authorized By: Kaveh Emdad, Lab Services Scientist

Results reported herein apply only to the sample matrices as received.

Results reported herein relate to the genetic material extracted from the sample matrix processed and included in the analysis.

Revision 2.2
Effective Date: 11/11/2021

Laboratory Comments

Submitter:
Report Generated:

Non-Detect (ND) Results

In sample(s) classified as non-detect, the host-associated fecal gene biomarker(s) was either not detected in test replicates, one replicate was detected at a cycle threshold greater than 35 and the other was not, or one replicate was detected at a cycle threshold less than 35 and the other was not after repeated analysis.

Detected Not Quantified (DNQ) Results

In sample(s) classified as Detected Not Quantified (DNQ), the host-associated fecal biomarker was detected in both test replicates but in quantities below the limit of quantification (LOQ, see below). This result indicates that fecal indicators associated with the respective host was present in the sample(s) but in low concentrations, and the confidence of such quantification will be lower than that declared by the definition of LOQ.

Quantifiable Results (ROQ)

Sample results are within the range of quantification of calibration curves (standard curves) of a validation qPCR method. For most qPCR assays, the range is 1E1 to 1E5 copies/reaction. Copy number measurements reported are relative, not absolute, quantification.

LOD (Limit of Detection, lower)

A general consensus was reached around the definition of the LOD as the lowest amount of analyte, which can be detected with more than a stated percentage of confidence (95%), but, not necessarily quantified as an exact value. It must be noted that LOD is not a limiting value and therefore, that Ct values below the LOD cannot automatically be considered as negative. From the definition of LOD, it is evident that values below LOD are absolutely valid in terms of microorganism presence. However, the probability of their repeated detection is lower than 95%.

LOQ (Limit of Quantification, lower)

The LOQ was defined as the smallest amount of analyte, which can be measured and quantified with defined precision and accuracy under the experimental conditions by the method under validation. Numerically, the LOQ is defined as the lowest concentration of analyte, which gives a predefined variability (coefficient of variation, CV) of under 25%.

Inhibition check

A 1:10 dilution of the original sample is analyzed together each time with the undiluted sample to evaluate the effect of PCR inhibition. If the sample is inhibited, where 1:10 dilution produces a high signal than undiluted sample, the 1:10 dilution results will be used for quantification. The use of 1:10 dilution sample results will be reflected in Analytical Volume(ul). For example, if the analytical volume for undiluted sample is 2ul, the analytical volume for 1:10 dilution will be 0.2ul.

Result Interpretations

The presence of the biomarker does not signify the presence or absence of that form of fecal pollution conclusively. The most reliable way to accurately test for contamination is to combine genetic testing with scientifically sound and adequate study design appropriate for the environmental quality questions to be answered or issues to be resolved.

Additional Testing

A portion of all samples has been frozen and will be archived for 3 months. The client is encouraged to perform additional tests on the sample(s) for other hosts suspected of contributing to the fecal contamination.

Qualification Assay Results (Detected/Non-Detected only)

Such results are only reported as Detected or Non-Detected without quantification. Non-Detected results are defined as stated above, and Detected results are defined as detected Ct in both replicate qPCR reactions.

Limitation of Damages – Repayment of Service Price

It is agreed that in the event of breach of any warranty or breach of contract, or negligence of LuminUltra Technologies Inc, as well as its agents or representatives, the liability of the company shall be limited to the repayment, to the purchaser (submitter), of the individual analysis price paid by him/her to LuminUltra Technologies Inc. The company shall not be liable for any damages, either direct or consequential. LuminUltra Technologies Inc provides analytical services on a PRIME CONTRACT BASIS ONLY. Terms are available upon request. The sample(s) cited in this report may be used for research purposes after an archiving period of 3 months from the date of this report. Research includes, but is not limited to internal validation studies and peer-reviewed research publications. Anonymity of the sample(s), including the exact geographic location will be maintained by assigning an arbitrary internal reference. These anonymous samples will only be grouped by state / province of origin for research purposes. The client must contact LuminUltra Technologies Inc in writing within 10 days from the date of this report if he/she does not wish for their submitted sample(s) to be used for any type of future research.

DNA Analytical Method Explanation

Water Samples: Each submitted water sample is filtered through 0.45 micron membrane filter(s). Each filter is placed in a separate, sterile 5ml disposable tube containing a unique mix of beads and lysis buffer. The sample is homogenized for and the DNA extracted per kit manufacturer's protocol. Deviations to these procedures may occur at the client's request.

Non-Water Samples: Each non-water sample submitted by the client is processed as per internal laboratory extraction procedures. An extracted DNA sample is proceed directly to PCR analysis. Details available upon request.

Amplifications to detect the target gene biomarker were run in a final reaction volume of 20ul sample extract, forward primer, reverse primer, probe and an optimized buffer. All assays are run in duplicate. Quantification is achieved by extrapolating target gene copy numbers from a standard curve generated from serial dilutions of known gene copy numbers.

For quality control purposes, a positive control and a negative control, were run alongside the sample(s) to ensure a properly functioning reaction and reveal any false negatives or false positives



805 Pinnacle Drive, Suite M, Linthicum Heights, MD 21090

Fecal Host Quantification ID Test Results Report

Detection and quantification of the fecal host associated gene biomarker by quantitative Polymerase Chain Reaction (qPCR) DNA analytical technology

ND: Not Detected; DNQ: Detected, Not Quantifiable

ROQ: Detected, Quantifiable

Submitter: Flatwoods Consulting Group

Report Generated: November 22, 2023

[illegible]

Reported Results Authorized By: Kaveh Emdad, Lab Services Scientist

Results reported herein apply only to the sample matrices as received.

