Biological Analysis Report

NW-DIST-2022-08-05-01

Florida DEP Laboratory
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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-2022-08-01-64**
Customer: **NW-DIST**
Project ID: **SMP**

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Date Certified: 15-SEP-2022 13:51

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: Wrights Creek upstream of HWY 2 **Collection Date/Time: 08/04/2022 10:59**

Field ID: G3NW0236 Matrix: W-SURF-FRH

Sample ID	Ref. Method	Component	Result	Code	Units	Batch ID	QC Codes
2346612	SM 9223 B Quanti-Tray	Escherichia Coli-Quanti-Tray	148		MPN/100 mL		

Sample Location: Sikes Creek upstream of Harris Stevenson Rd **Collection Date/Time: 08/04/2022 11:50**

Field ID: G3NW0450 Matrix: W-SURF-FRH

Sample ID	Ref. Method	Component	Result	Code	Units	Batch ID	QC Codes
2346613	SM 9223 B Quanti-Tray	Escherichia Coli-Quanti-Tray	109		MPN/100 mL		

Sample Location: Wrights Creek upstream CR 179 **Collection Date/Time: 08/04/2022 12:56**

Field ID: G3NW0228 Matrix: W-SURF-FRH

Sample ID	Ref. Method	Component	Result	Code	Units	Batch ID	QC Codes
2346614	SM 9223 B Quanti-Tray	Escherichia Coli-Quanti-Tray	160		MPN/100 mL		

Sample Location: Limestone Branch upstream of Lake Victor Rd **Collection Date/Time: 08/04/2022 13:45**

Field ID: G3NW0232 Matrix: W-SURF-FRH

Sample ID	Ref. Method	Component	Result	Code	Units	Batch ID	QC Codes
2346615	SM 9223 B Quanti-Tray	Escherichia Coli-Quanti-Tray	645		MPN/100 mL		
2346616	SOP-PCR-1.0	HF183-qPCR**	1.7E+03	U	TSC/100 mL	P417615	
	SOP-PCR-1.2	GULL2-qPCR**	1.8E+03	U	TSC/100 mL	P417615	
	SOP-PCR-1.3	GFD-qPCR**	360	U	TSC/100 mL	P417615	
	SOP-PCR-1.4	BacR-qPCR**	1.5E+03	U	TSC/100 mL	P417615	
	SOP-PCR-1.5	DG3-qPCR**	1.3E+03	U	TSC/100 mL	P417615	

Sample Location: Sikes Creek upstream of Bonifay Gritney Rd **Collection Date/Time: 08/04/2022 12:22**

Field ID: G3NW0159 Matrix: W-SURF-FRH

Sample ID	Ref. Method	Component	Result	Code	Units	Batch ID	QC Codes
2346599	SM 10200 H (mod.)	Chlorophyll-a, Corrected	1.0	I	ug/L	P418132	
		Phaeophytin-a	0.90	U	ug/L	P418132	
		Chlorophyll-a, Uncorrected	0.79	I	ug/L	P418132	
2346611	SM 9223 B Quanti-Tray	Escherichia Coli-Quanti-Tray	464		MPN/100 mL		

Ref. Method and Comment:
SM 10200 H (mod.): 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

Reference Method: SM 10200 H (mod.)
Batch ID: P418132

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

Quality Assurance Report
Method Blank Results

Reference Method: SOP-PCR-1.0
Batch ID: P417615

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

Reference Method: SOP-PCR-1.2
Batch ID: P417615

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

Reference Method: SOP-PCR-1.3
Batch ID: P417615

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

Reference Method: SOP-PCR-1.4
Batch ID: P417615

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

Reference Method: SOP-PCR-1.5
Batch ID: P417615

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

Quality Assurance Report
Laboratory Control Sample Accuracy

Reference Method: SM 10200 H (mod.)
Batch ID: P418132

Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
Chlorophyll-a, Corrected	88.5		P	84 - 116

Reference Method: SOP-PCR-1.0
Batch ID: P417615

Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
HF183-qPCR	64.7	83.4	P/P	50 - 175

Reference Method: SOP-PCR-1.2
Batch ID: P417615

Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
GULL2-qPCR	58.8	79.4	P/P	50 - 300

Reference Method: SOP-PCR-1.3
Batch ID: P417615

Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits

Quality Assurance Report
Laboratory Control Sample Accuracy

Reference Method: SOP-PCR-1.3
Batch ID: P417615

Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
GFD-qPCR	90.4	73.3	P/P	50 - 175

Reference Method: SOP-PCR-1.4
Batch ID: P417615

Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
BacR-qPCR	93.0	83.2	P/P	50 - 175

Reference Method: SOP-PCR-1.5
Batch ID: P417615

Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
DG3-qPCR	81.7	95.6	P/P	50 - 175

