

Biological Analysis Report

SO-DIST-2021-06-03-01

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

Overflow Analyses Performed By:
Sanders Laboratories
10090 Bavaria Road
Ft. Myers, FL 33913
DOH Accreditation E85457

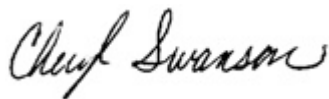
Event Description: **2021 SMP: Lee Coastal**
Request ID: **RQ-2021-05-31-47**
Customer: **SO-DIST**
Project ID: **SMP**

Send Reports to:
FL Dept. of Environmental Protection
FLDEP South District Office
2295 Victoria Ave. Suite 364
Fort Myers, FL 33901-3381
Attn: William Mullen

For additional information please contact
Cheryl Swanson - Administrator
Jacqueline Savage - Bench Biology & Aquatic
Toxicity
Puja Jasrotia, Ph.D. - Molecular Biology & Taxonomy
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Phone (850) 245-8177

Certified by: Cheryl A. Swanson, Program Administrator

Date Certified: 23-JUN-2021 13:49



NON-CONFORMANCE REPORT INCLUDED

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: Molecular and Overflow.

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: Punta Rassa Cove

Collection Date/Time: 06/01/2021 09:20

Field ID: G2SD0047

Matrix: W-SURF-SLT

Sample ID	Ref. Method	Component	Result	Code	Units	Batch ID	QC Codes
2251969	Enterolert/QT	ENTEROCOCCI-ENTEROLERT	22.4	J	MPN/100 mL		
	SM 9222 D	Fecal Coliforms-Membrane Filter	2.0	U	cfu/100 mL		

Ref. Method and Comment:
 Enterolert/QT: Improper dilution performed. See non-conformance report #8897.

Sample Location: Estero River @ Preserve

Collection Date/Time: 06/01/2021 11:15

Field ID: G1SD0104

Matrix: W-SURF-SLT

Sample ID	Ref. Method	Component	Result	Code	Units	Batch ID	QC Codes
2251967	SOP-PCR-1.0	HF183-qPCR**	1.7E+03	UQ	TSC/100 mL	P398979	
	SOP-PCR-1.2	GULL2-qPCR**	1.8E+03	UQ	TSC/100 mL	P398979	
	SOP-PCR-1.3	GFD-qPCR**	1.11E+04	Q	TSC/100 mL	P398979	
	SOP-PCR-1.4	BacR-qPCR**	1.5E+03	UQ	TSC/100 mL	P398979	
	SOP-PCR-1.5	DG3-qPCR**	1.3E+03	UQ	TSC/100 mL	P398979	
2251970	Enterolert/QT	ENTEROCOCCI-ENTEROLERT	2420	J	MPN/100 mL		

Ref. Method and Comment:
 SOP-PCR-1.2: Precision data unavailable for Gull2-qPCR component.
 SOP-PCR-1.3: Precision data unavailable for GFD-qPCR component.
 Enterolert/QT: All Quanti-Tray wells were positive. Result may be higher than reported. Improper dilution performed. See non-conformance report #8897.

Sample Location: Imperial River near US 41

Collection Date/Time: 06/01/2021 12:15

Field ID: G1SD0057

Matrix: W-SURF-SLT

Sample ID	Ref. Method	Component	Result	Code	Units	Batch ID	QC Codes
2251968	SOP-PCR-1.0	HF183-qPCR**	1.1E+03	UQ	TSC/100 mL	P398979	
	SOP-PCR-1.2	GULL2-qPCR**	1.2E+03	UQ	TSC/100 mL	P398979	
	SOP-PCR-1.3	GFD-qPCR**	7.5E+03	Q	TSC/100 mL	P398979	
	SOP-PCR-1.4	BacR-qPCR**	1.0E+03	UQ	TSC/100 mL	P398979	
	SOP-PCR-1.5	DG3-qPCR**	860	UQ	TSC/100 mL	P398979	
2251971	Enterolert/QT	ENTEROCOCCI-ENTEROLERT	2420	J	MPN/100 mL		

Ref. Method and Comment:
 SOP-PCR-1.2: Precision data unavailable for Gull2-qPCR component.
 SOP-PCR-1.3: Precision data unavailable for GFD-qPCR component.
 Enterolert/QT: All Quanti-Tray wells were positive. Result may be higher than reported. Improper dilution performed. See non-conformance report #8897.

Non-Conformance Report

NCR ID: 8872

Event(s)

Job(s)

Sample(s)

Test(s)

SO-DIST-2021-06-03-01

NCR Type: SHIPPING/RECEIVING

NCR Category: Samples arrived late

Observation: Samples arrived late, samples received within the acceptable temperature range.

Resolution: Customer (Curtis Musson) contacted, He asked that the short-hold analyses not be logged in.

