

Biological Analysis Report

CEN-DIST-2022-06-16-01

Florida Department of Environmental Protection
Central Laboratory
2600 Blair Stone Road
Tallahassee, FL 32399-2400
DOH Accreditation E31780

Event Description: **Soldier Creek**
Request ID: **RQ-2022-06-13-53**
Customer: **CEN-DIST**
Project ID: **SMP**

Send Reports to:
FL Dept. of Environmental Protection
3319 Maguire Blvd., Suite 232
Orlando, FL 32803-3767
Attn: Matt Bearden

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Certified by: Sarah Menz, Environmental Administrator

Date Certified: 28-JUL-2022 15:04

A handwritten signature in dark ink, appearing to be 'Sarah Menz', written in a cursive style.

Case Narrative

Unless otherwise noted, all samples included in this report were received in accordance with protocols referenced in Chapter 62-160, Florida Administrative Code (F.A.C.). Results published in this report pertain only to the samples as submitted to, and received by the laboratory. All times in this report are adjusted to the applicable Eastern Time Zone (EST or EDT).

Results for the following analytical groups are included in this report: Microbiology and Molecular.

Scientific notation may be used in reporting very large or small values. Values reported using scientific notation will take the form of the following example: 1.3E+03, which is equivalent to 1.3×10^3 or 1300.

Unless otherwise noted, analytical values for soil and sediment samples are reported on a dry weight basis, and analytical values for waste and tissue samples are reported on a wet weight basis.

Results for TNI accredited tests met requirements established by The NELAC Institute. A double asterisk (**) is used to indicate an analyte/matrix/method for which the laboratory is not TNI accredited by the Florida Department of Health Environmental Laboratory Certification Program or where accreditation for that field of testing is not applicable.

Any significant anomalies or deviations from established protocols are documented in Non-Conformance Reports, which, where appropriate, are included within this analytical report. Additional comments related to specific analytical tests may be included as remarks following the analytical results for each sample. Such comments and remarks are for informational purposes only and are not intended to convey judgement about the usability of the reported data.

A quality control report on the performance of the test method for the submitted samples is included. Uncertainty associated with the analytical results contained in this report can be estimated from the reported quality assurance results and from published quality control acceptance limits for each analytical test. Matrix quality control results (matrix spike recoveries and matrix sample precision) pertain only to the matrix sample tested and do not necessarily reflect test method performance for other samples.

Typical matrix quality control (QC) measurements may include matrix spike recovery, matrix spike duplicate recovery, matrix spike precision and matrix sample precision. Not all matrix QC results may be available or reportable; where they are not an explanation is provided. Typical reasons for unavailable QC results include, but are not limited to, a) insufficient matrix sample to perform some or all QC measurements; b) analyte concentration in the sample replicated was too low for a meaningful measurement of precision and c) analyte concentration in the matrix sample spiked was too high (relative to the amount of analyte spiked) for a meaningful measurement of recovery. Where matrix QC results are unavailable, other method performance metrics (e.g., LCS recovery, LCS precision, surrogate recovery) may be used to assess performance of the method. Comments explaining any missing QC measurements are not intended to convey any adverse conclusions about the quality of the reported data.

Precision is reported as relative percent difference unless otherwise noted.

Quality Control codes as defined below may be used in this report to indicate results that are associated with one or more quality control elements which did not fall within established test method criteria. Such results may be qualified as estimates using a J qualifier as required by 62-160 F.A.C. Explanations are included in the report for any results that were reported as estimates for other reasons.

QC Codes used in this report may include:

- LCS – Recovery for the batch Laboratory Control Sample (LCS) was outside existing control limits;
- MS – Recovery for the batch matrix spike (MS) was outside existing control limits;
- CCV – Recovery for a continuing calibration verification (CCV) standard was outside existing control limits;
- SUR – Recovery of a surrogate (SUR) for associated analytes was outside existing control limits;
- RPD – The precision, measured as relative percent difference (RPD), of batch replicate measurements was outside existing control limits;
- RSD – The precision, measured as relative standard deviation (RSD), of batch replicate measurements was outside existing control limits;
- SMP – Sample - used precision derived from replicate analyses of a sample;

The following data qualifiers are used, where applicable, in this report as specified in 62-160 F.A.C.

- A - Value reported is the mean of two or more determinations.
- B - Results based on colony counts outside the acceptable range.
- I - The reported value is between the laboratory method detection limit and the laboratory practical quantitation limit.
- J - Estimated value and/or the analysis did not meet established quality control criteria.
- K - Actual value is known to be less than value given.
- L - Actual value is known to be greater than value given.
- N - Presumptive evidence of presence of material.
- O - Sampled, but analysis lost or not performed.
- Q - Sample held beyond normal holding time.
- T - Value reported is less than the criterion of detection.
- U - Material was analyzed for but not detected. The reported value is the method detection limit for the sample analyzed.
- V - Analyte was detected in both sample and method blank.
- X - Too few individuals to calculate SCI value.
- Y - The laboratory analysis was from an unpreserved or improperly preserved sample. The data may not be accurate.
- Z - Colonies were too numerous to count (TNTC).

