

Biological Analysis Report

NW-DIST-2023-05-18-03

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

Overflow Analyses Performed By:
University of West Florida
11000 University Parkway
Pensacola, FL 32514
DOH Accreditation E71969

Event Description: **Holmes Run**
Request ID: **RQ-2023-05-15-42**
Customer: **NW-DIST**
Project ID: **SMP**

Send Reports to:
FL Dept. of Environmental Protection
160 W Government Street
Suite 208
Pensacola, FL 32502-5794
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Certified by: Sarah Menz, Environmental Administrator

Date Certified: 30-JUN-2023 13:37

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: Chlorophyll/Grain Size/BOD, 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: SIKES CREEK UPSTREAM OF HARRIS STEVENSON RD

Collection Date/Time: 05/17/2023 11:05

Field ID: G3NW0450

Matrix: W-SURF-FRH

Sample ID	Ref. Method	Component	Result	Code	Units	Batch ID	QC Codes
2410828	SM 9223 B Quanti-Tray	Escherichia Coli-Quanti-Tray	189		MPN/100 mL		

Sample Location: ANDERSON MILL CREEK

Collection Date/Time: 05/17/2023 12:00

Field ID: G3NW0224

Matrix: W-SURF-FRH

Sample ID	Ref. Method	Component	Result	Code	Units	Batch ID	QC Codes
2410825	SM 10150 B	Chlorophyll-a, Corrected	0.82	U	ug/L	P430668	
		Phaeophytin-a	0.90	U	ug/L	P430668	
		Chlorophyll-a, Uncorrected	0.98	I	ug/L	P430668	
2410829	SM 9223 B Quanti-Tray	Escherichia Coli-Quanti-Tray	408		MPN/100 mL		

Ref. Method and Comment:

SM 10150 B: Precision data is not available for at least one component due to the small amount of analyte in the QC sample. Refer to QA report for available precision data.

Sample Location: HUCKLEBERRY CREEK

Collection Date/Time: 05/17/2023 12:15

Field ID: G3NW0135

Matrix: W-SURF-FRH

Sample ID	Ref. Method	Component	Result	Code	Units	Batch ID	QC Codes
2410827	SM 9223 B Quanti-Tray	Escherichia Coli-Quanti-Tray	96		MPN/100 mL		
2410831	SOP-PCR-1.0	HF183-qPCR**	6.6E+03	U	TSC/100 mL	P429819	
	SOP-PCR-1.2	GULL2-qPCR**	7.2E+03	U	TSC/100 mL	P429819	
	SOP-PCR-1.3	GFD-qPCR**	1.4E+03	U	TSC/100 mL	P429819	
	SOP-PCR-1.4	BacR-qPCR**	6.1E+03	U	TSC/100 mL	P429819	
	SOP-PCR-1.5	DG3-qPCR**	5.1E+03	U	TSC/100 mL	P429819	

Sample Location: WEST SANDY CREEK ABOVE OLD LANDFILL RD

Collection Date/Time: 05/17/2023 12:35

Field ID: G3NW0054

Matrix: W-SURF-FRH

Sample ID	Ref. Method	Component	Result	Code	Units	Batch ID	QC Codes
2410830	SM 9223 B Quanti-Tray	Escherichia Coli-Quanti-Tray	158		MPN/100 mL		

Sample Location: CHOCTAWHATCHEE RIVER UPSTREAM HWY 90 BOAT RAMP

Collection Date/Time: 05/17/2023 10:35

Field ID: G3NW0052

Matrix: W-SURF-FRH

Sample ID	Ref. Method	Component	Result	Code	Units	Batch ID	QC Codes
2410824	SM 10150 B	Chlorophyll-a, Corrected	5.2		ug/L	P430668	
		Phaeophytin-a	0.90	U	ug/L	P430668	
		Chlorophyll-a, Uncorrected	5.7		ug/L	P430668	
2410826	SM 9223 B Quanti-Tray	Escherichia Coli-Quanti-Tray	96		MPN/100 mL		

Ref. Method and Comment:

SM 10150 B: Precision data is not available for at least one component due to the small amount of analyte in the QC sample. Refer to QA report for available precision data.

