

Biological Analysis Report

NW-DIST-2022-08-17-04

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: **Highway 20 Run**
Request ID: **RQ-2022-08-15-27**
Customer: **NW-DIST**
Project ID: **SMP**

Send Reports to:
FL Dept. of Environmental Protection
160 W Government Street
Suite 208
Pensacola, FL 32502-5794
Attn: Myranda Butler

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Certified by: Sarah Menz, Environmental Administrator

Date Certified: 28-SEP-2022 15:42

A handwritten signature in black 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, Microbiology, 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: Alaqua Creek at Park boat ramp

Collection Date/Time: 08/16/2022 10:33

Field ID: G3NW0163

Matrix: W-SURF-FRH

Sample ID	Ref. Method	Component	Result	Code	Units	Batch ID	QC Codes
2350136	Enterolert/QT	Enterococci-Quanti-Tray	359		MPN/100 mL		

Sample Location: Alaqua Creek below Portland Park

Collection Date/Time: 08/16/2022 11:10

Field ID: G3NW0443

Matrix: W-SURF-FRH

Sample ID	Ref. Method	Component	Result	Code	Units	Batch ID	QC Codes
2350137	Enterolert/QT	Enterococci-Quanti-Tray	187		MPN/100 mL		

Sample Location: Alaqua Bayou Center

Collection Date/Time: 08/16/2022 11:32

Field ID: G3NW0180

Matrix: W-SURF-SLT

Sample ID	Ref. Method	Component	Result	Code	Units	Batch ID	QC Codes
2350117	SM 10200 H (mod.)	Chlorophyll-a, Corrected	7.3	A	ug/L	P419075	
		Phaeophytin-a	1.8	U	ug/L	P419075	
		Chlorophyll-a, Uncorrected	7.1	A	ug/L	P419075	
2350131	Enterolert/QT	Enterococci-Quanti-Tray	31		MPN/100 mL		

Ref. Method and Comment:

SM 10200 H (mod.): Laboratory duplicate result is 1.8 U for Phaeophytin-a. Precision data is unavailable due to the small amount of analyte in the QC sample.

Sample Location: Boggy Bayou at Lincoln Park, Okaloosa County (also 21FLDOH)

Collection Date/Time: 08/16/2022 13:37

Field ID: OKALOOSA355

Matrix: W-SURF-SLT

Sample ID	Ref. Method	Component	Result	Code	Units	Batch ID	QC Codes
2350133	Enterolert/QT	Enterococci-Quanti-Tray	20		MPN/100 mL		
2350141	SOP-PCR-1.0	HF183-qPCR**	1.7E+03	U	TSC/100 mL	P418252	
	SOP-PCR-1.2	GULL2-qPCR**	1.8E+03	U	TSC/100 mL	P418252	
	SOP-PCR-1.3	GFD-qPCR**	430	I	TSC/100 mL	P418252	
	SOP-PCR-1.4	BacR-qPCR**	1.5E+03	U	TSC/100 mL	P418252	
	SOP-PCR-1.5	DG3-qPCR**	1.3E+03	U	TSC/100 mL	P418252	

Sample Location: Boggy Bayou Mouth

Collection Date/Time: 08/16/2022 14:02

Field ID: G3NW0186

Matrix: W-SURF-SLT

Sample ID	Ref. Method	Component	Result	Code	Units	Batch ID	QC Codes
2350118	SM 10200 H (mod.)	Chlorophyll-a, Corrected	4.7		ug/L	P419075	
		Phaeophytin-a	0.90	U	ug/L	P419075	
		Chlorophyll-a, Uncorrected	4.6		ug/L	P419075	
2350132	Enterolert/QT	Enterococci-Quanti-Tray	10	U	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.

Sample Location: Basin Bayou North End

Collection Date/Time: 08/16/2022 12:20

Field ID: G3NW0102

Matrix: W-SURF-FRH

Sample ID	Ref. Method	Component	Result	Code	Units	Batch ID	QC Codes
2350123	SM 9222 D	Fecal Coliforms-Membrane Filter	28	BQ	cfu/100 mL	P418372	
2350134	Enterolert/QT	Enterococci-Quanti-Tray	10		MPN/100 mL		

Ref. Method and Comment:
SM 9222 D: Laboratory duplicate result is 28 B. Precision data for this duplicate is unavailable due to the colony count falling outside of method acceptable range.

