

Biological Analysis Report

NW-DIST-2022-07-28-02

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: **Walton Washinton Run**
Request ID: **RQ-2022-07-25-44**
Customer: **NW-DIST**
Project ID: **SMP**

Send Reports to:
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160 W Government Street
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Date Certified: 25-AUG-2022 13:52



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: Pine Log Creek upstream of Highway 20

Collection Date/Time: 07/27/2022 10:57

Field ID: G3NW0454

Matrix: W-SURF-FRH

Sample ID	Ref. Method	Component	Result	Code	Units	Batch ID	QC Codes
2344363	SM 9223 B Quanti-Tray	Escherichia Coli-Quanti-Tray	52		MPN/100 mL		

Sample Location: Lafayette Creek

Collection Date/Time: 07/27/2022 12:00

Field ID: G3NW0455

Matrix: W-SURF-FRH

Sample ID	Ref. Method	Component	Result	Code	Units	Batch ID	QC Codes
2344364	SM 9223 B Quanti-Tray	Escherichia Coli-Quanti-Tray	187		MPN/100 mL		

Sample Location: Panther Creek upstream of SR 280

Collection Date/Time: 07/27/2022 12:40

Field ID: G3NW0061

Matrix: W-SURF-FRH

Sample ID	Ref. Method	Component	Result	Code	Units	Batch ID	QC Codes
2344359	SM 10200 H (mod.)	Chlorophyll-a, Corrected	2.8	U	ug/L	P417691	
		Phaeophytin-a	3.0	U	ug/L	P417691	
		Chlorophyll-a, Uncorrected	2.0	U	ug/L	P417691	
2344360	SM 9223 B Quanti-Tray	Escherichia Coli-Quanti-Tray	158		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: Bay Branch below Coy Burgess Loop

Collection Date/Time: 07/27/2022 13:10

Field ID: G3NW0231

Matrix: W-SURF-FRH

Sample ID	Ref. Method	Component	Result	Code	Units	Batch ID	QC Codes
2344361	SM 9223 B Quanti-Tray	Escherichia Coli-Quanti-Tray	199		MPN/100 mL		
2344365	SOP-PCR-1.0	HF183-qPCR**	3.3E+03	U	TSC/100 mL	P416899	
	SOP-PCR-1.2	GULL2-qPCR**	3.6E+03	U	TSC/100 mL	P416899	
	SOP-PCR-1.3	GFD-qPCR**	720	U	TSC/100 mL	P416899	
	SOP-PCR-1.4	BacR-qPCR**	3.0E+03	UJ	TSC/100 mL	P416899	MS
	SOP-PCR-1.5	DG3-qPCR**	2.6E+03	U	TSC/100 mL	P416899	

Ref. Method and Comment:

SOP-PCR-1.2: Precision data unavailable for Gull2-qPCR component.

SOP-PCR-1.3: Precision data unavailable for GFD-qPCR component.

SOP-PCR-1.4: Refer to the Lab Analysis Report for an explanation of QC Codes.

Sample Location: Bay Branch upstream of HWY 331

Collection Date/Time: 07/27/2022 13:45

Field ID: G3NW0171

Matrix: W-SURF-FRH

Sample ID	Ref. Method	Component	Result	Code	Units	Batch ID	QC Codes
2344362	SM 9223 B Quanti-Tray	Escherichia Coli-Quanti-Tray	243		MPN/100 mL		
2344366	SOP-PCR-1.0	HF183-qPCR**	3.3E+03	U	TSC/100 mL	P416899	
	SOP-PCR-1.2	GULL2-qPCR**	3.6E+03	U	TSC/100 mL	P416899	
	SOP-PCR-1.3	GFD-qPCR**	720	U	TSC/100 mL	P416899	
	SOP-PCR-1.4	BacR-qPCR**	3.0E+03	U	TSC/100 mL	P416899	
	SOP-PCR-1.5	DG3-qPCR**	2.6E+03	U	TSC/100 mL	P416899	

Ref. Method and Comment:

SOP-PCR-1.2: Precision data unavailable for Gull2-qPCR component.

SOP-PCR-1.3: Precision data unavailable for GFD-qPCR component.