Quality Assurance Report

Method Blank Results

Quality Assurance Report Method Blank Results

Reference Method: SM 10150 B
Batch ID: P430668

Component	Result	Code	Units
Chlorophyll-a, Corrected	0.82	U	ug/L
Chlorophyll-a, Uncorrected	0.60	U	ug/L
Phaeophytin-a	0.90	U	ug/L

Reference Method: SOP-PCR-1.0
Batch ID: P429819

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

Reference Method: SOP-PCR-1.2
Batch ID: P429819

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

Reference Method: SOP-PCR-1.3
Batch ID: P429819

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

Reference Method: SOP-PCR-1.4
Batch ID: P429819

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

Reference Method: SOP-PCR-1.5
Batch ID: P429819

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

Quality Assurance Report Laboratory Control Sample Accuracy

Reference Method: SM 10150 B
Batch ID: P430668

Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
Chlorophyll-a, Corrected	100		P	84 - 116

Reference Method: SOP-PCR-1.0
Batch ID: P429819

Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
HF183-qPCR	97.5	91.2	P/P	50 - 175

Quality Assurance Report Laboratory Control Sample Accuracy

Reference Method: SOP-PCR-1.2
Batch ID: P429819

Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
GULL2-qPCR	107	98.1	P/P	50 - 300

Reference Method: SOP-PCR-1.3
Batch ID: P429819

Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
GFD-qPCR	85.3	104	P/P	50 - 175

Reference Method: SOP-PCR-1.4
Batch ID: P429819

Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
BacR-qPCR	125	95.1	P/P	50 - 175

Reference Method: SOP-PCR-1.5
Batch ID: P429819

Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
DG3-qPCR	107	83.8	P/P	50 - 175

Quality Assurance Report Matrix Spike Accuracy

Reference Method: SOP-PCR-1.0
Batch ID: P429819

Spiked Sample	Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
2409346	HF183-qPCR	75.7	79.9	P/P	50 - 175
2409749	HF183-qPCR	96.9		P	50 - 175
2409831	HF183-qPCR	86.4		P	50 - 175
2410171	HF183-qPCR	92.7		P	50 - 175
2410500	HF183-qPCR	104		P	50 - 175
2410501	HF183-qPCR	84.2		P	50 - 175
2410831	HF183-qPCR	72.8		P	50 - 175
2411303	HF183-qPCR	83.9		P	50 - 175
2411335	HF183-qPCR	85.3		P	50 - 175
2411336	HF183-qPCR	84.2		P	50 - 175
2411817	HF183-qPCR	105		P	50 - 175
2412131	HF183-qPCR	95.5		P	50 - 175
2412132	HF183-qPCR	78.2		P	50 - 175

Reference Method: SOP-PCR-1.2
Batch ID: P429819

Spiked Sample	Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
2409346	GULL2-qPCR	85.8	84.3	P/P	50 - 300
2409749	GULL2-qPCR	69.8		P	50 - 300
2409831	GULL2-qPCR	64.9		P	50 - 300
2410500	GULL2-qPCR	77.6		P	50 - 300
2410501	GULL2-qPCR	84.7		P	50 - 300
2410831	GULL2-qPCR	89.4		P	50 - 300

Quality Assurance Report Matrix Spike Accuracy

Reference Method: SOP-PCR-1.2

Batch ID: P429819

Spiked Sample	Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
2411303	GULL2-qPCR	69.4		P	50 - 300
2411335	GULL2-qPCR	80.5		P	50 - 300
2411336	GULL2-qPCR	81.4		P	50 - 300
2411817	GULL2-qPCR	94.7		P	50 - 300
2412131	GULL2-qPCR	89.7		P	50 - 300
2412132	GULL2-qPCR	77.5		P	50 - 300