Sample Location: Rocky Bayou Fred Gannon State Park Collection Date/Time: 08/16/2022 13:10

Field ID: G3NW0230

Matrix: W-SURF-SLT

Sample ID	Ref. Method	Component	Result	Code	Units	Batch ID	QC Codes
2350124	SM 9222 D	Fecal Coliforms-Membrane Filter	5	BQ	cfu/100 mL	P418372	
2350135	Enterolert/QT	Enterococci-Quanti-Tray	10		MPN/100 mL		

Ref. Method and Comment:
SM 9222 D: Precision data for QC sample is unavailable due to the colony count falling outside of method acceptable range.

Quality Assurance Report
Method Blank Results

Reference Method: SM 10200 H (mod.)
Batch ID: P419075

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: SM 9222 D
Batch ID: P418372

Component	Result	Code	Units
Fecal Coliforms-Membrane Filter	2	U	cfu/100 mL

Reference Method: SOP-PCR-1.0
Batch ID: P418252

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

Reference Method: SOP-PCR-1.2
Batch ID: P418252

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

Reference Method: SOP-PCR-1.3
Batch ID: P418252

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

Reference Method: SOP-PCR-1.4
Batch ID: P418252

Component	Result	Code	Units
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Quality Assurance Report Method Blank Results

Reference Method: SOP-PCR-1.4
Batch ID: P418252

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

Reference Method: SOP-PCR-1.5
Batch ID: P418252

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: P419075

Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
Chlorophyll-a, Corrected	109		P	84 - 116

Reference Method: SOP-PCR-1.0
Batch ID: P418252

Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
HF183-qPCR	98.2	96.2	P/P	50 - 175

Reference Method: SOP-PCR-1.2
Batch ID: P418252

Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
GULL2-qPCR	98.0	105	P/P	50 - 300

Reference Method: SOP-PCR-1.3
Batch ID: P418252

Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
GFD-qPCR	69.1	101	P/P	50 - 175

Reference Method: SOP-PCR-1.4
Batch ID: P418252

Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
BacR-qPCR	89.5	99.2	P/P	50 - 175

Reference Method: SOP-PCR-1.5
Batch ID: P418252

Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
DG3-qPCR	114	110	P/P	50 - 175

Quality Assurance Report Matrix Spike Accuracy

Quality Assurance Report Matrix Spike Accuracy

Reference Method: SOP-PCR-1.0

Batch ID: P418252

Spiked Sample	Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
2349697	HF183-qPCR	78.0	83.4	P	50 - 175
2350037	HF183-qPCR	81.2		P	50 - 175
2350038	HF183-qPCR	84.2		P	50 - 175
2350039	HF183-qPCR	92.5		P	50 - 175
2350141	HF183-qPCR	82.8		P	50 - 175
2350219	HF183-qPCR	94.0		P/P	50 - 175
2350517	HF183-qPCR	96.9		P	50 - 175
2350518	HF183-qPCR	88.7		P	50 - 175
2350562	HF183-qPCR	97.4		P	50 - 175
2351305	HF183-qPCR	86.7		P	50 - 175
2351306	HF183-qPCR	81.8		P	50 - 175
2351307	HF183-qPCR	89.4		P	50 - 175
2351982	HF183-qPCR	110		P	50 - 175

Reference Method: SOP-PCR-1.2

Batch ID: P418252

Spiked Sample	Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
2349697	GULL2-qPCR	89.6	65.3	P	50 - 300
2350037	GULL2-qPCR	67.5		P	50 - 300
2350038	GULL2-qPCR	95.8		P	50 - 300
2350039	GULL2-qPCR	93.5		P	50 - 300
2350141	GULL2-qPCR	94.0		P	50 - 300
2350219	GULL2-qPCR	73.0		P/P	50 - 300
2350517	GULL2-qPCR	74.4		P	50 - 300
2350518	GULL2-qPCR	92.8		P	50 - 300
2350562	GULL2-qPCR	76.1		P	50 - 300
2351305	GULL2-qPCR	84.6		P	50 - 300
2351306	GULL2-qPCR	85.2		P	50 - 300
2351307	GULL2-qPCR	75.8		P	50 - 300
2351982	GULL2-qPCR	80.3		P	50 - 300

