

Biological Analysis Report

NW-DIST-2023-10-05-01

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-2023-10-02-62**
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: 17-NOV-2023 10:35

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: 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: 10/04/2023 10:30**

Field ID: G3NW0052

Matrix: W-SURF-FRH

Sample ID	Ref. Method	Component	Result	Code	Units	Batch ID	QC Codes
2443187	SM 10150 B	Chlorophyll-a, Corrected	2.8	I	ug/L	P436795	
		Phaeophytin-a	1.0	U	ug/L	P436795	
		Chlorophyll-a, Uncorrected	3.2		ug/L	P436795	
2443193	SM 9223 B Quanti-Tray	Escherichia Coli-Quanti-Tray	31		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: 10/04/2023 12:15**

Field ID: G3NW0135

Matrix: W-SURF-FRH

Sample ID	Ref. Method	Component	Result	Code	Units	Batch ID	QC Codes
2443194	SM 9223 B Quanti-Tray	Escherichia Coli-Quanti-Tray	109		MPN/100 mL		
2443197	SOP-PCR-1.0	HF183-qPCR**	3.3E+03	U	TSC/100 mL	P436033	
	SOP-PCR-1.2	GULL2-qPCR**	3.6E+03	U	TSC/100 mL	P436033	
	SOP-PCR-1.3	GFD-qPCR**	720	U	TSC/100 mL	P436033	
	SOP-PCR-1.4	BacR-qPCR**	3.0E+03	U	TSC/100 mL	P436033	LCS
	SOP-PCR-1.5	DG3-qPCR**	2.6E+03	U	TSC/100 mL	P436033	LCS

Sample Location: ANDERSON MILL CREEK **Collection Date/Time: 10/04/2023 11:55**

Field ID: G3NW0224

Matrix: W-SURF-FRH

Sample ID	Ref. Method	Component	Result	Code	Units	Batch ID	QC Codes
2443188	SM 10150 B	Chlorophyll-a, Corrected	1.6	U	ug/L	P436795	
		Phaeophytin-a	1.8	U	ug/L	P436795	
		Chlorophyll-a, Uncorrected	1.2	U	ug/L	P436795	
2443195	SM 9223 B Quanti-Tray	Escherichia Coli-Quanti-Tray	2359		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: WEST SANDY CREEK ABOVE OLD LANDFILL RD **Collection Date/Time: 10/04/2023 12:35**

Field ID: G3NW0054

Matrix: W-SURF-FRH

Sample ID	Ref. Method	Component	Result	Code	Units	Batch ID	QC Codes
2443196	SM 9223 B Quanti-Tray	Escherichia Coli-Quanti-Tray	96		MPN/100 mL		

Quality Assurance Report Method Blank Results

Reference Method: SM 10150 B
Batch ID: P436795

Component	Result	Code	Units
Chlorophyll-a, Corrected	0.96	U	ug/L
Chlorophyll-a, Uncorrected	0.70	U	ug/L
Phaeophytin-a	1.0	U	ug/L

Quality Assurance Report Method Blank Results

Reference Method: SOP-PCR-1.0
Batch ID: P436033

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

Reference Method: SOP-PCR-1.2
Batch ID: P436033

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

Reference Method: SOP-PCR-1.3
Batch ID: P436033

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

Reference Method: SOP-PCR-1.4
Batch ID: P436033

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

Reference Method: SOP-PCR-1.5
Batch ID: P436033

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: P436795

Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
Chlorophyll-a, Corrected	95.1		P	84 - 116

Reference Method: SOP-PCR-1.0
Batch ID: P436033

Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
HF183-qPCR	79.9	85.4	P/P	50 - 175

Reference Method: SOP-PCR-1.2
Batch ID: P436033

Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
GULL2-qPCR	96.6	79.3	P/P	50 - 175

Reference Method: SOP-PCR-1.3
Batch ID: P436033

Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
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Quality Assurance Report Laboratory Control Sample Accuracy

Reference Method: SOP-PCR-1.3
Batch ID: P436033

Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
GFD-qPCR	83.8	67.7	P/P	50 - 175

Reference Method: SOP-PCR-1.4
Batch ID: P436033

Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
BacR-qPCR	104	46.2	P/F	50 - 175

Reference Method: SOP-PCR-1.5
Batch ID: P436033

Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
DG3-qPCR	94.3	43.9	P/F	50 - 175

