

Biological Analysis Report

NW-DIST-2023-08-10-01

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-2023-08-07-63**
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: 21-SEP-2023 10:20

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: CHOCTAWHATCHEE RIVER UPSTREAM HWY 90 BOAT RAMP **Collection Date/Time: 08/09/2023 10:50**

Field ID: G3NW0052

Matrix: W-SURF-FRH

Sample ID	Ref. Method	Component	Result	Code	Units	Batch ID	QC Codes
2430091	SM 10150 B	Chlorophyll-a, Corrected	16		ug/L	P433781	
		Phaeophytin-a	2.5	I	ug/L	P433781	
		Chlorophyll-a, Uncorrected	18		ug/L	P433781	
2430093	SM 9223 B Quanti-Tray	Escherichia Coli-Quanti-Tray	41		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: ANDERSON MILL CREEK **Collection Date/Time: 08/09/2023 12:20**

Field ID: G3NW0224

Matrix: W-SURF-FRH

Sample ID	Ref. Method	Component	Result	Code	Units	Batch ID	QC Codes
2430092	SM 10150 B	Chlorophyll-a, Corrected	2.4	I	ug/L	P433781	
		Phaeophytin-a	1.8	U	ug/L	P433781	
		Chlorophyll-a, Uncorrected	2.3	I	ug/L	P433781	
2430096	SM 9223 B Quanti-Tray	Escherichia Coli-Quanti-Tray	435		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: 08/09/2023 12:35**

Field ID: G3NW0135

Matrix: W-SURF-FRH

Sample ID	Ref. Method	Component	Result	Code	Units	Batch ID	QC Codes
2430094	SM 9223 B Quanti-Tray	Escherichia Coli-Quanti-Tray	906		MPN/100 mL		
2430100	SOP-PCR-1.0	HF183-qPCR**	6.6E+03	U	TSC/100 mL	P433620	
	SOP-PCR-1.4	BacR-qPCR**	2.8E+04	I	TSC/100 mL	P433620	
	SOP-PCR-1.5	DG3-qPCR**	5.1E+03	U	TSC/100 mL	P433620	

Sample Location: SIKES CREEK UPSTREAM OF HARRIS STEVENSON RD **Collection Date/Time: 08/09/2023 11:30**

Field ID: G3NW0450

Matrix: W-SURF-FRH

Sample ID	Ref. Method	Component	Result	Code	Units	Batch ID	QC Codes
2430095	SM 9223 B Quanti-Tray	Escherichia Coli-Quanti-Tray	279		MPN/100 mL		

Sample Location: WEST SANDY CREEK ABOVE OLD LANDFILL RD **Collection Date/Time: 08/09/2023 12:55**

Field ID: G3NW0054

Matrix: W-SURF-FRH

Sample ID	Ref. Method	Component	Result	Code	Units	Batch ID	QC Codes
2430097	SM 9223 B Quanti-Tray	Escherichia Coli-Quanti-Tray	197		MPN/100 mL		

Quality Assurance Report

Method Blank Results

Quality Assurance Report Method Blank Results

Reference Method: SM 10150 B
Batch ID: P433781

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: P433620

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

Reference Method: SOP-PCR-1.4
Batch ID: P433620

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

Reference Method: SOP-PCR-1.5
Batch ID: P433620

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: P433781

Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
Chlorophyll-a, Corrected	95.8		P	84 - 116

Reference Method: SOP-PCR-1.0
Batch ID: P433620

Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
HF183-qPCR	94.6	87.4	P/P	50 - 175

Reference Method: SOP-PCR-1.4
Batch ID: P433620

Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
BacR-qPCR	94.6	73.0	P/P	50 - 175

Reference Method: SOP-PCR-1.5
Batch ID: P433620

Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
DG3-qPCR	97.7	69.6	P/P	50 - 175

Quality Assurance Report Matrix Spike Accuracy

Quality Assurance Report Matrix Spike Accuracy

Reference Method: SOP-PCR-1.0

Batch ID: P433620

Spiked Sample	Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
2429973	HF183-qPCR	86.2	96.0	P	50 - 175
2430004	HF183-qPCR	97.4		P/P	50 - 175
2430100	HF183-qPCR	88.2	96.0	P	50 - 175
2430429	HF183-qPCR	99.4		P	50 - 175
2430516	HF183-qPCR	92.6	96.0	P	50 - 175
2430517	HF183-qPCR	78.4		P	50 - 175
2430588	HF183-qPCR	101	96.0	P	50 - 175
2430589	HF183-qPCR	98.2		P	50 - 175
2430590	HF183-qPCR	117	96.0	P	50 - 175
2430591	HF183-qPCR	99.4		P	50 - 175
2430592	HF183-qPCR	102	96.0	P	50 - 175
2430593	HF183-qPCR	89.6		P	50 - 175
2430850	HF183-qPCR	91.7	96.0	P	50 - 175

Reference Method: SOP-PCR-1.4

Batch ID: P433620

Spiked Sample	Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
2429973	BacR-qPCR	76.1	73.1	P	50 - 175
2430004	BacR-qPCR	72.3		P/P	50 - 175
2430100	BacR-qPCR	79.0	73.1	P	50 - 175
2430429	BacR-qPCR	81.2		P	50 - 175
2430516	BacR-qPCR	70.7	73.1	P	50 - 175
2430517	BacR-qPCR	72.5		P	50 - 175
2430850	BacR-qPCR	85.2	73.1	P	50 - 175

