

Biological Analysis Report

NW-DIST-2022-07-13-01

Florida Department of Environmental Protection
Central 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-2022-07-11-35**
Customer: **NW-DIST**
Project ID: **SMP**

Send Reports to:
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160 W Government Street
Suite 208
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Certified by: Sarah Menz, Environmental Administrator

Date Certified: 24-AUG-2022 15:21

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, 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: Basin Bayou North End

Collection Date/Time: 07/12/2022 12:30

Field ID: G3NW0102

Matrix: W-SURF-FRH

Sample ID	Ref. Method	Component	Result	Code	Units	Batch ID	QC Codes
2341216	SM 9222 D	Fecal Coliforms-Membrane Filter	3	BQ	cfu/100 mL	P416702	
2341224	Enterolert/QT	Enterococci-Quanti-Tray	10		MPN/100 mL		

Sample Location: Rocky Bayou Fred Gannon State Park

Collection Date/Time: 07/12/2022 13:15

Field ID: G3NW0230

Matrix: W-SURF-SLT

Sample ID	Ref. Method	Component	Result	Code	Units	Batch ID	QC Codes
2341217	SM 9222 D	Fecal Coliforms-Membrane Filter	52	AQ	cfu/100 mL	P416702	
2341225	Enterolert/QT	Enterococci-Quanti-Tray	20		MPN/100 mL		

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

Collection Date/Time: 07/12/2022 13:45

Field ID: OKALOOSA355

Matrix: W-SURF-SLT

Sample ID	Ref. Method	Component	Result	Code	Units	Batch ID	QC Codes
2341223	Enterolert/QT	Enterococci-Quanti-Tray	63		MPN/100 mL		
2341229	SOP-PCR-1.0	HF183-qPCR**	1.1E+03	U	TSC/100 mL	P416493	
	SOP-PCR-1.2	GULL2-qPCR**	1.2E+03	U	TSC/100 mL	P416493	
	SOP-PCR-1.3	GFD-qPCR**	1.6E+03		TSC/100 mL	P416493	
	SOP-PCR-1.4	BacR-qPCR**	1.0E+03	U	TSC/100 mL	P416493	
	SOP-PCR-1.5	DG3-qPCR**	860	U	TSC/100 mL	P416493	

Sample Location: Boggy Bayou Mouth

Collection Date/Time: 07/12/2022 14:00

Field ID: G3NW0186

Matrix: W-SURF-SLT

Sample ID	Ref. Method	Component	Result	Code	Units	Batch ID	QC Codes
2341214	SM 10200 H (mod.)	Chlorophyll-a, Corrected	3.5	I	ug/L	P416892	
		Phaeophytin-a	1.4	U	ug/L	P416892	
		Chlorophyll-a, Uncorrected	3.4		ug/L	P416892	
2341221	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: Black Creek

Collection Date/Time: 07/12/2022 10:20

Field ID: G3NW0131

Matrix: W-SURF-FRH

Sample ID	Ref. Method	Component	Result	Code	Units	Batch ID	QC Codes
2341228	SM 9223 B Quanti-Tray	Escherichia Coli-Quanti-Tray	175		MPN/100 mL		

Sample Location: Alaqua Creek at Park Boat Ramp

Collection Date/Time: 07/12/2022 11:00

Field ID: G3NW0163

Matrix: W-SURF-FRH

Sample ID	Ref. Method	Component	Result	Code	Units	Batch ID	QC Codes
2341226	Enterolert/QT	Enterococci-Quanti-Tray	246		MPN/100 mL		

Sample Location: Alaqua Creek Below Portland Park

Collection Date/Time: 07/12/2022 11:30

Field ID: G3NW0443

Matrix: W-SURF-FRH

Sample ID	Ref. Method	Component	Result	Code	Units	Batch ID	QC Codes
2341227	Enterolert/QT	Enterococci-Quanti-Tray	355		MPN/100 mL		

Sample Location: Alaqua Bayou Center

Collection Date/Time: 07/12/2022 11:45

Field ID: G3NW0180

Matrix: W-SURF-SLT

Sample ID	Ref. Method	Component	Result	Code	Units	Batch ID	QC Codes
2341215	SM 10200 H (mod.)	Chlorophyll-a, Corrected	14		ug/L	P416892	
		Phaeophytin-a	0.90	U	ug/L	P416892	
		Chlorophyll-a, Uncorrected	14		ug/L	P416892	
2341222	Enterolert/QT	Enterococci-Quanti-Tray	10		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: P416892

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

Reference Method: SM 9222 D
Batch ID: P416702

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

Reference Method: SOP-PCR-1.0
Batch ID: P416493

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

Reference Method: SOP-PCR-1.2
Batch ID: P416493

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

Reference Method: SOP-PCR-1.3
Batch ID: P416493

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

Reference Method: SOP-PCR-1.4
Batch ID: P416493

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

Quality Assurance Report Method Blank Results

Reference Method: SOP-PCR-1.5
Batch ID: P416493

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: P416892

Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
Chlorophyll-a, Corrected	90.0		P	84 - 116