Quality Assurance Report Method Blank Results

Reference Method: SM 10200 H (mod.)
Batch ID: P417691

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: P416899

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

Reference Method: SOP-PCR-1.2
Batch ID: P416899

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

Reference Method: SOP-PCR-1.3
Batch ID: P416899

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

Reference Method: SOP-PCR-1.4
Batch ID: P416899

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

Reference Method: SOP-PCR-1.5
Batch ID: P416899

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: P417691

Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
Chlorophyll-a, Corrected	88.8		P	84 - 116

Reference Method: SOP-PCR-1.0
Batch ID: P416899

Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
HF183-qPCR	95.4	79.4	P/P	50 - 175

Quality Assurance Report Laboratory Control Sample Accuracy

Reference Method: SOP-PCR-1.2
Batch ID: P416899

Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
GULL2-qPCR	86.4	79.8	P/P	50 - 300

Reference Method: SOP-PCR-1.3
Batch ID: P416899

Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
GFD-qPCR	89.7	80.3	P/P	50 - 175

Reference Method: SOP-PCR-1.4
Batch ID: P416899

Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
BacR-qPCR	98.8	75.5	P/P	50 - 175

Reference Method: SOP-PCR-1.5
Batch ID: P416899

Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
DG3-qPCR	75.1	78.6	P/P	50 - 175

Quality Assurance Report Matrix Spike Accuracy

Reference Method: SOP-PCR-1.0
Batch ID: P416899

Spiked Sample	Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
2342444	HF183-qPCR	50.6	59.3	P/P	50 - 175
2342735	HF183-qPCR	99.8		P	50 - 175
2342736	HF183-qPCR	88.7		P	50 - 175
2343219	HF183-qPCR	90.5		P	50 - 175
2343224	HF183-qPCR	86.5		P	50 - 175
2344365	HF183-qPCR	73.1		P	50 - 175
2344366	HF183-qPCR	72.5		P	50 - 175
2344708	HF183-qPCR	94.2		P	50 - 175
2344763	HF183-qPCR	128		P	50 - 175
2344764	HF183-qPCR	83.9		P	50 - 175
2345085	HF183-qPCR	89.4		P	50 - 175
2345123	HF183-qPCR	92.2		P	50 - 175

Reference Method: SOP-PCR-1.2
Batch ID: P416899

Spiked Sample	Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
2343219	GULL2-qPCR	83.8		P	50 - 300
2344365	GULL2-qPCR	56.9		P	50 - 300
2344366	GULL2-qPCR	60.5		P	50 - 300
2344708	GULL2-qPCR	88.6		P	50 - 300
2345085	GULL2-qPCR	95.9		P	50 - 300
2345123	GULL2-qPCR	77.0		P	50 - 300

Quality Assurance Report Matrix Spike Accuracy

Reference Method: SOP-PCR-1.3
Batch ID: P416899

Spiked Sample	Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
2343219	GFD-qPCR	99.0		P	50 - 175
2344365	GFD-qPCR	120		P	50 - 175
2344366	GFD-qPCR	100		P	50 - 175
2344708	GFD-qPCR	95.2		P	50 - 175
2345085	GFD-qPCR	99.0		P	50 - 175
2345123	GFD-qPCR	89.6		P	50 - 175

Reference Method: SOP-PCR-1.4
Batch ID: P416899

Spiked Sample	Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
2342444	BacR-qPCR	91.1	91.2	P/P	50 - 175
2342735	BacR-qPCR	69.7		P	50 - 175
2342736	BacR-qPCR	83.1		P	50 - 175
2343219	BacR-qPCR	74.8		P	50 - 175
2343224	BacR-qPCR	59.0		P	50 - 175
2344365	BacR-qPCR	47.3		F	50 - 175
2344366	BacR-qPCR	52.3		P	50 - 175
2344708	BacR-qPCR	81.9		P	50 - 175
2344763	BacR-qPCR	81.3		P	50 - 175
2344764	BacR-qPCR	84.8		P	50 - 175
2345085	BacR-qPCR	78.2		P	50 - 175
2345123	BacR-qPCR	82.2		P	50 - 175

Reference Method: SOP-PCR-1.5
Batch ID: P416899

Spiked Sample	Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
2342444	DG3-qPCR	97.6	97.9	P/P	50 - 175
2342735	DG3-qPCR	71.7		P	50 - 175
2342736	DG3-qPCR	93.9		P	50 - 175
2343219	DG3-qPCR	99.7		P	50 - 175
2343224	DG3-qPCR	94.8		P	50 - 175
2344365	DG3-qPCR	67.1		P	50 - 175
2344366	DG3-qPCR	71.7		P	50 - 175
2344708	DG3-qPCR	84.9		P	50 - 175
2344763	DG3-qPCR	86.3		P	50 - 175
2344764	DG3-qPCR	89.2		P	50 - 175
2345085	DG3-qPCR	88.4		P	50 - 175
2345123	DG3-qPCR	93.9		P	50 - 175

Quality Assurance Report Precision

Reference Method: SOP-PCR-1.0
Batch ID: P416899

Replicated Lab Sample	Component	% RSD/RPD	Sample/Spike/LCS*	Pass/Fail	Control Limits
2342444	HF183-qPCR	5.93	Spike	P	0 - 25