Reference Method: SOP-PCR-1.3

Batch ID: P429819

Spiked Sample	Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
2409346	GFD-qPCR	86.3	93.2	P/P	50 - 175
2409749	GFD-qPCR	95.2		P	50 - 175
2409831	GFD-qPCR	95.9		P	50 - 175
2410500	GFD-qPCR	90.4		P	50 - 175
2410501	GFD-qPCR	93.6		P	50 - 175
2410831	GFD-qPCR	94.7		P	50 - 175
2411303	GFD-qPCR	102		P	50 - 175
2411335	GFD-qPCR	83.9		P	50 - 175
2411336	GFD-qPCR	82.9		P	50 - 175
2411817	GFD-qPCR	93.4		P	50 - 175
2412131	GFD-qPCR	84.8		P	50 - 175
2412132	GFD-qPCR	82.9		P	50 - 175

Reference Method: SOP-PCR-1.4

Batch ID: P429819

Spiked Sample	Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
2409346	BacR-qPCR	95.8	96.8	P/P	50 - 175
2409749	BacR-qPCR	88.8		P	50 - 175
2409831	BacR-qPCR	68.9		P	50 - 175
2410171	BacR-qPCR	103		P	50 - 175
2410500	BacR-qPCR	84.7		P	50 - 175
2410501	BacR-qPCR	83.6		P	50 - 175
2410831	BacR-qPCR	90.2		P	50 - 175
2411303	BacR-qPCR	77.3		P	50 - 175
2411335	BacR-qPCR	86.5		P	50 - 175
2411336	BacR-qPCR	80.1		P	50 - 175
2411817	BacR-qPCR	97.2		P	50 - 175
2412131	BacR-qPCR	78.4		P	50 - 175
2412132	BacR-qPCR	78.2		P	50 - 175

Reference Method: SOP-PCR-1.5

Batch ID: P429819

Spiked Sample	Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
2409346	DG3-qPCR	81.7	88.9	P/P	50 - 175
2409749	DG3-qPCR	90.7		P	50 - 175
2409831	DG3-qPCR	81.6		P	50 - 175
2410171	DG3-qPCR	89.1		P	50 - 175
2410500	DG3-qPCR	91.4		P	50 - 175
2410501	DG3-qPCR	101		P	50 - 175

Quality Assurance Report Matrix Spike Accuracy

Reference Method: SOP-PCR-1.5
 Batch ID: P429819

Spiked Sample	Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
2410831	DG3-qPCR	90.6		P	50 - 175
2411303	DG3-qPCR	86.9		P	50 - 175
2411335	DG3-qPCR	90.6		P	50 - 175
2411336	DG3-qPCR	102		P	50 - 175
2411817	DG3-qPCR	111		P	50 - 175
2412131	DG3-qPCR	107		P	50 - 175
2412132	DG3-qPCR	92.2		P	50 - 175

Quality Assurance Report Precision

Reference Method: SOP-PCR-1.0
 Batch ID: P429819

Replicated Lab Sample	Component	% RSD/RPD	Sample/Spike/LCS*	Pass/Fail	Control Limits
2409346	HF183-qPCR	5.35	Spike	P	0 - 25

Reference Method: SOP-PCR-1.2
 Batch ID: P429819

Replicated Lab Sample	Component	% RSD/RPD	Sample/Spike/LCS*	Pass/Fail	Control Limits
2409346	GULL2-qPCR	1.73	Spike	P	0 - 25

Reference Method: SOP-PCR-1.3
 Batch ID: P429819

Replicated Lab Sample	Component	% RSD/RPD	Sample/Spike/LCS*	Pass/Fail	Control Limits
2409346	GFD-qPCR	7.68	Spike	P	0 - 25

Reference Method: SOP-PCR-1.4
 Batch ID: P429819

Replicated Lab Sample	Component	% RSD/RPD	Sample/Spike/LCS*	Pass/Fail	Control Limits
2409346	BacR-qPCR	1.06	Spike	P	0 - 25

