

Biological Analysis Report

NW-DIST-2022-12-02-01

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: **Walton Washinton Run**
Request ID: **RQ-2022-11-21-07**
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: Puja Jasrotia, Environmental Administrator

Date Certified: 11-JAN-2023 09:49

A handwritten signature in black ink, appearing to be 'Puja Jasrotia', with a horizontal line extending from the end of the signature.

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: Bay Branch below Coy Burgess Loop

Collection Date/Time: 11/30/2022 11:40

Field ID: G3NW0231

Matrix: W-SURF-FRH

Sample ID	Ref. Method	Component	Result	Code	Units	Batch ID	QC Codes
2372419	SM 9223 B Quanti-Tray	Escherichia Coli-Quanti-Tray	1904		MPN/100 mL		
2372420	SOP-PCR-1.0	HF183-purified-qPCR**	550	UJ	TSC/100 mL	P423407	LCS, SUR
	SOP-PCR-1.2	GULL2-purified-qPCR**	600	UJ	TSC/100 mL	P423407	LCS, SUR
	SOP-PCR-1.3	GFD-purified-qPCR**	2.46E+03	J	TSC/100 mL	P423407	LCS, SUR
	SOP-PCR-1.4	BacR-purified-qPCR**	510	UJ	TSC/100 mL	P423407	LCS, SUR
	SOP-PCR-1.5	DG3-purified-qPCR**	430	UJ	TSC/100 mL	P423407	LCS, SUR

Ref. Method and Comment:

SOP-PCR-1.0: Precision data unavailable for HF183-purified-qPCR component. Refer to the Lab Analysis Report for an explanation of QC Codes.

SOP-PCR-1.2: Precision data unavailable for GULL2-purified-qPCR component. Refer to the Lab Analysis Report for an explanation of QC Codes.

SOP-PCR-1.3: Precision data unavailable for GFD-purified-qPCR component. Refer to the Lab Analysis Report for an explanation of QC Codes.

SOP-PCR-1.4: Precision data unavailable for BacR-purified-qPCR component. Refer to the Lab Analysis Report for an explanation of QC Codes.

SOP-PCR-1.5: Precision data unavailable for DG3-purified-qPCR component. Refer to the Lab Analysis Report for an explanation of QC Codes.

Sample Location: Panther Creek upstream of SR280

Collection Date/Time: 11/30/2022 12:10

Field ID: G3NW0061

Matrix: W-SURF-FRH

Sample ID	Ref. Method	Component	Result	Code	Units	Batch ID	QC Codes
2372417	SM 10200 H (mod.)	Chlorophyll-a, Corrected	2.8	U	ug/L	P423392	
		Phaeophytin-a	3.0	U	ug/L	P423392	
		Chlorophyll-a, Uncorrected	2.0	U	ug/L	P423392	
2372418	SM 9223 B Quanti-Tray	Escherichia Coli-Quanti-Tray	15531		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: P423392

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: P423407

Component	Result	Code	Units
HF183-purified-qPCR	550	U	TSC/100 mL

Reference Method: SOP-PCR-1.2

Batch ID: P423407

Component	Result	Code	Units
GULL2-purified-qPCR	600	U	TSC/100 mL

Reference Method: SOP-PCR-1.3

Batch ID: P423407

Component	Result	Code	Units
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Quality Assurance Report Method Blank Results

Reference Method: SOP-PCR-1.3
Batch ID: P423407

Component	Result	Code	Units
GFD-purified-qPCR	120	U	TSC/100 mL

Reference Method: SOP-PCR-1.4
Batch ID: P423407

Component	Result	Code	Units
BacR-purified-qPCR	510	U	TSC/100 mL

Reference Method: SOP-PCR-1.5
Batch ID: P423407

Component	Result	Code	Units
DG3-purified-qPCR	430	U	TSC/100 mL

Quality Assurance Report Laboratory Control Sample Accuracy

Reference Method: SM 10200 H (mod.)
Batch ID: P423392

Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
Chlorophyll-a, Corrected	96.6		P	84 - 116

Reference Method: SOP-PCR-1.0
Batch ID: P423407

Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
HF183-purified-qPCR	111	3.69	P/F	50 - 175

Reference Method: SOP-PCR-1.2
Batch ID: P423407

Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
GULL2-purified-qPCR	106	2.42	P/F	50 - 300

Reference Method: SOP-PCR-1.3
Batch ID: P423407

Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
GFD-purified-qPCR	102	3.05	P/F	50 - 175

Reference Method: SOP-PCR-1.4
Batch ID: P423407

Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
BacR-purified-qPCR	99.9	1.66	P/F	50 - 175

Reference Method: SOP-PCR-1.5
Batch ID: P423407

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

Reference Method: SOP-PCR-1.5
Batch ID: P423407

Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
DG3-purified-qPCR	108	1.85	P/F	50 - 175

Quality Assurance Report Matrix Spike Accuracy

Reference Method: SOP-PCR-1.0
Batch ID: P423407

Spiked Sample	Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
2370771	HF183-purified-qPCR	103		P	50 - 175
2370961	HF183-purified-qPCR	103		P	50 - 175
2371766	HF183-purified-qPCR	94.3		P	50 - 175
2372420	HF183-purified-qPCR	98.4		P	50 - 175
2373386	HF183-purified-qPCR	98.7		P	50 - 175