Reference Method: SOP-PCR-1.5

Batch ID: P433620

Spiked Sample	Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
2429973	DG3-qPCR	96.9	96.3	P	50 - 175
2430004	DG3-qPCR	102		P/P	50 - 175
2430100	DG3-qPCR	88.5	96.3	P	50 - 175
2430429	DG3-qPCR	94.1		P	50 - 175
2430516	DG3-qPCR	84.5	96.3	P	50 - 175
2430517	DG3-qPCR	84.9		P	50 - 175
2430588	DG3-qPCR	104	96.3	P	50 - 175
2430589	DG3-qPCR	99.5		P	50 - 175
2430590	DG3-qPCR	108	96.3	P	50 - 175
2430591	DG3-qPCR	102		P	50 - 175
2430592	DG3-qPCR	110	96.3	P	50 - 175
2430593	DG3-qPCR	101		P	50 - 175
2430850	DG3-qPCR	89.1	96.3	P	50 - 175

Quality Assurance Report Precision

Quality Assurance Report Precision

Reference Method: SM 10150 B
 Batch ID: P433781

Replicated Lab Sample	Component	% RSD/RPD	Sample/Spike/LCS*	Pass/Fail	Control Limits
2430101	Chlorophyll-a, Corrected	3.41	Sample	P	0 - 25
2430101	Chlorophyll-a, Uncorrected	2.82	Sample	P	0 - 25

Reference Method: SOP-PCR-1.0
 Batch ID: P433620

Replicated Lab Sample	Component	% RSD/RPD	Sample/Spike/LCS*	Pass/Fail	Control Limits
2430004	HF183-qPCR	1.48	Spike	P	0 - 25

Reference Method: SOP-PCR-1.4
 Batch ID: P433620

Replicated Lab Sample	Component	% RSD/RPD	Sample/Spike/LCS*	Pass/Fail	Control Limits
2430004	BacR-qPCR	1.00	Spike	P	0 - 25

Reference Method: SOP-PCR-1.5
 Batch ID: P433620

Replicated Lab Sample	Component	% RSD/RPD	Sample/Spike/LCS*	Pass/Fail	Control Limits
2430004	DG3-qPCR	5.33	Spike	P	0 - 25

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

Quality Assurance Report Surrogates

Lab Sample ID: 2430100
 Field Sample ID: G3NW0135

Reference Method	Surrogate	% Rec.	Pass/Fail	Control Limits
SOP-PCR-1.0	SKETA-qPCR	120	P	50 - 300
SOP-PCR-1.4	SKETA-qPCR	126	P	50 - 300
SOP-PCR-1.5	SKETA-qPCR	109	P	50 - 300

Quality Assurance Report Summary

Ref. Method	Analyte	LCS % Recovery		MS % Recovery			Precision		
							LCS	SMP	MS
SM 10150 B	Chlorophyll-a, Corrected	95.8						3.41	
	Chlorophyll-a, Uncorrected							2.82	
SOP-PCR-1.0	HF183-qPCR	87.4	94.6	86.2	97.4	96.0			1.48
SOP-PCR-1.4	BacR-qPCR	73.0 94.6		88.2					
				76.1 72.3 73.1 79.0					1.00

Quality Assurance Report
Summary

Ref. Method	Analyte	LCS % Recovery		MS % Recovery			Precision	
							LCS	SMP
SOP-PCR-1.5	DG3-qPCR	69.6	97.7	96.9	102	96.3		5.33
				88.5				

Reference Method Descriptions

Method	Description	Associated Samples
SM 10150 B	Phytoplankton chlorophyll-a corrected, uncorrected, and phaeophytin by spectrophotometry	2430091, 2430092
SM 9223 B Quanti-Tray	E. coli by Colilert-18/QT by UWF in Pensacola	2430093, 2430094, 2430095, 2430096, 2430097
SOP-PCR-1.0	Detection and quantification of human-specific HF183 Bacteroides genetic marker with quantitative polymerase chain reaction	2430100
SOP-PCR-1.4	Detection and quantification of Ruminant specific Bacteroidetes BacR genetic marker with quantitative polymerase chain reaction	2430100
SOP-PCR-1.5	Detection and quantification of canine-specific DG3 Bacteroides genetic marker with quantitative polymerase chain reaction	2430100

Preparation and Analysis Log

Ref. Method	Received Date	Prep Date/Time	Prepared By	Analysis Date/Time	Analyzed By	Associated Samples
SM 10150 B	08/10/2023	08/10/2023 14:11	Tynan A Branch	08/11/2023 09:57	Megan Higbee	2430091, 2430092
SM 9223 B Quanti-Tray	08/09/2023	08/09/2023 15:30		08/10/2023 11:37	UWF Lab	2430093, 2430094, 2430095, 2430096, 2430097
SOP-PCR-1.0	08/10/2023	08/10/2023 15:00	Claudia Castro	08/16/2023 17:07	Madeline Gruver	2430100
SOP-PCR-1.4	08/10/2023	08/10/2023 15:00	Claudia Castro	08/17/2023 15:21	Madeline Gruver	2430100
SOP-PCR-1.5	08/10/2023	08/10/2023 15:00	Claudia Castro	08/18/2023 10:39	Roberta Tatti	2430100