

Biological Analysis Report

NW-DIST-2023-09-06-01

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: **Freeport Run**
Request ID: **RQ-2023-09-04-53**
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

For additional information please contact
Cheryl Swanson - Administrator
Sarah Menz, Ph.D. - Bench Biology & Aquatic
Toxicity
Puja Jasrotia, Ph.D. - Molecular Biology & Taxonomy
Thekkekalathil Chandrasekhar, Ph.D, QA Officer
Phone (850) 245-8177

Certified by: Sarah Menz, Environmental Administrator

Date Certified: 20-OCT-2023 11:33

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: 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: SEVEN RUNS UPSTREAM OF HWY 81

Collection Date/Time: 09/05/2023 10:50

Field ID: G3NW0116

Matrix: W-SURF-FRH

Sample ID	Ref. Method	Component	Result	Code	Units	Batch ID	QC Codes
2434232	SM 9223 B Quanti-Tray	Escherichia Coli-Quanti-Tray	282		MPN/100 mL		

Sample Location: LITTLE BLACK CREEK UPSTREAM OF BLACK CREEK

Collection Date/Time: 09/05/2023 11:15

Field ID: G3NW0200

Matrix: W-SURF-FRH

Sample ID	Ref. Method	Component	Result	Code	Units	Batch ID	QC Codes
2434231	SM 9223 B Quanti-Tray	Escherichia Coli-Quanti-Tray	41		MPN/100 mL		
2434239	SOP-PCR-1.0	HF183-qPCR**	1.7E+03	U	TSC/100 mL	P434608	
	SOP-PCR-1.2	GULL2-qPCR**	1.8E+03	U	TSC/100 mL	P434608	
	SOP-PCR-1.3	GFD-qPCR**	360	U	TSC/100 mL	P434608	
	SOP-PCR-1.4	BacR-qPCR**	1.5E+03	U	TSC/100 mL	P434608	
	SOP-PCR-1.5	DG3-qPCR**	1.3E+03	U	TSC/100 mL	P434608	LCS

Sample Location: MOUTH OF MULLET CREEK AT HWY 20

Collection Date/Time: 09/05/2023 11:50

Field ID: G3NW0137

Matrix: W-SURF-FRH

Sample ID	Ref. Method	Component	Result	Code	Units	Batch ID	QC Codes
2434233	SM 9223 B Quanti-Tray	Escherichia Coli-Quanti-Tray	794		MPN/100 mL		

Sample Location: OPEN BRANCH ABOVE EGLIN 374

Collection Date/Time: 09/05/2023 12:35

Field ID: G3NW0105

Matrix: W-SURF-FRH

Sample ID	Ref. Method	Component	Result	Code	Units	Batch ID	QC Codes
2434234	SM 9223 B Quanti-Tray	Escherichia Coli-Quanti-Tray	31		MPN/100 mL		

Quality Assurance Report Method Blank Results

Reference Method: SOP-PCR-1.0
Batch ID: P434608

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

Reference Method: SOP-PCR-1.2
Batch ID: P434608

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

Reference Method: SOP-PCR-1.3
Batch ID: P434608

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

Quality Assurance Report Method Blank Results

Reference Method: SOP-PCR-1.4
Batch ID: P434608

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

Reference Method: SOP-PCR-1.5
Batch ID: P434608

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

Quality Assurance Report Laboratory Control Sample Accuracy

Reference Method: SOP-PCR-1.0
Batch ID: P434608

Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
HF183-qPCR	87.0	76.4	P/P	50 - 175

Reference Method: SOP-PCR-1.2
Batch ID: P434608

Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
GULL2-qPCR	84.9	59.0	P/P	50 - 300

Reference Method: SOP-PCR-1.3
Batch ID: P434608

Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
GFD-qPCR	83.5	56.3	P/P	50 - 175

Reference Method: SOP-PCR-1.4
Batch ID: P434608

Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
BacR-qPCR	108	69.2	P/P	50 - 175

Reference Method: SOP-PCR-1.5
Batch ID: P434608

Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
DG3-qPCR	48.1	66.1	F/P	50 - 175

Quality Assurance Report Matrix Spike Accuracy

Reference Method: SOP-PCR-1.0
Batch ID: P434608

Spiked Sample	Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
2434239	HF183-qPCR	74.6	79.8	P/P	50 - 175
2435446	HF183-qPCR	81.5		P	50 - 175
2435447	HF183-qPCR	97.5		P	50 - 175

