

Biological Analysis Report

SW-DIST-2022-09-14-02

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

Overflow Analyses Performed By:
Benchmark EnvironAnalytical, Inc.
1711 12th Street East
Palmetto, FL 34221
DOH Accreditation E84167

Event Description: **Big Slough**
Request ID: **RQ-2022-09-12-53**
Customer: **SW-DIST**
Project ID: **SMP**

Send Reports to:
FL Dept. of Environmental Protection
FL Dept. of Environmental Protection
13051 N. Telecom Parkway
Temple Terrace, FL 33637-0926
Attn: Ben Paswater

For additional information please contact
Cheryl Swanson - Administrator
Sarah Menz, Ph.D. - Bench Biology & Aquatic
Toxicity
Puja Jasrotia, Ph.D. - Molecular Biology & Taxonomy
Thekkekalathil Chandrasekhar, Ph.D, QA Officer
Phone (850) 245-8177

Certified by: Cheryl A. Swanson, Program Administrator

Date Certified: 25-OCT-2022 15:35



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: Big Slough Canal (Potable Portion) @ Route 72 Collection Date/Time: 09/13/2022 11:25

Field ID: G3SW0115					Matrix: W-SURF-FRH		
Sample ID	Ref. Method	Component	Result	Code	Units	Batch ID	QC Codes
2356445	SOP-PCR-1.0	HF183-qPCR**	6.6E+03	U	TSC/100 mL	P419488	
	SOP-PCR-1.2	GULL2-qPCR**	7.2E+03	U	TSC/100 mL	P419488	
	SOP-PCR-1.3	GFD-qPCR**	2.6E+03	I	TSC/100 mL	P419488	
	SOP-PCR-1.4	BacR-qPCR**	6.1E+03	U	TSC/100 mL	P419488	
	SOP-PCR-1.5	DG3-qPCR**	5.1E+03	U	TSC/100 mL	P419488	
2356446	SM 9223 B Quanti-Tray	Escherichia Coli-Quanti-Tray	120		MPN/100 mL		

Quality Assurance Report Method Blank Results

Reference Method: SOP-PCR-1.0
 Batch ID: P419488

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

Reference Method: SOP-PCR-1.2
 Batch ID: P419488

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

Reference Method: SOP-PCR-1.3
 Batch ID: P419488

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

Reference Method: SOP-PCR-1.4
 Batch ID: P419488

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

Reference Method: SOP-PCR-1.5
 Batch ID: P419488

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: P419488

Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
HF183-qPCR	90.4	87.1	P/P	50 - 175

Quality Assurance Report Laboratory Control Sample Accuracy

Reference Method: SOP-PCR-1.2
Batch ID: P419488

Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
GULL2-qPCR	156	132	P/P	50 - 300

Reference Method: SOP-PCR-1.3
Batch ID: P419488

Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
GFD-qPCR	95.7	87.2	P/P	50 - 175

Reference Method: SOP-PCR-1.4
Batch ID: P419488

Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
BacR-qPCR	103	76.9	P/P	50 - 175

Reference Method: SOP-PCR-1.5
Batch ID: P419488

Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
DG3-qPCR	91.3	78.1	P/P	50 - 175

Quality Assurance Report Matrix Spike Accuracy

Reference Method: SOP-PCR-1.0
Batch ID: P419488

Spiked Sample	Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
2356017	HF183-qPCR	93.9	91.2	P/P	50 - 175
2356147	HF183-qPCR	82.0		P	50 - 175
2356445	HF183-qPCR	86.6		P	50 - 175
2356571	HF183-qPCR	77.9		P	50 - 175

Reference Method: SOP-PCR-1.2
Batch ID: P419488

Spiked Sample	Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
2356017	GULL2-qPCR	147	147	P/P	50 - 300
2356147	GULL2-qPCR	87.9		P	50 - 300
2356445	GULL2-qPCR	113		P	50 - 300
2356523	GULL2-qPCR	152		P	50 - 300
2356524	GULL2-qPCR	134		P	50 - 300
2356525	GULL2-qPCR	119		P	50 - 300
2356526	GULL2-qPCR	153		P	50 - 300
2356527	GULL2-qPCR	114		P	50 - 300
2356528	GULL2-qPCR	51.6		P	50 - 300
2356529	GULL2-qPCR	117		P	50 - 300
2356530	GULL2-qPCR	143		P	50 - 300
2356531	GULL2-qPCR	124		P	50 - 300
2356571	GULL2-qPCR	143		P	50 - 300

Quality Assurance Report Matrix Spike Accuracy

Reference Method: SOP-PCR-1.3
Batch ID: P419488

Spiked Sample	Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
2356017	GFD-qPCR	105	108	P/P	50 - 175
2356147	GFD-qPCR	92.9		P	50 - 175
2356445	GFD-qPCR	120		P	50 - 175
2356523	GFD-qPCR	106		P	50 - 175
2356524	GFD-qPCR	99.7		P	50 - 175
2356525	GFD-qPCR	108		P	50 - 175
2356526	GFD-qPCR	101		P	50 - 175
2356527	GFD-qPCR	91.9		P	50 - 175
2356528	GFD-qPCR	110		P	50 - 175
2356529	GFD-qPCR	85.5		P	50 - 175
2356530	GFD-qPCR	108		P	50 - 175
2356531	GFD-qPCR	97.2		P	50 - 175
2356571	GFD-qPCR	95.0		P	50 - 175

Reference Method: SOP-PCR-1.4
Batch ID: P419488

Spiked Sample	Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
2356017	BacR-qPCR	86.1	82.6	P/P	50 - 175
2356147	BacR-qPCR	57.1		P	50 - 175
2356445	BacR-qPCR	90.6		P	50 - 175

