

Biological Analysis Report

SE-DIST-2023-04-25-02

Florida DEP Laboratory
2600 Blair Stone Road
Tallahassee, FL 32399-2400
DOH Accreditation E31780

Event Description: **Highlands Run**
Request ID: **RQ-2023-04-10-29**
Customer: **SE-DIST**
Project ID: **SMP**

Send Reports to:
FL Dept. of Environmental Protection
FL Dept. of Environmental Protection
2199 South Rock Road
Fort Pierce, FL 34945
Attn: Jordan Skaggs

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Certified by: Sarah Menz, Environmental Administrator

Date Certified: 07-JUN-2023 10:47

A handwritten signature in dark ink, appearing to be 'Sarah Menz', with a long, sweeping horizontal stroke extending to the right.

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: KISSIMMEE TRIBUTARY AT US 98 EAST OF BUCKHORN

Collection Date/Time: 04/24/2023 10:45

Field ID: G4SE0155

Matrix: W-SURF-FRH

Sample ID	Ref. Method	Component	Result	Code	Units	Batch ID	QC Codes
2402671	SM 9223 B Quanti-Tray	Escherichia Coli-Quanti-Tray	133.4	Q	MPN/100 mL	P429056	
2402672	SOP-PCR-1.0	HF183-qPCR**	2.2E+03	U	TSC/100 mL	P428772	
	SOP-PCR-1.2	GULL2-qPCR**	2.4E+03	U	TSC/100 mL	P428772	
	SOP-PCR-1.3	GFD-qPCR**	480	U	TSC/100 mL	P428772	
	SOP-PCR-1.4	BacR-qPCR**	2.0E+03	U	TSC/100 mL	P428772	
	SOP-PCR-1.5	DG3-qPCR**	1.7E+03	U	TSC/100 mL	P428772	

Quality Assurance Report
Method Blank Results

Reference Method: SM 9223 B Quanti-Tray
Batch ID: P429056

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

Reference Method: SOP-PCR-1.0
Batch ID: P428772

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

Reference Method: SOP-PCR-1.2
Batch ID: P428772

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

Reference Method: SOP-PCR-1.3
Batch ID: P428772

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

Reference Method: SOP-PCR-1.4
Batch ID: P428772

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

Reference Method: SOP-PCR-1.5
Batch ID: P428772

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

Quality Assurance Report
Laboratory Control Sample Accuracy

Quality Assurance Report Laboratory Control Sample Accuracy

Reference Method: SOP-PCR-1.0
Batch ID: P428772

Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
HF183-qPCR	89.9	98.5	P/P	50 - 175

Reference Method: SOP-PCR-1.2
Batch ID: P428772

Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
GULL2-qPCR	106	92.7	P/P	50 - 300

Reference Method: SOP-PCR-1.3
Batch ID: P428772

Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
GFD-qPCR	96.2	93.4	P/P	50 - 175

Reference Method: SOP-PCR-1.4
Batch ID: P428772

Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
BacR-qPCR	95.0	114	P/P	50 - 175

Reference Method: SOP-PCR-1.5
Batch ID: P428772

Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
DG3-qPCR	101	107	P/P	50 - 175

Quality Assurance Report Matrix Spike Accuracy

Reference Method: SOP-PCR-1.0
Batch ID: P428772

Spiked Sample	Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
2401737	HF183-qPCR	88.8	100	P/P	50 - 175
2401791	HF183-qPCR	97.9		P	50 - 175
2401809	HF183-qPCR	82.1		P	50 - 175
2401824	HF183-qPCR	79.9		P	50 - 175
2401942	HF183-qPCR	84.3		P	50 - 175
2401943	HF183-qPCR	92.7		P	50 - 175
2402492	HF183-qPCR	78.9		P	50 - 175
2402493	HF183-qPCR	81.1		P	50 - 175
2402566	HF183-qPCR	81.6		P	50 - 175
2402655	HF183-qPCR	119		P	50 - 175
2402672	HF183-qPCR	78.3		P	50 - 175
2403004	HF183-qPCR	79.7		P	50 - 175
2403005	HF183-qPCR	74.2		P	50 - 175