Authorized by/Date: Joshua Ayres 6/3/2021

Non-Conformance Report

NCR ID: 8897

<u>Event(s)</u>	<u>Job(s)</u>	<u>Sample(s)</u>	<u>Test(s)</u>
SO-DIST-2021-06-03-01	TLH-2021-06-03-62	2251969	SD-SND-ENQ
SO-DIST-2021-06-03-01	TLH-2021-06-03-62	2251970	SD-SND-ENQ
SO-DIST-2021-06-03-01	TLH-2021-06-03-62	2251971	SD-SND-ENQ

NCR Type: ANALYSIS

NCR Category: Analytical Measurement

Observation: Marine sample was not diluted at least tenfold according to the method.

Resolution: J-qualified samples. Customer (William Mullen) has been notified.

Authorized by/Date: Cheryl A. Swanson 6/21/2021

The Non-Conformance Report details exceptions or problems encountered with the events/jobs/samples/test.
Please address questions to:

Chemistry Colin Wright (850) 245-8085
Biology Cheryl Swanson (850) 245-8177

Quality Assurance Report Method Blank Results

Reference Method: SOP-PCR-1.0

Batch ID: P398979

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

Reference Method: SOP-PCR-1.2

Batch ID: P398979

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

Reference Method: SOP-PCR-1.3

Batch ID: P398979

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

Reference Method: SOP-PCR-1.4

Batch ID: P398979

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

Reference Method: SOP-PCR-1.5

Batch ID: P398979

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: P398979

Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
HF183-qPCR	85.1	102	P/P	50 - 175

Reference Method: SOP-PCR-1.2
Batch ID: P398979

Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
GULL2-qPCR	128	172	P/P	50 - 300

Reference Method: SOP-PCR-1.3
Batch ID: P398979

Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
GFD-qPCR	94.5	90.9	P/P	50 - 175

Reference Method: SOP-PCR-1.4
Batch ID: P398979

Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
BacR-qPCR	95.8	68.8	P/P	50 - 175

Reference Method: SOP-PCR-1.5
Batch ID: P398979

Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
DG3-qPCR	68.5	135	P/P	50 - 175

Quality Assurance Report Matrix Spike Accuracy

Reference Method: SOP-PCR-1.0
Batch ID: P398979

Spiked Sample	Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
2251013	HF183-qPCR	84.9	85.8	P/P	50 - 175
2251014	HF183-qPCR	88.0		P	50 - 175
2251015	HF183-qPCR	77.4		P	50 - 175
2251967	HF183-qPCR	88.3		P	50 - 175
2251968	HF183-qPCR	98.1		P	50 - 175
2252019	HF183-qPCR	83.9		P	50 - 175
2252602	HF183-qPCR	75.2		P	50 - 175
2252778	HF183-qPCR	77.5		P	50 - 175
2252959	HF183-qPCR	71.6		P	50 - 175

Reference Method: SOP-PCR-1.2
Batch ID: P398979

Spiked Sample	Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
2251015	GULL2-qPCR	109		P	50 - 300
2251967	GULL2-qPCR	112		P	50 - 300
2251968	GULL2-qPCR	118		P	50 - 300
2252778	GULL2-qPCR	110		P	50 - 300

Quality Assurance Report Matrix Spike Accuracy

Reference Method: SOP-PCR-1.2
 Batch ID: P398979

Spiked Sample	Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
2252959	GULL2-qPCR	95.4		P	50 - 300

Reference Method: SOP-PCR-1.3
 Batch ID: P398979

Spiked Sample	Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
2251015	GFD-qPCR	90.9		P	50 - 175
2251967	GFD-qPCR	98.2		P	50 - 175
2251968	GFD-qPCR	88.7		P	50 - 175
2252778	GFD-qPCR	81.7		P	50 - 175
2252959	GFD-qPCR	88.8		P	50 - 175

Reference Method: SOP-PCR-1.4
 Batch ID: P398979

Spiked Sample	Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
2251013	BacR-qPCR	64.9	74.0	P/P	50 - 175
2251014	BacR-qPCR	64.6		P	50 - 175
2251015	BacR-qPCR	65.3		P	50 - 175
2251967	BacR-qPCR	67.9		P	50 - 175
2251968	BacR-qPCR	68.6		P	50 - 175
2252019	BacR-qPCR	65.1		P	50 - 175
2252602	BacR-qPCR	68.0		P	50 - 175
2252778	BacR-qPCR	70.3		P	50 - 175
2252959	BacR-qPCR	50.2		P	50 - 175

Reference Method: SOP-PCR-1.5
 Batch ID: P398979

Spiked Sample	Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
2251013	DG3-qPCR	91.9	81.8	P/P	50 - 175
2251014	DG3-qPCR	86.2		P	50 - 175
2251015	DG3-qPCR	80.4		P	50 - 175
2251967	DG3-qPCR	93.0		P	50 - 175
2251968	DG3-qPCR	80.4		P	50 - 175
2252019	DG3-qPCR	66.6		P	50 - 175
2252602	DG3-qPCR	67.4		P	50 - 175
2252778	DG3-qPCR	67.2		P	50 - 175
2252959	DG3-qPCR	57.8		P	50 - 175