Reference Method: SOP-PCR-1.5
 Batch ID: P429819

Replicated Lab Sample	Component	% RSD/RPD	Sample/Spike/LCS*	Pass/Fail	Control Limits
2409346	DG3-qPCR	8.36	Spike	P	0 - 25

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

Quality Assurance Report Surrogates

Quality Assurance Report Surrogates

Lab Sample ID: 2410831
 Field Sample ID: G3NW0135

Reference Method	Surrogate	% Rec.	Pass/Fail	Control Limits
SOP-PCR-1.0	SKETA-qPCR	114	P	50 - 300
SOP-PCR-1.2	SKETA-qPCR	108	P	50 - 300
SOP-PCR-1.3	SKETA-qPCR	110	P	50 - 300
SOP-PCR-1.4	SKETA-qPCR	116	P	50 - 300
SOP-PCR-1.5	SKETA-qPCR	106	P	50 - 300

Quality Assurance Report Summary

Ref. Method	Analyte	LCS % Recovery		MS % Recovery			LCS	Precision SMP	MS
SM 10150 B	Chlorophyll-a, Corrected	100							
SOP-PCR-1.0	HF183-qPCR	91.2	97.5	75.7	79.9	96.9			5.35
SOP-PCR-1.2	GULL2-qPCR	98.1	107	85.8	84.3	69.8			1.73
SOP-PCR-1.3	GFD-qPCR	104	85.3	86.3	93.2	95.2			7.68
SOP-PCR-1.4	BacR-qPCR	95.1	125	95.8	96.8	88.8			1.06
SOP-PCR-1.5	DG3-qPCR	83.8	107	81.7	88.9	90.7			8.36

Reference Method Descriptions

Method	Description	Associated Samples
SM 10150 B	Phytoplankton chlorophyll-a corrected, uncorrected, and phaeophytin by spectrophotometry	2410824, 2410825
SM 9223 B Quanti-Tray	E. coli by Colilert-18/QT by UWF in Pensacola	2410826, 2410827, 2410828, 2410829, 2410830
SOP-PCR-1.0	Detection and quantification of human-specific HF183 Bacteroides genetic marker with quantitative polymerase chain reaction	2410831
SOP-PCR-1.2	Detection and quantification of coastal bird specific Catellicoccus marimammalium Gull2 genetic marker with quantitative polymerase chain reaction	2410831
SOP-PCR-1.3	Detection and quantification of bird specific Helicobacter GFD genetic marker with quantitative polymerase chain reaction	2410831
SOP-PCR-1.4	Detection and quantification of Ruminant specific Bacteroidetes BacR genetic marker with quantitative polymerase chain reaction	2410831
SOP-PCR-1.5	Detection and quantification of canine-specific DG3 Bacteroides genetic marker with quantitative polymerase chain reaction	2410831

Preparation and Analysis Log

Ref. Method	Received Date	Prep Date/Time	Prepared By	Analysis Date/Time	Analyzed By	Associated Samples
SM 10150 B	05/18/2023	05/18/2023 13:36	Megan Higbee	05/31/2023 10:24	Tynan A Branch	2410824, 2410825
SM 9223 B Quanti-Tray	05/17/2023	05/17/2023 15:35		05/18/2023 11:40	UWF Lab	2410826, 2410827, 2410828, 2410829, 2410830
SOP-PCR-1.0	05/18/2023	05/18/2023 14:20	Madeline Gruver	06/06/2023 12:25	Jennifer Mihalic	2410831
SOP-PCR-1.2	05/18/2023	05/18/2023 14:20	Madeline Gruver	06/14/2023 12:10	Jennifer Mihalic	2410831
SOP-PCR-1.3	05/18/2023	05/18/2023 14:20	Madeline Gruver	06/13/2023 15:38	Madeline Gruver	2410831
SOP-PCR-1.4	05/18/2023	05/18/2023 14:20	Madeline Gruver	06/07/2023 12:37	Jennifer Mihalic	2410831
SOP-PCR-1.5	05/18/2023	05/18/2023 14:20	Madeline Gruver	06/07/2023 15:13	Jennifer Mihalic	2410831