Quality Assurance Report Matrix Spike Accuracy

Reference Method: SOP-PCR-1.0
Batch ID: P436033

Spiked Sample	Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
2443197	HF183-qPCR	83.3	80.6	P/P	50 - 175
2443379	HF183-qPCR	87.6		P	50 - 175
2443415	HF183-qPCR	87.7		P	50 - 175
2443447	HF183-qPCR	83.1		P	50 - 175
2444335	HF183-qPCR	77.0		P	50 - 175
2444337	HF183-qPCR	58.5		P	50 - 175
2444617	HF183-qPCR	96.0		P	50 - 175
2444690	HF183-qPCR	99.6		P	50 - 175
2444691	HF183-qPCR	79.8		P	50 - 175
2444692	HF183-qPCR	91.5		P	50 - 175
2444960	HF183-qPCR	87.5		P	50 - 175

Reference Method: SOP-PCR-1.2
Batch ID: P436033

Spiked Sample	Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
2443197	GULL2-qPCR	90.2	101	P/P	50 - 175
2443379	GULL2-qPCR	94.1		P	50 - 175
2443447	GULL2-qPCR	79.3		P	50 - 175
2444337	GULL2-qPCR	72.8		P	50 - 175

Reference Method: SOP-PCR-1.3
Batch ID: P436033

Spiked Sample	Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
2443197	GFD-qPCR	101	90.3	P/P	50 - 175
2443379	GFD-qPCR	90.8		P	50 - 175
2443447	GFD-qPCR	84.4		P	50 - 175
2444337	GFD-qPCR	81.3		P	50 - 175

Quality Assurance Report Matrix Spike Accuracy

Reference Method: SOP-PCR-1.4
Batch ID: P436033

Spiked Sample	Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
2443197	BacR-qPCR	85.7	88.2	P/P	50 - 175
2443379	BacR-qPCR	89.4		P	50 - 175
2443415	BacR-qPCR	77.0		P	50 - 175
2443447	BacR-qPCR	77.1		P	50 - 175
2444335	BacR-qPCR	72.2		P	50 - 175
2444337	BacR-qPCR	78.9		P	50 - 175
2444617	BacR-qPCR	77.8		P	50 - 175
2444690	BacR-qPCR	88.8		P	50 - 175
2444691	BacR-qPCR	82.1		P	50 - 175
2444692	BacR-qPCR	73.1		P	50 - 175
2444960	BacR-qPCR	61.0		P	50 - 175

Reference Method: SOP-PCR-1.5
Batch ID: P436033

Spiked Sample	Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
2443197	DG3-qPCR	92.6	98.9	P/P	50 - 175
2443379	DG3-qPCR	93.6		P	50 - 175
2443415	DG3-qPCR	88.8		P	50 - 175
2443447	DG3-qPCR	79.1		P	50 - 175
2444335	DG3-qPCR	68.7		P	50 - 175
2444337	DG3-qPCR	63.4		P	50 - 175
2444617	DG3-qPCR	92.3		P	50 - 175
2444690	DG3-qPCR	90.4		P	50 - 175
2444691	DG3-qPCR	83.1		P	50 - 175
2444692	DG3-qPCR	74.6		P	50 - 175
2444960	DG3-qPCR	67.7		P	50 - 175

Quality Assurance Report Precision

Reference Method: SM 10150 B
Batch ID: P436795

Replicated Lab Sample	Component	% RSD/RPD	Sample/Spike/LCS*	Pass/Fail	Control Limits
2443378	Chlorophyll-a, Uncorrected	6.92	Sample	P	0 - 25

Reference Method: SOP-PCR-1.0
Batch ID: P436033

Replicated Lab Sample	Component	% RSD/RPD	Sample/Spike/LCS*	Pass/Fail	Control Limits
2443197	HF183-qPCR	3.33	Spike	P	0 - 25

Reference Method: SOP-PCR-1.2
Batch ID: P436033

Replicated Lab Sample	Component	% RSD/RPD	Sample/Spike/LCS*	Pass/Fail	Control Limits
2443197	GULL2-qPCR	11.2	Spike	P	0 - 25

Quality Assurance Report Precision

Reference Method: SOP-PCR-1.3
Batch ID: P436033

Replicated Lab Sample	Component	% RSD/RPD	Sample/Spike/LCS*	Pass/Fail	Control Limits
2443197	GFD-qPCR	10.9	Spike	P	0 - 25

Reference Method: SOP-PCR-1.4
Batch ID: P436033

Replicated Lab Sample	Component	% RSD/RPD	Sample/Spike/LCS*	Pass/Fail	Control Limits
2443197	BacR-qPCR	2.88	Spike	P	0 - 25