Quality Assurance Report Precision

Reference Method: SOP-PCR-1.0
 Batch ID: P398979

Replicated Lab Sample	Component	% RSD/RPD	Sample/Spike/LCS*	Pass/Fail	Control Limits
2251013	HF183-qPCR	1.10	Spike	P	0 - 25

Quality Assurance Report Precision

Reference Method: SOP-PCR-1.4
 Batch ID: P398979

Replicated Lab Sample	Component	% RSD/RPD	Sample/Spike/LCS*	Pass/Fail	Control Limits
2251013	BacR-qPCR	13.1	Spike	P	0 - 25

Reference Method: SOP-PCR-1.5
 Batch ID: P398979

Replicated Lab Sample	Component	% RSD/RPD	Sample/Spike/LCS*	Pass/Fail	Control Limits
2251013	DG3-qPCR	11.6	Spike	P	0 - 25

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

Quality Assurance Report Surrogates

Lab Sample ID: 2251967
 Field Sample ID: G1SD0104

Reference Method	Surrogate	% Rec.	Pass/Fail	Control Limits
SOP-PCR-1.0	SKETA-qPCR	106	P	50 - 300
SOP-PCR-1.2	SKETA-qPCR	110	P	50 - 300
SOP-PCR-1.3	SKETA-qPCR	105	P	50 - 300
SOP-PCR-1.4	SKETA-qPCR	101	P	50 - 300
SOP-PCR-1.5	SKETA-qPCR	100	P	50 - 300

Lab Sample ID: 2251968
 Field Sample ID: G1SD0057

Reference Method	Surrogate	% Rec.	Pass/Fail	Control Limits
SOP-PCR-1.0	SKETA-qPCR	93.9	P	50 - 300
SOP-PCR-1.2	SKETA-qPCR	93.5	P	50 - 300
SOP-PCR-1.3	SKETA-qPCR	96.4	P	50 - 300
SOP-PCR-1.4	SKETA-qPCR	99.6	P	50 - 300
SOP-PCR-1.5	SKETA-qPCR	91.8	P	50 - 300

Quality Assurance Report Summary

Ref. Method	Analyte	LCS % Recovery		MS % Recovery			LCS	Precision SMP	MS
SOP-PCR-1.0	HF183-qPCR	102	85.1	84.9	85.8	88.0			1.10
				77.4					
SOP-PCR-1.2	GULL2-qPCR	172	128	109	112	118			
				110					
SOP-PCR-1.3	GFD-qPCR	90.9	94.5	90.9	98.2	88.7			
				81.7					
SOP-PCR-1.4	BacR-qPCR	68.8	95.8	64.9	74.0	64.6			13.1
				65.3					
SOP-PCR-1.5	DG3-qPCR	135	68.5	91.9	81.8	86.2			11.6
				80.4					

Reference Method Descriptions

Method	Description	Associated Samples
Enterolert/QT	Enterococci by Enterolert/QT by Sanders in Fort Myers	2251969, 2251970, 2251971
SM 9222 D	Fecal coliforms by MF by Sanders in Fort Myers	2251969
SOP-PCR-1.0	Detection and quantification of human-specific HF183 Bacteroides genetic marker with quantitative polymerase chain reaction	2251967, 2251968
SOP-PCR-1.2	Detection and quantification of coastal bird specific Catellicoccus marimammalium Gull2 genetic marker with quantitative polymerase chain reaction	2251967, 2251968
SOP-PCR-1.3	Detection and quantification of bird specific Helicobacter GFD genetic marker with quantitative polymerase chain reaction	2251967, 2251968
SOP-PCR-1.4	Detection and quantification of Ruminant specific Bacteroidetes BacR genetic marker with quantitative polymerase chain reaction	2251967, 2251968
SOP-PCR-1.5	Detection and quantification of canine-specific DG3 Bacteroides genetic marker with quantitative polymerase chain reaction	2251967, 2251968

Preparation and Analysis Log

Ref. Method	Received Date	Prep Date/Time	Prepared By	Analysis Date/Time	Analyzed By	Associated Samples
Enterolert/QT	06/01/2021	06/01/2021 14:48		06/02/2021 16:34	Sanders-Fort Myers	2251969, 2251970, 2251971
SM 9222 D	06/01/2021	06/01/2021 15:37		06/02/2021 14:13	Sanders-Fort Myers	2251969
SOP-PCR-1.0	06/03/2021	06/03/2021 14:20	DeAsia Armster	06/14/2021 14:07	DeAsia Armster	2251967, 2251968
SOP-PCR-1.2	06/03/2021	06/03/2021 14:20	DeAsia Armster	06/17/2021 10:14	DeAsia Armster	2251967, 2251968
SOP-PCR-1.3	06/03/2021	06/03/2021 14:20	DeAsia Armster	06/17/2021 08:24	Mailin Lopez	2251967, 2251968
SOP-PCR-1.4	06/03/2021	06/03/2021 14:20	DeAsia Armster	06/10/2021 10:42	DeAsia Armster	2251967, 2251968
SOP-PCR-1.5	06/03/2021	06/03/2021 14:20	DeAsia Armster	06/16/2021 09:39	DeAsia Armster	2251967, 2251968