Quality Assurance Report
Matrix Spike Accuracy

Reference Method: SOP-PCR-1.0
Batch ID: P417615

Spiked Sample	Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
2345962	HF183-qPCR	87.9	87.9	P/P	50 - 175
2346162	HF183-qPCR	87.4		P	50 - 175
2346393	HF183-qPCR	82.5		P	50 - 175
2346587	HF183-qPCR	94.6		P	50 - 175
2346598	HF183-qPCR	96.3		P	50 - 175
2346616	HF183-qPCR	102		P	50 - 175
2347375	HF183-qPCR	84.4		P	50 - 175
2347737	HF183-qPCR	92.4		P	50 - 175
2347738	HF183-qPCR	103		P	50 - 175
2347739	HF183-qPCR	101		P	50 - 175
2347740	HF183-qPCR	110		P	50 - 175
2347741	HF183-qPCR	116		P	50 - 175
2347742	HF183-qPCR	101		P	50 - 175

Reference Method: SOP-PCR-1.2
Batch ID: P417615

Spiked Sample	Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
2345962	GULL2-qPCR	79.3	78.3	P/P	50 - 300
2346162	GULL2-qPCR	75.8		P	50 - 300
2346393	GULL2-qPCR	90.8		P	50 - 300
2346616	GULL2-qPCR	70.4		P	50 - 300
2347375	GULL2-qPCR	87.6		P	50 - 300
2347737	GULL2-qPCR	62.9		P	50 - 300
2347738	GULL2-qPCR	70.5		P	50 - 300
2347739	GULL2-qPCR	82.3		P	50 - 300
2347740	GULL2-qPCR	88.9		P	50 - 300
2347741	GULL2-qPCR	83.9		P	50 - 300
2347742	GULL2-qPCR	82.5		P	50 - 300

Quality Assurance Report
Matrix Spike Accuracy

Reference Method: SOP-PCR-1.3
Batch ID: P417615

Spiked Sample	Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
2345962	GFD-qPCR	106	102	P/P	50 - 175
2346162	GFD-qPCR	85.7		P	50 - 175
2346393	GFD-qPCR	103		P	50 - 175
2346616	GFD-qPCR	103		P	50 - 175
2347375	GFD-qPCR	93.7		P	50 - 175
2347737	GFD-qPCR	88.4		P	50 - 175
2347738	GFD-qPCR	97.1		P	50 - 175
2347739	GFD-qPCR	92.3		P	50 - 175
2347740	GFD-qPCR	95.1		P	50 - 175
2347741	GFD-qPCR	92.3		P	50 - 175
2347742	GFD-qPCR	93.3		P	50 - 175

Reference Method: SOP-PCR-1.4
Batch ID: P417615

Spiked Sample	Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
2345962	BacR-qPCR	71.6	75.4	P/P	50 - 175
2346162	BacR-qPCR	74.2		P	50 - 175
2346393	BacR-qPCR	70.2		P	50 - 175
2346587	BacR-qPCR	93.2		P	50 - 175
2346598	BacR-qPCR	73.8		P	50 - 175
2346616	BacR-qPCR	62.7		P	50 - 175
2347375	BacR-qPCR	86.6		P	50 - 175

Reference Method: SOP-PCR-1.5
Batch ID: P417615

Spiked Sample	Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
2345962	DG3-qPCR	94.5	93.2	P/P	50 - 175
2346162	DG3-qPCR	89.6		P	50 - 175
2346393	DG3-qPCR	90.7		P	50 - 175
2346587	DG3-qPCR	102		P	50 - 175
2346598	DG3-qPCR	102		P	50 - 175
2346616	DG3-qPCR	108		P	50 - 175
2347375	DG3-qPCR	102		P	50 - 175
2347737	DG3-qPCR	94.0		P	50 - 175
2347738	DG3-qPCR	98.0		P	50 - 175
2347739	DG3-qPCR	103		P	50 - 175
2347740	DG3-qPCR	103		P	50 - 175
2347741	DG3-qPCR	111		P	50 - 175
2347742	DG3-qPCR	102		P	50 - 175

Quality Assurance Report
Precision

Reference Method: SOP-PCR-1.0
Batch ID: P417615

Replicated Lab Sample	Component	% RSD/RPD	Sample/Spike/LCS*	Pass/Fail	Control Limits
2345962	HF183-qPCR	0.0893	Spike	P	0 - 25

Quality Assurance Report
Precision

Reference Method: SOP-PCR-1.2
Batch ID: P417615

Replicated Lab Sample	Component	% RSD/RPD	Sample/Spike/LCS*	Pass/Fail	Control Limits
2345962	GULL2-qPCR	1.28	Spike	P	0 - 25