Quality control information from overflow laboratories may not be included in this report. Please refer to the associated report from the overflow laboratory for additional information.

Sample Location: Soldier Creek @ 150m Upstream of SR Hwy 419 Collection Date/Time: 06/15/2022 10:35

Field ID: G2CE0071

Matrix: W-SURF-FRH

Sample ID	Ref. Method	Component	Result	Code	Units	Batch ID	QC Codes
2335125	SM 9223 B Quanti-Tray	Escherichia Coli-Quanti-Tray	1050.0	Q	MPN/100 mL	P415422	
2335126	SOP-PCR-1.0	HF183-qPCR**	1.7E+03	U	TSC/100 mL	P415219	
	SOP-PCR-1.2	GULL2-qPCR**	1.8E+03	U	TSC/100 mL	P415219	
	SOP-PCR-1.3	GFD-qPCR**	360	U	TSC/100 mL	P415219	
	SOP-PCR-1.4	BacR-qPCR**	1.5E+03	U	TSC/100 mL	P415219	
	SOP-PCR-1.5	DG3-qPCR**	1.3E+03	U	TSC/100 mL	P415219	
2335347	Enterolert/QT	Enterococci-Quanti-Tray	1735.5	AQ	MPN/100 mL	P415422	

Quality Assurance Report Method Blank Results

Reference Method: Enterolert/QT
Batch ID: P415422

Component	Result	Code	Units
Enterococci-Quanti-Tray	1.0	U	MPN/100 mL

Reference Method: SM 9223 B Quanti-Tray
Batch ID: P415422

Component	Result	Code	Units
Escherichia Coli-Quanti-Tray	1.0	U	MPN/100 mL

Reference Method: SOP-PCR-1.0
Batch ID: P415219

Component	Result	Code	Units
HF183-qPCR	3.3E+03	U	TSC/100 mL

Reference Method: SOP-PCR-1.2
Batch ID: P415219

Component	Result	Code	Units
GULL2-qPCR	3.6E+03	U	TSC/100 mL

Reference Method: SOP-PCR-1.3
Batch ID: P415219

Component	Result	Code	Units
GFD-qPCR	720	U	TSC/100 mL

Reference Method: SOP-PCR-1.4
Batch ID: P415219

Component	Result	Code	Units
BacR-qPCR	3.0E+03	U	TSC/100 mL

Reference Method: SOP-PCR-1.5
Batch ID: P415219

Component	Result	Code	Units
DG3-qPCR	2.6E+03	U	TSC/100 mL

Quality Assurance Report Laboratory Control Sample Accuracy

Reference Method: SOP-PCR-1.0
Batch ID: P415219

Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
HF183-qPCR	77.9	74.9	P/P	50 - 175

Reference Method: SOP-PCR-1.2
Batch ID: P415219

Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
GULL2-qPCR	104	73.4	P/P	50 - 300

Reference Method: SOP-PCR-1.3
Batch ID: P415219

Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
GFD-qPCR	93.6	71.4	P/P	50 - 175

Reference Method: SOP-PCR-1.4
Batch ID: P415219

Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
BacR-qPCR	98.4	76.4	P/P	50 - 175

Reference Method: SOP-PCR-1.5
Batch ID: P415219

Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
DG3-qPCR	74.9	77.0	P/P	50 - 175

Quality Assurance Report Matrix Spike Accuracy

Reference Method: SOP-PCR-1.0
Batch ID: P415219

Spiked Sample	Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
2334570	HF183-qPCR	87.6	90.3	P/P	50 - 175
2334977	HF183-qPCR	92.3		P	50 - 175
2334978	HF183-qPCR	96.1		P	50 - 175
2335095	HF183-qPCR	92.1		P	50 - 175
2335126	HF183-qPCR	87.5		P	50 - 175
2335192	HF183-qPCR	81.8		P	50 - 175
2335340	HF183-qPCR	86.3		P	50 - 175
2335422	HF183-qPCR	83.6		P	50 - 175
2335808	HF183-qPCR	83.5		P	50 - 175
2335825	HF183-qPCR	81.3		P	50 - 175
2335837	HF183-qPCR	86.6		P	50 - 175
2336006	HF183-qPCR	89.9		P	50 - 175
2336214	HF183-qPCR	83.5		P	50 - 175

Reference Method: SOP-PCR-1.2
Batch ID: P415219

Spiked Sample	Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
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Quality Assurance Report Matrix Spike Accuracy