Reference Method: SOP-PCR-1.5
Batch ID: P419488

Spiked Sample	Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
2356017	DG3-qPCR	80.3	75.0	P/P	50 - 175
2356147	DG3-qPCR	70.3		P	50 - 175
2356445	DG3-qPCR	84.5		P	50 - 175
2356523	DG3-qPCR	93.5		P	50 - 175
2356524	DG3-qPCR	89.5		P	50 - 175
2356525	DG3-qPCR	77.7		P	50 - 175
2356526	DG3-qPCR	94.3		P	50 - 175
2356527	DG3-qPCR	95.0		P	50 - 175
2356528	DG3-qPCR	62.3		P	50 - 175
2356529	DG3-qPCR	85.8		P	50 - 175
2356530	DG3-qPCR	107		P	50 - 175
2356531	DG3-qPCR	86.6		P	50 - 175
2356571	DG3-qPCR	94.8		P	50 - 175

Quality Assurance Report Precision

Reference Method: SOP-PCR-1.0
Batch ID: P419488

Replicated Lab Sample	Component	% RSD/RPD	Sample/Spike/LCS*	Pass/Fail	Control Limits
2356017	HF183-qPCR	2.87	Spike	P	0 - 25

Quality Assurance Report Precision

Reference Method: SOP-PCR-1.2
Batch ID: P419488

Replicated Lab Sample	Component	% RSD/RPD	Sample/Spike/LCS*	Pass/Fail	Control Limits
2356017	GULL2-qPCR	0.368	Spike	P	0 - 25

Reference Method: SOP-PCR-1.3
Batch ID: P419488

Replicated Lab Sample	Component	% RSD/RPD	Sample/Spike/LCS*	Pass/Fail	Control Limits
2356017	GFD-qPCR	2.92	Spike	P	0 - 25

Reference Method: SOP-PCR-1.4
Batch ID: P419488

Replicated Lab Sample	Component	% RSD/RPD	Sample/Spike/LCS*	Pass/Fail	Control Limits
2356017	BacR-qPCR	4.14	Spike	P	0 - 25

Reference Method: SOP-PCR-1.5
Batch ID: P419488

Replicated Lab Sample	Component	% RSD/RPD	Sample/Spike/LCS*	Pass/Fail	Control Limits
2356017	DG3-qPCR	6.80	Spike	P	0 - 25

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

Quality Assurance Report Surrogates

Lab Sample ID: 2356445
Field Sample ID: G3SW0115

Reference Method	Surrogate	% Rec.	Pass/Fail	Control Limits
SOP-PCR-1.0	SKETA-qPCR	78.3	P	50 - 300
SOP-PCR-1.2	SKETA-qPCR	144	P	50 - 300
SOP-PCR-1.3	SKETA-qPCR	116	P	50 - 300
SOP-PCR-1.4	SKETA-qPCR	109	P	50 - 300
SOP-PCR-1.5	SKETA-qPCR	92.8	P	50 - 300

Quality Assurance Report Summary

Ref. Method	Analyte	LCS % Recovery		MS % Recovery			Precision SMP	MS
SOP-PCR-1.0	HF183-qPCR	87.1	90.4	93.9	91.2	82.0		2.87
SOP-PCR-1.2	GULL2-qPCR	132	156	147	147	87.9		0.368
SOP-PCR-1.3	GFD-qPCR	87.2	95.7	105	108	92.9		2.92

Quality Assurance Report Summary

Ref. Method	Analyte	LCS % Recovery		MS % Recovery			LCS	Precision SMP	MS
SOP-PCR-1.4	BacR-qPCR	76.9	103	86.1	82.6	57.1			4.14
SOP-PCR-1.5	DG3-qPCR	78.1	91.3	80.3	75.0	70.3			6.80
				90.6					
				84.5					

Reference Method Descriptions

Method	Description	<u>Associated Samples</u>
SM 9223 B Quanti-Tray	E. coli by Colilert/QT by BEA in Palmetto	2356446
SOP-PCR-1.0	Detection and quantification of human-specific HF183 Bacteroides genetic marker with quantitative polymerase chain reaction	2356445
SOP-PCR-1.2	Detection and quantification of coastal bird specific Catellicoccus marimammalium Gull2 genetic marker with quantitative polymerase chain reaction	2356445
SOP-PCR-1.3	Detection and quantification of bird specific Helicobacter GFD genetic marker with quantitative polymerase chain reaction	2356445
SOP-PCR-1.4	Detection and quantification of Ruminant specific Bacteroidetes BacR genetic marker with quantitative polymerase chain reaction	2356445
SOP-PCR-1.5	Detection and quantification of canine-specific DG3 Bacteroides genetic marker with quantitative polymerase chain reaction	2356445

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	09/13/2022	09/13/2022 16:50		09/14/2022 17:56	Benchmark EnviroAnalytical - Palmetto	2356446
SOP-PCR-1.0	09/14/2022	09/14/2022 13:50	Claudia Castro	09/29/2022 11:29	Roberta Tatti	2356445
SOP-PCR-1.2	09/14/2022	09/14/2022 13:50	Claudia Castro	09/28/2022 09:14	DeAsia Armster	2356445
SOP-PCR-1.3	09/14/2022	09/14/2022 13:50	Claudia Castro	09/28/2022 12:45	Roberta Tatti	2356445
SOP-PCR-1.4	09/14/2022	09/14/2022 13:50	Claudia Castro	10/03/2022 12:13	DeAsia Armster	2356445
SOP-PCR-1.5	09/14/2022	09/14/2022 13:50	Claudia Castro	09/21/2022 09:18	DeAsia Armster	2356445