Reference Method: SOP-PCR-1.3
Batch ID: P417615

Replicated Lab Sample	Component	% RSD/RPD	Sample/Spike/LCS*	Pass/Fail	Control Limits
2345962	GFD-qPCR	4.24	Spike	P	0 - 25

Reference Method: SOP-PCR-1.4
Batch ID: P417615

Replicated Lab Sample	Component	% RSD/RPD	Sample/Spike/LCS*	Pass/Fail	Control Limits
2345962	BacR-qPCR	5.23	Spike	P	0 - 25

Reference Method: SOP-PCR-1.5
Batch ID: P417615

Replicated Lab Sample	Component	% RSD/RPD	Sample/Spike/LCS*	Pass/Fail	Control Limits
2345962	DG3-qPCR	1.35	Spike	P	0 - 25

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

Quality Assurance Report
Surrogates

Lab Sample ID: 2346616
Field Sample ID: G3NW0232

Reference Method	Surrogate	% Rec.	Pass/Fail	Control Limits
SOP-PCR-1.0	SKETA-qPCR	103	P	50 - 300
SOP-PCR-1.2	SKETA-qPCR	80.2	P	50 - 300
SOP-PCR-1.3	SKETA-qPCR	87.6	P	50 - 300
SOP-PCR-1.4	SKETA-qPCR	83.8	P	50 - 300
SOP-PCR-1.5	SKETA-qPCR	95.6	P	50 - 300

Quality Assurance Report
Summary

Ref. Method	Analyte	LCS % Recovery	MS % Recovery	Precision		
				LCS	SMP	MS
SM 10200 H (mod.)	Chlorophyll-a, Corrected	88.5				
SOP-PCR-1.0	HF183-qPCR	83.4 64.7	87.9 87.9 87.4 82.5			0.0893
SOP-PCR-1.2	GULL2-qPCR	79.4 58.8	79.3 78.3 75.8 90.8			1.28

Quality Assurance Report
Summary

Ref. Method	Analyte	LCS % Recovery	MS % Recovery	Precision		
				LCS	SMP	MS
SOP-PCR-1.3	GFD-qPCR	73.3 90.4	106 102 85.7 103			4.24
SOP-PCR-1.4	BacR-qPCR	83.2 93.0	71.6 75.4 74.2 70.2			5.23
SOP-PCR-1.5	DG3-qPCR	95.6 81.7	94.5 93.2 89.6 90.7			1.35

Reference Method Descriptions

Method	Description	Associated Samples
SM 10200 H (mod.)	Phytoplankton chlorophyll-a corrected, uncorrected, and phaeophytin by spectrophotometry	2346599
SM 9223 B Quanti-Tray	E. coli by Colilert-18/QT by UWF in Pensacola	2346611, 2346612, 2346613, 2346614, 2346615
SOP-PCR-1.0	Detection and quantification of human-specific HF183 Bacteroides genetic marker with quantitative polymerase chain reaction	2346616
SOP-PCR-1.2	Detection and quantification of coastal bird specific Catellicoccus marimammalium Gull2 genetic marker with quantitative polymerase chain reaction	2346616
SOP-PCR-1.3	Detection and quantification of bird specific Helicobacter GFD genetic marker with quantitative polymerase chain reaction	2346616
SOP-PCR-1.4	Detection and quantification of Ruminant specific Bacteroidetes BacR genetic marker with quantitative polymerase chain reaction	2346616
SOP-PCR-1.5	Detection and quantification of canine-specific DG3 Bacteroides genetic marker with quantitative polymerase chain reaction	2346616

Preparation and Analysis Log

Ref. Method	Received Date	Prep Date/Time	Prepared By	Analysis Date/Time	Analyzed By	Associated Samples
SM 10200 H (mod.)	08/05/2022	08/05/2022 14:16	Ethan Wickline	08/11/2022 11:06	Carina A. Tull	2346599
SM 9223 B Quanti-Tray	08/04/2022	08/04/2022 17:00		08/05/2022 12:56	UWF Lab	2346611, 2346612, 2346613, 2346614, 2346615
SOP-PCR-1.0	08/05/2022	08/05/2022 13:50	Claudia Castro	08/29/2022 14:25	DeAsia Armster	2346616
SOP-PCR-1.2	08/05/2022	08/05/2022 13:50	Claudia Castro	09/01/2022 10:48	Roberta Tatti	2346616
SOP-PCR-1.3	08/05/2022	08/05/2022 13:50	Claudia Castro	09/02/2022 09:19	DeAsia Armster	2346616
SOP-PCR-1.4	08/05/2022	08/05/2022 13:50	Claudia Castro	08/30/2022 09:34	DeAsia Armster	2346616
SOP-PCR-1.5	08/05/2022	08/05/2022 13:50	Claudia Castro	08/31/2022 09:46	Roberta Tatti	2346616