Reference Method: SOP-PCR-1.2
Batch ID: P428772

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: P428772

Spiked Sample	Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
2401737	GULL2-qPCR	71.6	75.6	P/P	50 - 300
2401791	GULL2-qPCR	66.4		P	50 - 300
2401809	GULL2-qPCR	81.2		P	50 - 300
2401824	GULL2-qPCR	52.5		P	50 - 300
2401942	GULL2-qPCR	83.6		P	50 - 300
2401943	GULL2-qPCR	55.3		P	50 - 300
2402566	GULL2-qPCR	69.1		P	50 - 300
2402672	GULL2-qPCR	85.0		P	50 - 300
2403004	GULL2-qPCR	71.9		P	50 - 300
2403005	GULL2-qPCR	75.2		P	50 - 300

Reference Method: SOP-PCR-1.3
Batch ID: P428772

Spiked Sample	Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
2401737	GFD-qPCR	98.5	102	P/P	50 - 175
2401791	GFD-qPCR	114		P	50 - 175
2401809	GFD-qPCR	111		P	50 - 175
2401824	GFD-qPCR	89.9		P	50 - 175
2401942	GFD-qPCR	94.9		P	50 - 175
2401943	GFD-qPCR	81.5		P	50 - 175
2402566	GFD-qPCR	101		P	50 - 175
2402672	GFD-qPCR	92.6		P	50 - 175
2403004	GFD-qPCR	85.9		P	50 - 175
2403005	GFD-qPCR	90.8		P	50 - 175

Reference Method: SOP-PCR-1.4
Batch ID: P428772

Spiked Sample	Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
2401737	BacR-qPCR	86.0	88.6	P/P	50 - 175
2401791	BacR-qPCR	91.0		P	50 - 175
2401809	BacR-qPCR	79.8		P	50 - 175
2401824	BacR-qPCR	81.8		P	50 - 175
2401942	BacR-qPCR	85.8		P	50 - 175
2401943	BacR-qPCR	70.7		P	50 - 175
2402492	BacR-qPCR	79.5		P	50 - 175
2402493	BacR-qPCR	90.6		P	50 - 175
2402566	BacR-qPCR	82.0		P	50 - 175
2402655	BacR-qPCR	58.7		P	50 - 175
2402672	BacR-qPCR	82.7		P	50 - 175
2403004	BacR-qPCR	74.9		P	50 - 175
2403005	BacR-qPCR	72.9		P	50 - 175

Reference Method: SOP-PCR-1.5
Batch ID: P428772

Spiked Sample	Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
2401737	DG3-qPCR	93.9	92.4	P/P	50 - 175
2401791	DG3-qPCR	93.1		P	50 - 175
2401809	DG3-qPCR	87.7		P	50 - 175
2401824	DG3-qPCR	86.7		P	50 - 175

Quality Assurance Report Matrix Spike Accuracy

Reference Method: SOP-PCR-1.5
 Batch ID: P428772

Spiked Sample	Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
2401942	DG3-qPCR	101		P	50 - 175
2401943	DG3-qPCR	77.3		P	50 - 175
2402492	DG3-qPCR	79.4		P	50 - 175
2402493	DG3-qPCR	83.0		P	50 - 175
2402566	DG3-qPCR	87.9		P	50 - 175
2402655	DG3-qPCR	77.1		P	50 - 175
2402672	DG3-qPCR	85.0		P	50 - 175
2403004	DG3-qPCR	89.5		P	50 - 175
2403005	DG3-qPCR	79.8		P	50 - 175

Quality Assurance Report Precision

Reference Method: SOP-PCR-1.0
 Batch ID: P428772

Replicated Lab Sample	Component	% RSD/RPD	Sample/Spike/LCS*	Pass/Fail	Control Limits
2401737	HF183-qPCR	12.3	Spike	P	0 - 25

Reference Method: SOP-PCR-1.2
 Batch ID: P428772

Replicated Lab Sample	Component	% RSD/RPD	Sample/Spike/LCS*	Pass/Fail	Control Limits
2401737	GULL2-qPCR	5.31	Spike	P	0 - 25