Reference Method: SOP-PCR-1.0
Batch ID: P416493

Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
HF183-qPCR	69.4	82.6	P/P	50 - 175

Reference Method: SOP-PCR-1.2
Batch ID: P416493

Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
GULL2-qPCR	94.1	81.9	P/P	50 - 300

Reference Method: SOP-PCR-1.3
Batch ID: P416493

Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
GFD-qPCR	86.3	71.5	P/P	50 - 175

Reference Method: SOP-PCR-1.4
Batch ID: P416493

Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
BacR-qPCR	101	67.9	P/P	50 - 175

Reference Method: SOP-PCR-1.5
Batch ID: P416493

Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
DG3-qPCR	110	72.5	P/P	50 - 175

Quality Assurance Report Matrix Spike Accuracy

Reference Method: SOP-PCR-1.0
Batch ID: P416493

Spiked Sample	Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
2340643	HF183-qPCR	84.5	87.3	P/P	50 - 175
2340644	HF183-qPCR	86.9		P	50 - 175
2340645	HF183-qPCR	82.6		P	50 - 175

Quality Assurance Report Matrix Spike Accuracy

Reference Method: SOP-PCR-1.0

Batch ID: P416493

Spiked Sample	Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
2340836	HF183-qPCR	88.6		P	50 - 175
2340837	HF183-qPCR	91.0		P	50 - 175
2341229	HF183-qPCR	74.2		P	50 - 175
2341751	HF183-qPCR	92.3		P	50 - 175
2341934	HF183-qPCR	80.4		P	50 - 175

Reference Method: SOP-PCR-1.2

Batch ID: P416493

Spiked Sample	Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
2340643	GULL2-qPCR	94.1	91.9	P/P	50 - 300
2340644	GULL2-qPCR	90.6		P	50 - 300
2340645	GULL2-qPCR	90.2		P	50 - 300
2341229	GULL2-qPCR	75.8		P	50 - 300
2341751	GULL2-qPCR	83.3		P	50 - 300
2341934	GULL2-qPCR	82.8		P	50 - 300

Reference Method: SOP-PCR-1.3

Batch ID: P416493

Spiked Sample	Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
2340643	GFD-qPCR	87.5	96.2	P/P	50 - 175
2340644	GFD-qPCR	94.8		P	50 - 175
2340645	GFD-qPCR	95.6		P	50 - 175
2341229	GFD-qPCR	87.7		P	50 - 175
2341751	GFD-qPCR	100		P	50 - 175
2341934	GFD-qPCR	97.4		P	50 - 175

Reference Method: SOP-PCR-1.4

Batch ID: P416493

Spiked Sample	Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
2340643	BacR-qPCR	87.3	94.5	P/P	50 - 175
2340644	BacR-qPCR	83.1		P	50 - 175
2340645	BacR-qPCR	76.9		P	50 - 175
2340836	BacR-qPCR	82.5		P	50 - 175
2340837	BacR-qPCR	80.6		P	50 - 175
2341229	BacR-qPCR	73.4		P	50 - 175
2341751	BacR-qPCR	93.0		P	50 - 175
2341934	BacR-qPCR	79.1		P	50 - 175

Reference Method: SOP-PCR-1.5

Batch ID: P416493

Spiked Sample	Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
2340643	DG3-qPCR	102	98.0	P/P	50 - 175
2340644	DG3-qPCR	103		P	50 - 175
2340645	DG3-qPCR	94.0		P	50 - 175
2340836	DG3-qPCR	93.7		P	50 - 175
2340837	DG3-qPCR	96.8		P	50 - 175
2341229	DG3-qPCR	90.2		P	50 - 175
2341751	DG3-qPCR	92.1		P	50 - 175

Quality Assurance Report Matrix Spike Accuracy

Reference Method: SOP-PCR-1.5
Batch ID: P416493

Spiked Sample	Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
2341934	DG3-qPCR	88.6		P	50 - 175

Quality Assurance Report Precision

Reference Method: SM 10200 H (mod.)
Batch ID: P416892

Replicated Lab Sample	Component	% RSD/RPD	Sample/Spike/LCS*	Pass/Fail	Control Limits
2341108	Chlorophyll-a, Corrected	7.42	Sample	P	0 - 25
2341108	Chlorophyll-a, Uncorrected	6.52	Sample	P	0 - 25

Reference Method: SM 9222 D
Batch ID: P416702

Replicated Lab Sample	Component	% RSD/RPD	Sample/Spike/LCS*	Pass/Fail	Control Limits
2341217	Fecal Coliforms-Membrane Filter	23.1	Sample	P	0 - 50

Reference Method: SOP-PCR-1.0
Batch ID: P416493

Replicated Lab Sample	Component	% RSD/RPD	Sample/Spike/LCS*	Pass/Fail	Control Limits
2340643	HF183-qPCR	3.27	Spike	P	0 - 25

Reference Method: SOP-PCR-1.2
Batch ID: P416493

Replicated Lab Sample	Component	% RSD/RPD	Sample/Spike/LCS*	Pass/Fail	Control Limits
2340643	GULL2-qPCR	2.33	Spike	P	0 - 25