Reference Method: SOP-PCR-1.2

Batch ID: P415219

Spiked Sample	Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
2334570	GULL2-qPCR	112	105	P/P	50 - 300
2335095	GULL2-qPCR	51.8		P	50 - 300
2335126	GULL2-qPCR	86.2		P	50 - 300
2335340	GULL2-qPCR	78.6		P	50 - 300
2335422	GULL2-qPCR	87.2		P	50 - 300
2335808	GULL2-qPCR	82.8		P	50 - 300
2335825	GULL2-qPCR	84.0		P	50 - 300
2335837	GULL2-qPCR	69.6		P	50 - 300
2336006	GULL2-qPCR	84.6		P	50 - 300
2336214	GULL2-qPCR	93.2		P	50 - 300

Reference Method: SOP-PCR-1.3

Batch ID: P415219

Spiked Sample	Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
2334570	GFD-qPCR	93.4	93.6	P/P	50 - 175
2335095	GFD-qPCR	78.8		P	50 - 175
2335126	GFD-qPCR	96.4		P	50 - 175
2335340	GFD-qPCR	85.9		P	50 - 175
2335422	GFD-qPCR	88.7		P	50 - 175
2335808	GFD-qPCR	91.5		P	50 - 175
2335825	GFD-qPCR	87.8		P	50 - 175
2335837	GFD-qPCR	92.4		P	50 - 175
2336006	GFD-qPCR	96.6		P	50 - 175
2336214	GFD-qPCR	87.1		P	50 - 175

Reference Method: SOP-PCR-1.4

Batch ID: P415219

Spiked Sample	Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
2334570	BacR-qPCR	88.4	89.9	P/P	50 - 175
2334977	BacR-qPCR	80.2		P	50 - 175
2334978	BacR-qPCR	95.3		P	50 - 175
2335095	BacR-qPCR	53.5		P	50 - 175
2335126	BacR-qPCR	73.5		P	50 - 175
2335340	BacR-qPCR	73.2		P	50 - 175
2335422	BacR-qPCR	71.8		P	50 - 175
2335808	BacR-qPCR	65.5		P	50 - 175
2335825	BacR-qPCR	76.0		P	50 - 175
2335837	BacR-qPCR	72.4		P	50 - 175
2336006	BacR-qPCR	87.5		P	50 - 175
2336214	BacR-qPCR	102		P	50 - 175

Reference Method: SOP-PCR-1.5

Batch ID: P415219

Spiked Sample	Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
2334570	DG3-qPCR	93.4	83.9	P/P	50 - 175
2334977	DG3-qPCR	89.3		P	50 - 175
2334978	DG3-qPCR	82.6		P	50 - 175
2335095	DG3-qPCR	71.9		P	50 - 175
2335126	DG3-qPCR	86.1		P	50 - 175

Quality Assurance Report Matrix Spike Accuracy

Reference Method: SOP-PCR-1.5
Batch ID: P415219

Spiked Sample	Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
2335340	DG3-qPCR	79.1		P	50 - 175
2335422	DG3-qPCR	82.8		P	50 - 175
2335808	DG3-qPCR	86.0		P	50 - 175
2335825	DG3-qPCR	81.4		P	50 - 175
2335837	DG3-qPCR	84.7		P	50 - 175
2336006	DG3-qPCR	85.3		P	50 - 175
2336214	DG3-qPCR	85.3		P	50 - 175

Quality Assurance Report Precision

Reference Method: Enterolert/QT
Batch ID: P415422

Replicated Lab Sample	Component	% RSD/RPD	Sample/Spike/LCS*	Pass/Fail	Control Limits
2335347	Enterococci-Quanti-Tray	21.0	Sample	P	0 - 80

Reference Method: SM 9223 B Quanti-Tray
Batch ID: P415422

Replicated Lab Sample	Component	% RSD/RPD	Sample/Spike/LCS*	Pass/Fail	Control Limits
2335089	Escherichia Coli-Quanti-Tray	39.7	Sample	P	0 - 80
2335243	Escherichia Coli-Quanti-Tray	19.1	Sample	P	0 - 80

Reference Method: SOP-PCR-1.0
Batch ID: P415219

Replicated Lab Sample	Component	% RSD/RPD	Sample/Spike/LCS*	Pass/Fail	Control Limits
2334570	HF183-qPCR	3.02	Spike	P	0 - 25

Reference Method: SOP-PCR-1.2
Batch ID: P415219

Replicated Lab Sample	Component	% RSD/RPD	Sample/Spike/LCS*	Pass/Fail	Control Limits
2334570	GULL2-qPCR	6.34	Spike	P	0 - 25

Reference Method: SOP-PCR-1.3
Batch ID: P415219

Replicated Lab Sample	Component	% RSD/RPD	Sample/Spike/LCS*	Pass/Fail	Control Limits
2334570	GFD-qPCR	0.171	Spike	P	0 - 25