Reference Method: SOP-PCR-1.3

Batch ID: P418252

Spiked Sample	Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
2349697	GFD-qPCR	92.7	93.6	P	50 - 175
2350037	GFD-qPCR	91.4		P	50 - 175
2350038	GFD-qPCR	96.4		P	50 - 175
2350039	GFD-qPCR	176		F	50 - 175
2350141	GFD-qPCR	96.2		P	50 - 175
2350219	GFD-qPCR	95.8		P/P	50 - 175
2350517	GFD-qPCR	94.8		P	50 - 175
2350518	GFD-qPCR	91.3		P	50 - 175
2350562	GFD-qPCR	93.0		P	50 - 175
2351305	GFD-qPCR	92.3		P	50 - 175
2351306	GFD-qPCR	93.1		P	50 - 175
2351307	GFD-qPCR	90.0		P	50 - 175
2351982	GFD-qPCR	90.9		P	50 - 175

Quality Assurance Report Matrix Spike Accuracy

Reference Method: SOP-PCR-1.4
Batch ID: P418252

Spiked Sample	Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
2349697	BacR-qPCR	70.1		P	50 - 175
2350037	BacR-qPCR	61.4		P	50 - 175
2350038	BacR-qPCR	79.9		P	50 - 175
2350039	BacR-qPCR	77.3		P	50 - 175
2350141	BacR-qPCR	74.0		P	50 - 175
2350219	BacR-qPCR	67.6	67.8	P/P	50 - 175
2350517	BacR-qPCR	67.1		P	50 - 175
2350518	BacR-qPCR	79.2		P	50 - 175
2350562	BacR-qPCR	79.3		P	50 - 175
2351305	BacR-qPCR	72.5		P	50 - 175
2351306	BacR-qPCR	62.4		P	50 - 175
2351307	BacR-qPCR	67.8		P	50 - 175
2351982	BacR-qPCR	76.8		P	50 - 175

Reference Method: SOP-PCR-1.5
Batch ID: P418252

Spiked Sample	Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
2349697	DG3-qPCR	109		P	50 - 175
2350037	DG3-qPCR	103		P	50 - 175
2350038	DG3-qPCR	114		P	50 - 175
2350039	DG3-qPCR	116		P	50 - 175
2350141	DG3-qPCR	114		P	50 - 175
2350219	DG3-qPCR	106	108	P/P	50 - 175
2350517	DG3-qPCR	115		P	50 - 175
2350518	DG3-qPCR	111		P	50 - 175
2350562	DG3-qPCR	110		P	50 - 175
2351305	DG3-qPCR	107		P	50 - 175
2351306	DG3-qPCR	103		P	50 - 175
2351307	DG3-qPCR	101		P	50 - 175
2351982	DG3-qPCR	116		P	50 - 175

Quality Assurance Report Precision

Reference Method: SM 10200 H (mod.)
Batch ID: P419075

Replicated Lab Sample	Component	% RSD/RPD	Sample/Spike/LCS*	Pass/Fail	Control Limits
2350117	Chlorophyll-a, Corrected	2.45	Sample	P	0 - 25
2350117	Chlorophyll-a, Uncorrected	2.73	Sample	P	0 - 25

Reference Method: SOP-PCR-1.0
Batch ID: P418252

Replicated Lab Sample	Component	% RSD/RPD	Sample/Spike/LCS*	Pass/Fail	Control Limits
2350219	HF183-qPCR	11.9	Spike	P	0 - 25

Quality Assurance Report Precision

Reference Method: SOP-PCR-1.2
Batch ID: P418252

Replicated Lab Sample	Component	% RSD/RPD	Sample/Spike/LCS*	Pass/Fail	Control Limits
2350219	GULL2-qPCR	11.1	Spike	P	0 - 25

Reference Method: SOP-PCR-1.3
Batch ID: P418252

Replicated Lab Sample	Component	% RSD/RPD	Sample/Spike/LCS*	Pass/Fail	Control Limits
2350219	GFD-qPCR	2.31	Spike	P	0 - 25

Reference Method: SOP-PCR-1.4
Batch ID: P418252

Replicated Lab Sample	Component	% RSD/RPD	Sample/Spike/LCS*	Pass/Fail	Control Limits
2350219	BacR-qPCR	0.233	Spike	P	0 - 25

Reference Method: SOP-PCR-1.5
Batch ID: P418252

Replicated Lab Sample	Component	% RSD/RPD	Sample/Spike/LCS*	Pass/Fail	Control Limits
2350219	DG3-qPCR	1.94	Spike	P	0 - 25