Quality Assurance Report Precision

Reference Method: SOP-PCR-1.4
Batch ID: P416899

Replicated Lab Sample	Component	% RSD/RPD	Sample/Spike/LCS*	Pass/Fail	Control Limits
2342444	BacR-qPCR	0.178	Spike	P	0 - 25

Reference Method: SOP-PCR-1.5
Batch ID: P416899

Replicated Lab Sample	Component	% RSD/RPD	Sample/Spike/LCS*	Pass/Fail	Control Limits
2342444	DG3-qPCR	0.363	Spike	P	0 - 25

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

Quality Assurance Report Surrogates

Lab Sample ID: 2344365
Field Sample ID: G3NW0231

Reference Method	Surrogate	% Rec.	Pass/Fail	Control Limits
SOP-PCR-1.0	SKETA-qPCR	61.9	P	50 - 300
SOP-PCR-1.2	SKETA-qPCR	103	P	50 - 300
SOP-PCR-1.3	SKETA-qPCR	99.2	P	50 - 300
SOP-PCR-1.4	SKETA-qPCR	102	P	50 - 300
SOP-PCR-1.5	SKETA-qPCR	62.0	P	50 - 300

Lab Sample ID: 2344366
Field Sample ID: G3NW0171

Reference Method	Surrogate	% Rec.	Pass/Fail	Control Limits
SOP-PCR-1.0	SKETA-qPCR	55.6	P	50 - 300
SOP-PCR-1.2	SKETA-qPCR	110	P	50 - 300
SOP-PCR-1.3	SKETA-qPCR	107	P	50 - 300
SOP-PCR-1.4	SKETA-qPCR	101	P	50 - 300
SOP-PCR-1.5	SKETA-qPCR	68.1	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	88.8							
SOP-PCR-1.0	HF183-qPCR	79.4	95.4	50.6	59.3	99.8			5.93
				88.7					
SOP-PCR-1.2	GULL2-qPCR	79.8	86.4	83.8	56.9	60.5			
				88.6					
SOP-PCR-1.3	GFD-qPCR	80.3	89.7	99.0	120	100			
				95.2					
SOP-PCR-1.4	BacR-qPCR	75.5	98.8	91.1	91.2	69.7			0.178
				83.1					
SOP-PCR-1.5	DG3-qPCR	78.6	75.1	97.6	97.9	71.7			0.363
				93.9					

Reference Method Descriptions

Method	Description	<u>Associated Samples</u>
SM 10200 H (mod.)	Phytoplankton chlorophyll-a corrected, uncorrected, and phaeophytin by spectrophotometry	2344359
SM 9223 B Quanti-Tray	E. coli by Colilert-18/QT by UWF in Pensacola	2344360, 2344361, 2344362, 2344363, 2344364
SOP-PCR-1.0	Detection and quantification of human-specific HF183 Bacteroides genetic marker with quantitative polymerase chain reaction	2344365, 2344366
SOP-PCR-1.2	Detection and quantification of coastal bird specific Catellicoccus marimammalium Gull2 genetic marker with quantitative polymerase chain reaction	2344365, 2344366
SOP-PCR-1.3	Detection and quantification of bird specific Helicobacter GFD genetic marker with quantitative polymerase chain reaction	2344365, 2344366
SOP-PCR-1.4	Detection and quantification of Ruminant specific Bacteroidetes BacR genetic marker with quantitative polymerase chain reaction	2344365, 2344366
SOP-PCR-1.5	Detection and quantification of canine-specific DG3 Bacteroides genetic marker with quantitative polymerase chain reaction	2344365, 2344366

Preparation and Analysis Log

Ref. Method	Received Date	Prep Date/Time	Prepared By	Analysis Date/Time	Analyzed By	Associated Samples
SM 10200 H (mod.)	07/28/2022	07/28/2022 13:51	Carina A. Tull	08/01/2022 14:33	Carina A. Tull	2344359
SM 9223 B Quanti-Tray	07/27/2022	07/27/2022 16:25		07/28/2022 12:15	UWF Lab	2344360, 2344361, 2344362, 2344363, 2344364
SOP-PCR-1.0	07/28/2022	07/28/2022 13:15	Claudia Castro	08/10/2022 14:26	DeAsia Armster	2344365, 2344366
SOP-PCR-1.2	07/28/2022	07/28/2022 13:15	Claudia Castro	08/12/2022 11:20	DeAsia Armster	2344365, 2344366
SOP-PCR-1.3	07/28/2022	07/28/2022 13:15	Claudia Castro	08/12/2022 02:28	Roberta Tatti	2344365, 2344366
SOP-PCR-1.4	07/28/2022	07/28/2022 13:15	Claudia Castro	08/11/2022 10:27	Roberta Tatti	2344365, 2344366
SOP-PCR-1.5	07/28/2022	07/28/2022 13:15	Claudia Castro	08/11/2022 12:48	DeAsia Armster	2344365, 2344366