Results reported herein relate to the genetic material extracted from the sample matrix processed and included in the analysis.

Revision 2.2

Effective Date: 11/11/2021



805 Pinnacle Drive, Suite M, Linthicum Heights, MD 21090

Fecal Host Quantification ID Test Results Report

Sample Processing and Analysis Information

Submitter: Flatwoods Consulting Group

Report Generated: November 22, 2023

[illegible]

Reported Results Authorized By: Kaveh Emdad, Lab Services Scientist

Results reported herein apply only to the sample matrices as received.

Results reported herein relate to the genetic material extracted from the sample matrix processed and included in the analysis.

Revision 2.2

Effective Date: 11/11/2021



qPCR Analysis QAQC information

Submitter: Flatwoods Consulting Group
Report Generated: November 22, 2023

[illegible]

Reported Results Authorized By: Kaveh Emdad, Lab Services Scientist

Results reported herein apply only to the sample matrices as received.

Results reported herein relate to the genetic material extracted from the sample matrix processed and included in the analysis.

Revision 2.2
Effective Date: 11/11/2021

Laboratory Comments

Submitter: Flatwoods Consulting Group
Report Generated: November 22, 2023

Non-Detect (ND) Results

In sample(s) classified as non-detect, the host-associated fecal gene biomarker(s) was either not detected in test replicates, one replicate was detected at a cycle threshold greater than 35 and the other was not, or one replicate was detected at a cycle threshold less than 35 and the other was not after repeated analysis.

Detected Not Quantified (DNQ) Results

In sample(s) classified as Detected Not Quantified (DNQ), the host-associated fecal biomarker was detected in both test replicates but in quantities below the limit of quantification (LOQ, see below). This result indicates that fecal indicators associated with the respective host was present in the sample(s) but in low concentrations, and the confidence of such quantification will be lower than that declared by the definition of LOQ.

Quantifiable Results (ROQ)

Sample results are within the range of quantification of calibration curves (standard curves) of a validation qPCR method. For most qPCR assays, the range is 1E1 to 1E5 copies/reaction. Copy number measurements reported are relative, not absolute, quantification.

LOD (Limit of Detection, lower)

A general consensus was reached around the definition of the LOD as the lowest amount of analyte, which can be detected with more than a stated percentage of confidence (95%), but, not necessarily quantified as an exact value. It must be noted that LOD is not a limiting value and therefore, that Ct values below the LOD cannot automatically be considered as negative. From the definition of LOD, it is evident that values below LOD are absolutely valid in terms of microorganism presence. However, the probability of their repeated detection is lower than 95%.

LOQ (Limit of Quantification, lower)

The LOQ was defined as the smallest amount of analyte, which can be measured and quantified with defined precision and accuracy under the experimental conditions by the method under validation. Numerically, the LOQ is defined as the lowest concentration of analyte, which gives a predefined variability (coefficient of variation, CV) of under 25%.

Inhibition check

A 1:10 dilution of the original sample is analyzed together each time with the undiluted sample to evaluate the effect of PCR inhibition. If the sample is inhibited, where 1:10 dilution produces a high signal than undiluted sample, the 1:10 dilution results will be used for quantification. The use of 1:10 dilution sample results will be reflected in Analytical Volume(ul). For example, if the analytical volume for undiluted sample is 2ul, the analytical volume for 1:10 dilution will be 0.2ul.

Fecal Reference Samples

The client is encouraged to submit fecal samples from suspected sources in the surrounding area in order to gain a better understanding of the concentration of the host-associated biomarker with the regional population. A more precise interpretation would be available to the client with the submittal of such baseline samples.

Result Interpretations

The presence of the biomarker does not signify the presence or absence of that form of fecal pollution conclusively. The most reliable way to accurately test for contamination is to combine genetic testing with scientifically sound and adequate study design appropriate for the environmental quality questions to be answered or issues to be resolved.

Additional Testing

A portion of all samples has been frozen and will be archived for 3 months. The client is encouraged to perform additional tests on the sample(s) for other hosts suspected of contributing to the fecal contamination.

Qualification Assay Results (Detected/Non-Detected only)

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DNA Analytical Method Explanation

Water Samples: Each submitted water sample is filtered through 0.45 micron membrane filter(s). Each filter is placed in a separate, sterile 2ml disposable tube containing a unique mix of beads and lysis buffer. The sample is homogenized for and the DNA extracted per kit manufacturer's protocol. Deviations to these procedures may occur at the client's request.

Non-Water Samples: Each non-water sample submitted by the client is processed as per internal laboratory extraction procedures. An extracted DNA sample is proceed directly to PCR analysis. Details available upon request.

Amplifications to detect the target gene biomarker were run in a final reaction volume of 20ul sample extract, forward primer, reverse primer, probe and an optimized buffer. All assays are run in duplicate. Quantification is achieved by extrapolating target gene copy numbers from a standard curve generated from serial dilutions of known gene copy numbers.

For quality control purposes, a positive control and a negative control, were run alongside the sample(s) to ensure a properly functioning reaction and reveal any false negatives or false positives