Reference Method: SOP-PCR-1.5
Batch ID: P436033

Replicated Lab Sample	Component	% RSD/RPD	Sample/Spike/LCS*	Pass/Fail	Control Limits
2443197	DG3-qPCR	6.53	Spike	P	0 - 25

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

Quality Assurance Report Surrogates

Lab Sample ID: 2443197
Field Sample ID: G3NW0135

Reference Method	Surrogate	% Rec.	Pass/Fail	Control Limits
SOP-PCR-1.0	SKETA-qPCR	90.0	P	50 - 300
SOP-PCR-1.0	SKETA-qPCR	91.5	P	50 - 300
SOP-PCR-1.2	SKETA-qPCR	104	P	50 - 300
SOP-PCR-1.2	SKETA-qPCR	107	P	50 - 300
SOP-PCR-1.3	SKETA-qPCR	106	P	50 - 300
SOP-PCR-1.3	SKETA-qPCR	103	P	50 - 300
SOP-PCR-1.4	SKETA-qPCR	106	P	50 - 300
SOP-PCR-1.4	SKETA-qPCR	106	P	50 - 300
SOP-PCR-1.5	SKETA-qPCR	103	P	50 - 300
SOP-PCR-1.5	SKETA-qPCR	104	P	50 - 300

Quality Assurance Report Summary

Ref. Method	Analyte	LCS % Recovery		MS % Recovery			LCS	Precision SMP	MS
SM 10150 B	Chlorophyll-a, Corrected	95.1							
	Chlorophyll-a, Uncorrected							6.92	
SOP-PCR-1.0	HF183-qPCR	85.4	79.9	83.3	80.6	87.6			3.33
				87.7					
SOP-PCR-1.2	GULL2-qPCR	79.3	96.6	90.2	101	94.1			11.2
				79.3					
SOP-PCR-1.3	GFD-qPCR	67.7	83.8	101	90.3	90.8			10.9
				84.4					

Quality Assurance Report Summary

Ref. Method	Analyte	LCS % Recovery		MS % Recovery			Precision SMP	MS
SOP-PCR-1.4	BacR-qPCR	46.2	104	85.7	88.2	89.4		2.88
SOP-PCR-1.5	DG3-qPCR	43.9	94.3	88.8	79.1	68.7		6.53
				77.0				
				92.6				

Reference Method Descriptions

Method	Description	Associated Samples
SM 10150 B	Phytoplankton chlorophyll-a corrected, uncorrected, and phaeophytin by spectrophotometry	2443187, 2443188
SM 9223 B Quanti-Tray	E. coli by Colilert-18/QT by UWF in Pensacola	2443193, 2443194, 2443195, 2443196
SOP-PCR-1.0	Detection and quantification of human-specific HF183 Bacteroides genetic marker with quantitative polymerase chain reaction	2443197
SOP-PCR-1.2	Detection and quantification of coastal bird specific Catellicoccus marimammalium Gull2 genetic marker with quantitative polymerase chain reaction	2443197
SOP-PCR-1.3	Detection and quantification of bird specific Helicobacter GFD genetic marker with quantitative polymerase chain reaction	2443197
SOP-PCR-1.4	Detection and quantification of Ruminant specific Bacteroidetes BacR genetic marker with quantitative polymerase chain reaction	2443197
SOP-PCR-1.5	Detection and quantification of canine-specific DG3 Bacteroides genetic marker with quantitative polymerase chain reaction	2443197

Preparation and Analysis Log

Ref. Method	Received Date	Prep Date/Time	Prepared By	Analysis Date/Time	Analyzed By	Associated Samples
SM 10150 B	10/05/2023	10/05/2023 13:41	Tynan A Branch	10/19/2023 15:13	Keanu Thijm	2443187, 2443188
SM 9223 B Quanti-Tray	10/04/2023	10/04/2023 15:15		10/05/2023 11:50	UWF Lab	2443193, 2443194, 2443195, 2443196
SOP-PCR-1.0	10/05/2023	10/05/2023 14:35	Claudia Castro	10/30/2023 09:23	Roberta Tatti	2443197
SOP-PCR-1.2	10/05/2023	10/05/2023 14:35	Claudia Castro	11/03/2023 09:55	Roberta Tatti	2443197
SOP-PCR-1.3	10/05/2023	10/05/2023 14:35	Claudia Castro	11/06/2023 09:09	Roberta Tatti	2443197
SOP-PCR-1.4	10/05/2023	10/05/2023 14:35	Claudia Castro	11/02/2023 09:43	Roberta Tatti	2443197
SOP-PCR-1.5	10/05/2023	10/05/2023 14:35	Claudia Castro	11/02/2023 10:57	Roberta Tatti	2443197