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

Quality Assurance Report Surrogates

Lab Sample ID: 2350141
Field Sample ID: OKALOOSA355

Reference Method	Surrogate	% Rec.	Pass/Fail	Control Limits
SOP-PCR-1.0	SKETA-qPCR	120	P	50 - 300
SOP-PCR-1.2	SKETA-qPCR	133	P	50 - 300
SOP-PCR-1.3	SKETA-qPCR	138	P	50 - 300
SOP-PCR-1.4	SKETA-qPCR	123	P	50 - 300
SOP-PCR-1.5	SKETA-qPCR	115	P	50 - 300

Quality Assurance Report Summary

Ref. Method	Analyte	LCS % Recovery		MS % Recovery			LCS	Precision SMP	MS
SM 10200 H (mod.)	Chlorophyll-a, Corrected	109						2.45	
	Chlorophyll-a, Uncorrected							2.73	
SOP-PCR-1.0	HF183-qPCR	96.2	98.2	96.9	88.7	97.4			11.9
				86.7					
SOP-PCR-1.2	GULL2-qPCR	105	98.0	89.6	67.5	95.8			11.1
				93.5					

Quality Assurance Report Summary

Ref. Method	Analyte	LCS % Recovery		MS % Recovery			LCS	Precision SMP	MS
SOP-PCR-1.3	GFD-qPCR	101	69.1	92.7	91.4	96.4			2.31
SOP-PCR-1.4	BacR-qPCR	89.5	99.2	74.0	67.6	67.8			0.233
SOP-PCR-1.5	DG3-qPCR	110	114	109	103	114			1.94
				176					
				67.1					
				116					

Reference Method Descriptions

Method	Description	Associated Samples
Enterolert/QT	Enterococci by Enterolert/QT by UWF in Pensacola	2350131, 2350132, 2350133, 2350134, 2350135, 2350136, 2350137
SM 10200 H (mod.)	Phytoplankton chlorophyll-a corrected, uncorrected, and phaeophytin by spectrophotometry	2350117, 2350118
SM 9222 D	Fecal coliforms by membrane filter method - non-chlorinated water systems	2350123, 2350124
SOP-PCR-1.0	Detection and quantification of human-specific HF183 Bacteroides genetic marker with quantitative polymerase chain reaction	2350141
SOP-PCR-1.2	Detection and quantification of coastal bird specific Catellicoccus marimammalium Gull2 genetic marker with quantitative polymerase chain reaction	2350141
SOP-PCR-1.3	Detection and quantification of bird specific Helicobacter GFD genetic marker with quantitative polymerase chain reaction	2350141
SOP-PCR-1.4	Detection and quantification of Ruminant specific Bacteroidetes BacR genetic marker with quantitative polymerase chain reaction	2350141
SOP-PCR-1.5	Detection and quantification of canine-specific DG3 Bacteroides genetic marker with quantitative polymerase chain reaction	2350141

Preparation and Analysis Log

Ref. Method	Received Date	Prep Date/Time	Prepared By	Analysis Date/Time	Analyzed By	Associated Samples
Enterolert/QT	08/16/2022	08/16/2022 16:35		08/17/2022 17:01	UWF Lab	2350131, 2350132, 2350133, 2350134, 2350135, 2350136, 2350137
SM 10200 H (mod.)	08/17/2022	08/17/2022 14:06	Ethan Wickline	08/25/2022 09:45	Carina A. Tull	2350117, 2350118
SM 9222 D	08/17/2022	08/17/2022 15:21	Jillian G Hanley	08/18/2022 14:57	Jillian G Hanley	2350123, 2350124
SOP-PCR-1.0	08/17/2022	08/17/2022 14:30	Claudia Castro	09/16/2022 12:14	Roberta Tatti	2350141
SOP-PCR-1.2	08/17/2022	08/17/2022 14:30	Claudia Castro	09/20/2022 13:26	DeAsia Armster	2350141
SOP-PCR-1.3	08/17/2022	08/17/2022 14:30	Claudia Castro	09/21/2022 11:36	Roberta Tatti	2350141
SOP-PCR-1.4	08/17/2022	08/17/2022 14:30	Claudia Castro	09/19/2022 12:58	Roberta Tatti	2350141
SOP-PCR-1.5	08/17/2022	08/17/2022 14:30	Claudia Castro	09/19/2022 14:37	Roberta Tatti	2350141