Reference Method: SOP-PCR-1.3
Batch ID: P416493

Replicated Lab Sample	Component	% RSD/RPD	Sample/Spike/LCS*	Pass/Fail	Control Limits
2340643	GFD-qPCR	9.46	Spike	P	0 - 25

Reference Method: SOP-PCR-1.4
Batch ID: P416493

Replicated Lab Sample	Component	% RSD/RPD	Sample/Spike/LCS*	Pass/Fail	Control Limits
2340643	BacR-qPCR	7.90	Spike	P	0 - 25

Quality Assurance Report Precision

Reference Method: SOP-PCR-1.5
 Batch ID: P416493

Replicated Lab Sample	Component	% RSD/RPD	Sample/Spike/LCS*	Pass/Fail	Control Limits
2340643	DG3-qPCR	4.02	Spike	P	0 - 25

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

Quality Assurance Report Surrogates

Lab Sample ID: 2341229
 Field Sample ID: OKALOOSA355

Reference Method	Surrogate	% Rec.	Pass/Fail	Control Limits
SOP-PCR-1.0	SKETA-qPCR	103	P	50 - 300
SOP-PCR-1.2	SKETA-qPCR	105	P	50 - 300
SOP-PCR-1.3	SKETA-qPCR	110	P	50 - 300
SOP-PCR-1.4	SKETA-qPCR	105	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	90.0						7.42	
	Chlorophyll-a, Uncorrected							6.52	
SM 9222 D	Fecal Coliforms-Membrane Filter							23.1	
SOP-PCR-1.0	HF183-qPCR	82.6	69.4	84.5	87.3	86.9			3.27
SOP-PCR-1.2	GULL2-qPCR	81.9	94.1	94.1	91.9	90.6			2.33
SOP-PCR-1.3	GFD-qPCR	71.5	86.3	87.5	96.2	94.8			9.46
SOP-PCR-1.4	BacR-qPCR	67.9	101	87.3	94.5	83.1			7.90
SOP-PCR-1.5	DG3-qPCR	72.5	110	90.2	92.1	88.6			4.02

Reference Method Descriptions

Method	Description	Associated Samples
Enterolert/QT	Enterococci by Enterolert/QT by UWF in Pensacola	2341221, 2341222, 2341223, 2341224, 2341225, 2341226, 2341227
SM 10200 H (mod.)	Phytoplankton chlorophyll-a corrected, uncorrected, and phaeophytin by spectrophotometry	2341214, 2341215
SM 9222 D	Fecal coliforms by membrane filter method - non-chlorinated water systems	2341216, 2341217
SM 9223 B Quanti-Tray	E. coli by Colilert-18/QT by UWF in Pensacola	2341228
SOP-PCR-1.0	Detection and quantification of human-specific HF183 Bacteroides genetic marker with quantitative polymerase chain reaction	2341229
SOP-PCR-1.2	Detection and quantification of coastal bird specific Catellicoccus marimammalium Gull2 genetic marker with quantitative polymerase chain reaction	2341229

Reference Method Descriptions

Method	Description	<u>Associated Samples</u>
SOP-PCR-1.3	Detection and quantification of bird specific Helicobacter GFD genetic marker with quantitative polymerase chain reaction	2341229
SOP-PCR-1.4	Detection and quantification of Ruminant specific Bacteroidetes BacR genetic marker with quantitative polymerase chain reaction	2341229
SOP-PCR-1.5	Detection and quantification of canine-specific DG3 Bacteroides genetic marker with quantitative polymerase chain reaction	2341229

Preparation and Analysis Log

Ref. Method	Received Date	Prep Date/Time	Prepared By	Analysis Date/Time	Analyzed By	Associated Samples
Enterolert/QT	07/12/2022	07/12/2022 16:25		07/13/2022 16:50	UWF Lab	2341221, 2341222, 2341223, 2341224, 2341225, 2341226, 2341227
SM 10200 H (mod.)	07/13/2022	07/13/2022 16:31	Jillian G Hanley	07/19/2022 10:36	Ethan Wickline	2341214, 2341215
SM 9222 D	07/13/2022	07/13/2022 15:33	Amber S. Eells	07/14/2022 13:47	Austin Nolan	2341216, 2341217
SM 9223 B Quanti-Tray	07/12/2022	07/12/2022 17:55		07/13/2022 12:50	UWF Lab	2341228
SOP-PCR-1.0	07/13/2022	07/13/2022 14:40	Claudia Castro	08/05/2022 13:11	DeAsia Armster	2341229
SOP-PCR-1.2	07/13/2022	07/13/2022 14:40	Claudia Castro	08/09/2022 12:09	Roberta Tatti	2341229
SOP-PCR-1.3	07/13/2022	07/13/2022 14:40	Claudia Castro	08/10/2022 12:30	Roberta Tatti	2341229
SOP-PCR-1.4	07/13/2022	07/13/2022 14:40	Claudia Castro	08/08/2022 13:31	Roberta Tatti	2341229
SOP-PCR-1.5	07/13/2022	07/13/2022 14:40	Claudia Castro	08/09/2022 11:01	Roberta Tatti	2341229