Reference Method: SOP-PCR-1.2
Batch ID: P423407

Spiked Sample	Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
2370961	GULL2-purified-qPCR	104		P	50 - 300
2371766	GULL2-purified-qPCR	102		P	50 - 300
2372420	GULL2-purified-qPCR	102		P	50 - 300
2373386	GULL2-purified-qPCR	108		P	50 - 300

Reference Method: SOP-PCR-1.3
Batch ID: P423407

Spiked Sample	Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
2370961	GFD-purified-qPCR	104		P	50 - 175
2371766	GFD-purified-qPCR	96.7		P	50 - 175
2372420	GFD-purified-qPCR	98.3		P	50 - 175
2373386	GFD-purified-qPCR	100		P	50 - 175

Reference Method: SOP-PCR-1.4
Batch ID: P423407

Spiked Sample	Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
2370771	BacR-purified-qPCR	105		P	50 - 175
2370961	BacR-purified-qPCR	102		P	50 - 175
2371766	BacR-purified-qPCR	94.5		P	50 - 175
2372420	BacR-purified-qPCR	88.3		P	50 - 175
2373386	BacR-purified-qPCR	87.5		P	50 - 175

Reference Method: SOP-PCR-1.5
Batch ID: P423407

Spiked Sample	Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
2370771	DG3-purified-qPCR	101		P	50 - 175
2370961	DG3-purified-qPCR	107		P	50 - 175
2371766	DG3-purified-qPCR	105		P	50 - 175
2372420	DG3-purified-qPCR	108		P	50 - 175

Quality Assurance Report Matrix Spike Accuracy

Reference Method: SOP-PCR-1.5
 Batch ID: P423407

Spiked Sample	Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
2373386	DG3-purified-qPCR	109		P	50 - 175

Quality Assurance Report Surrogates

Lab Sample ID: 2372420
 Field Sample ID: G3NW0231

Reference Method	Surrogate	% Rec.	Pass/Fail	Control Limits
SOP-PCR-1.0	SKETA-qPCR	4.78	F	50 - 300
SOP-PCR-1.2	SKETA-qPCR	6.50	F	30 - 300
SOP-PCR-1.3	SKETA-qPCR	5.28	F	30 - 300
SOP-PCR-1.4	SKETA-qPCR	6.18	F	30 - 300
SOP-PCR-1.5	SKETA-qPCR	5.01	F	50 - 300

Quality Assurance Report Summary

Ref. Method	Analyte	LCS % Recovery		MS % Recovery			LCS	Precision SMP	MS
SM 10200 H (mod.)	Chlorophyll-a, Corrected	96.6							
SOP-PCR-1.0	HF183-purified-qPCR	3.69	111	103	103	94.3			
				98.4					
SOP-PCR-1.2	GULL2-purified-qPCR	2.42	106	104	102	102			
				108					
SOP-PCR-1.3	GFD-purified-qPCR	3.05	102	104	96.7	98.3			
				100					
SOP-PCR-1.4	BacR-purified-qPCR	1.66	99.9	105	102	94.5			
				88.3					
SOP-PCR-1.5	DG3-purified-qPCR	1.85	108	101	107	105			
				108					

Reference Method Descriptions

Method	Description	Associated Samples
SM 10200 H (mod.)	Phytoplankton chlorophyll-a corrected, uncorrected, and phaeophytin by spectrophotometry	2372417
SM 9223 B Quanti-Tray	E. coli by Colilert-18/QT by UWF in Pensacola	2372418, 2372419
SOP-PCR-1.0	Detection and quantification of human-specific HF183 Bacteroides genetic marker with quantitative polymerase chain reaction	2372420
SOP-PCR-1.2	Detection and quantification of coastal bird specific Catellicoccus marimammalium Gull2 genetic marker with quantitative polymerase chain reaction	2372420
SOP-PCR-1.3	Detection and quantification of bird specific Helicobacter GFD genetic marker with quantitative polymerase chain reaction	2372420
SOP-PCR-1.4	Detection and quantification of Ruminant specific Bacteroidetes BacR genetic marker with quantitative polymerase chain reaction	2372420
SOP-PCR-1.5	Detection and quantification of canine-specific DG3 Bacteroides genetic marker with quantitative polymerase chain reaction	2372420

Preparation and Analysis Log

Ref. Method	Received Date	Prep Date/Time	Prepared By	Analysis Date/Time	Analyzed By	Associated Samples
SM 10200 H (mod.)	12/02/2022	12/02/2022 09:44	Aidan Cronin	12/08/2022 15:19	Megan Higbee	2372417
SM 9223 B Quanti-Tray	11/30/2022	11/30/2022 15:25		12/01/2022 11:51	UWF Lab	2372418, 2372419
SOP-PCR-1.0	12/02/2022	12/02/2022 10:15	DeAsia Armster	12/14/2022 14:19	Roberta Tatti	2372420
SOP-PCR-1.2	12/02/2022	12/02/2022 10:15	DeAsia Armster	12/16/2022 14:53	DeAsia Armster	2372420
SOP-PCR-1.3	12/02/2022	12/02/2022 10:15	DeAsia Armster	12/16/2022 10:56	Roberta Tatti	2372420
SOP-PCR-1.4	12/02/2022	12/02/2022 10:15	DeAsia Armster	12/15/2022 08:44	DeAsia Armster	2372420
SOP-PCR-1.5	12/02/2022	12/02/2022 10:15	DeAsia Armster	12/15/2022 12:12	Roberta Tatti	2372420