Quality Assurance Report Precision

Reference Method: SOP-PCR-1.4
 Batch ID: P415219

Replicated Lab Sample	Component	% RSD/RPD	Sample/Spike/LCS*	Pass/Fail	Control Limits
2334570	BacR-qPCR	1.67	Spike	P	0 - 25

Reference Method: SOP-PCR-1.5
 Batch ID: P415219

Replicated Lab Sample	Component	% RSD/RPD	Sample/Spike/LCS*	Pass/Fail	Control Limits
2334570	DG3-qPCR	10.7	Spike	P	0 - 25

* Sample, spike and/or laboratory control sample precision (LCS) is reported.

Quality Assurance Report Surrogates

Lab Sample ID: 2335126
 Field Sample ID: G2CE0071

Reference Method	Surrogate	% Rec.	Pass/Fail	Control Limits
SOP-PCR-1.0	SKETA-qPCR	85.7	P	50 - 300
SOP-PCR-1.2	SKETA-qPCR	118	P	50 - 300
SOP-PCR-1.3	SKETA-qPCR	85.3	P	50 - 300
SOP-PCR-1.4	SKETA-qPCR	93.7	P	50 - 300
SOP-PCR-1.5	SKETA-qPCR	96.6	P	50 - 300

Quality Assurance Report Summary

Ref. Method	Analyte	LCS % Recovery		MS % Recovery			LCS	Precision SMP		MS
Enterolert/QT	Enterococci-Quanti-Tray							21.0		
SM 9223 B Quanti-Tray	Escherichia Coli-Quanti-Tray							39.7	19.1	
SOP-PCR-1.0	HF183-qPCR	74.9	77.9	87.6	90.3	92.3				3.02
				96.1						
SOP-PCR-1.2	GULL2-qPCR	73.4	104	112	105	51.8				6.34
				86.2						
SOP-PCR-1.3	GFD-qPCR	71.4	93.6	93.4	93.6	78.8				0.171
				96.4						
SOP-PCR-1.4	BacR-qPCR	76.4	98.4	65.5	76.0	72.4				1.67
				87.5						
SOP-PCR-1.5	DG3-qPCR	77.0	74.9	93.4	83.9	89.3				10.7
				82.6						

Reference Method Descriptions

Method	Description	Associated Samples
Enterolert/QT	Enterococci by Enterolert Quanti-Tray method - non-chlorinated water systems	2335347
SM 9223 B Quanti-Tray	Escherichia coli by Colilert 18 Quanti-Tray method - non-chlorinated water systems	2335125
SOP-PCR-1.0	Detection and quantification of human-specific HF183 Bacteroides genetic marker with quantitative polymerase chain reaction	2335126

Reference Method Descriptions

Method	Description	<u>Associated Samples</u>
SOP-PCR-1.2	Detection and quantification of coastal bird specific <i>Catelliboccus marimammalium</i> Gull2 genetic marker with quantitative polymerase chain reaction	2335126
SOP-PCR-1.3	Detection and quantification of bird specific <i>Helicobacter</i> GFD genetic marker with quantitative polymerase chain reaction	2335126
SOP-PCR-1.4	Detection and quantification of Ruminant specific <i>Bacteroidetes</i> BacR genetic marker with quantitative polymerase chain reaction	2335126
SOP-PCR-1.5	Detection and quantification of canine-specific DG3 <i>Bacteroides</i> genetic marker with quantitative polymerase chain reaction	2335126

Preparation and Analysis Log

Ref. Method	Received Date	Prep Date/Time	Prepared By	Analysis Date/Time	Analyzed By	Associated Samples
Enterolert/QT	06/16/2022	06/16/2022 15:04	Jillian G Hanley	06/17/2022 15:19	Tynan A Branch	2335347
SM 9223 B Quanti-Tray	06/16/2022	06/16/2022 16:23	Jillian G Hanley	06/17/2022 11:49	Jillian G Hanley	2335125
SOP-PCR-1.0	06/16/2022	06/16/2022 13:05	Claudia Castro	07/19/2022 09:43	DeAsia Armster	2335126
SOP-PCR-1.2	06/16/2022	06/16/2022 13:05	Claudia Castro	07/22/2022 14:58	DeAsia Armster	2335126
SOP-PCR-1.3	06/16/2022	06/16/2022 13:05	Claudia Castro	07/25/2022 10:07	DeAsia Armster	2335126
SOP-PCR-1.4	06/16/2022	06/16/2022 13:05	Claudia Castro	07/21/2022 08:23	DeAsia Armster	2335126
SOP-PCR-1.5	06/16/2022	06/16/2022 13:05	Claudia Castro	07/21/2022 14:06	DeAsia Armster	2335126