Reference Method: SOP-PCR-1.3
 Batch ID: P428772

Replicated Lab Sample	Component	% RSD/RPD	Sample/Spike/LCS*	Pass/Fail	Control Limits
2401737	GFD-qPCR	3.12	Spike	P	0 - 25

Reference Method: SOP-PCR-1.4
 Batch ID: P428772

Replicated Lab Sample	Component	% RSD/RPD	Sample/Spike/LCS*	Pass/Fail	Control Limits
2401737	BacR-qPCR	3.02	Spike	P	0 - 25

Reference Method: SOP-PCR-1.5
 Batch ID: P428772

Replicated Lab Sample	Component	% RSD/RPD	Sample/Spike/LCS*	Pass/Fail	Control Limits
2401737	DG3-qPCR	1.63	Spike	P	0 - 25

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

Quality Assurance Report Surrogates

Lab Sample ID: 2402672
 Field Sample ID: G4SE0155

Reference Method	Surrogate	% Rec.	Pass/Fail	Control Limits
SOP-PCR-1.0	SKETA-qPCR	114	P	50 - 300
SOP-PCR-1.2	SKETA-qPCR	96.2	P	50 - 300
SOP-PCR-1.3	SKETA-qPCR	100	P	50 - 300
SOP-PCR-1.4	SKETA-qPCR	111	P	50 - 300
SOP-PCR-1.5	SKETA-qPCR	117	P	50 - 300

Quality Assurance Report Summary

Ref. Method	Analyte	LCS % Recovery		MS % Recovery			LCS	Precision SMP	MS
SOP-PCR-1.0	HF183-qPCR	98.5	89.9	88.8	100	97.9			12.3
SOP-PCR-1.2	GULL2-qPCR	92.7	106	71.6	75.6	66.4			5.31
SOP-PCR-1.3	GFD-qPCR	93.4	96.2	98.5	102	114			3.12
SOP-PCR-1.4	BacR-qPCR	114	95.0	86.0	88.6	91.0			3.02
SOP-PCR-1.5	DG3-qPCR	107	101	85.0	89.5	79.8			1.63

Reference Method Descriptions

Method	Description	Associated Samples
SM 9223 B Quanti-Tray	Escherichia coli by Colilert 18 Quanti-Tray method - non-chlorinated water systems	2402671
SOP-PCR-1.0	Detection and quantification of human-specific HF183 Bacteroides genetic marker with quantitative polymerase chain reaction	2402672
SOP-PCR-1.2	Detection and quantification of coastal bird specific Catellicoccus marimammalium Gull2 genetic marker with quantitative polymerase chain reaction	2402672
SOP-PCR-1.3	Detection and quantification of bird specific Helicobacter GFD genetic marker with quantitative polymerase chain reaction	2402672
SOP-PCR-1.4	Detection and quantification of Ruminant specific Bacteroidetes BacR genetic marker with quantitative polymerase chain reaction	2402672
SOP-PCR-1.5	Detection and quantification of canine-specific DG3 Bacteroides genetic marker with quantitative polymerase chain reaction	2402672

Preparation and Analysis Log

Ref. Method	Received Date	Prep Date/Time	Prepared By	Analysis Date/Time	Analyzed By	Associated Samples
SM 9223 B Quanti-Tray	04/25/2023	04/25/2023 16:34	Aidan Cronin	04/26/2023 13:18	Aidan Cronin	2402671
SOP-PCR-1.0	04/25/2023	04/25/2023 14:10	Claudia Castro	05/11/2023 09:51	Roberta Tatti	2402672
SOP-PCR-1.2	04/25/2023	04/25/2023 14:10	Claudia Castro	05/16/2023 14:10	Jennifer Mihalic	2402672
SOP-PCR-1.3	04/25/2023	04/25/2023 14:10	Claudia Castro	05/17/2023 16:17	Jennifer Mihalic	2402672
SOP-PCR-1.4	04/25/2023	04/25/2023 14:10	Claudia Castro	05/12/2023 10:29	Roberta Tatti	2402672
SOP-PCR-1.5	04/25/2023	04/25/2023 14:10	Claudia Castro	05/12/2023 12:08	Roberta Tatti	2402672