Quality Assurance Report Matrix Spike Accuracy

Reference Method: SOP-PCR-1.0

Batch ID: P434608

Spiked Sample	Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
2435448	HF183-qPCR	85.0		P	50 - 175
2435679	HF183-qPCR	83.4		P	50 - 175
2435726	HF183-qPCR	104		P	50 - 175
2435727	HF183-qPCR	92.2		P	50 - 175
2435728	HF183-qPCR	93.7		P	50 - 175
2435729	HF183-qPCR	95.1		P	50 - 175
2435730	HF183-qPCR	101		P	50 - 175
2435731	HF183-qPCR	88.0		P	50 - 175
2436362	HF183-qPCR	81.4		P	50 - 175

Reference Method: SOP-PCR-1.2

Batch ID: P434608

Spiked Sample	Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
2434239	GULL2-qPCR	60.1	66.1	P/P	50 - 300
2435446	GULL2-qPCR	93.5		P	50 - 300
2435447	GULL2-qPCR	90.3		P	50 - 300
2435448	GULL2-qPCR	91.9		P	50 - 300
2435679	GULL2-qPCR	55.5		P	50 - 300
2435726	GULL2-qPCR	73.2		P	50 - 300
2435727	GULL2-qPCR	78.3		P	50 - 300
2435728	GULL2-qPCR	76.7		P	50 - 300
2435729	GULL2-qPCR	86.3		P	50 - 300
2435730	GULL2-qPCR	74.1		P	50 - 300
2435731	GULL2-qPCR	80.2		P	50 - 300

Reference Method: SOP-PCR-1.3

Batch ID: P434608

Spiked Sample	Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
2434239	GFD-qPCR	87.7	87.9	P/P	50 - 175
2435446	GFD-qPCR	88.8		P	50 - 175
2435447	GFD-qPCR	85.7		P	50 - 175
2435448	GFD-qPCR	88.6		P	50 - 175
2435679	GFD-qPCR	77.1		P	50 - 175
2435726	GFD-qPCR	70.1		P	50 - 175
2435727	GFD-qPCR	83.5		P	50 - 175
2435728	GFD-qPCR	86.1		P	50 - 175
2435729	GFD-qPCR	86.6		P	50 - 175
2435730	GFD-qPCR	84.9		P	50 - 175
2435731	GFD-qPCR	77.4		P	50 - 175

Reference Method: SOP-PCR-1.4

Batch ID: P434608

Spiked Sample	Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
2434239	BacR-qPCR	62.8	58.5	P/P	50 - 175
2435446	BacR-qPCR	90.9		P	50 - 175
2435447	BacR-qPCR	89.9		P	50 - 175
2435448	BacR-qPCR	87.0		P	50 - 175
2435679	BacR-qPCR	59.8		P	50 - 175
2436362	BacR-qPCR	65.1		P	50 - 175

Quality Assurance Report Matrix Spike Accuracy

Reference Method: SOP-PCR-1.5
 Batch ID: P434608

Spiked Sample	Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
2434239	DG3-qPCR	83.2	80.3	P/P	50 - 175
2435446	DG3-qPCR	91.1		P	50 - 175
2435447	DG3-qPCR	87.9		P	50 - 175
2435448	DG3-qPCR	91.4		P	50 - 175
2435679	DG3-qPCR	86.1		P	50 - 175
2435726	DG3-qPCR	105		P	50 - 175
2435727	DG3-qPCR	106		P	50 - 175
2435728	DG3-qPCR	100		P	50 - 175
2435729	DG3-qPCR	99.6		P	50 - 175
2435730	DG3-qPCR	104		P	50 - 175
2435731	DG3-qPCR	92.5		P	50 - 175
2436362	DG3-qPCR	86.1		P	50 - 175

Quality Assurance Report Precision

Reference Method: SOP-PCR-1.0
 Batch ID: P434608

Replicated Lab Sample	Component	% RSD/RPD	Sample/Spike/LCS*	Pass/Fail	Control Limits
2434239	HF183-qPCR	6.69	Spike	P	0 - 25

Reference Method: SOP-PCR-1.2
 Batch ID: P434608

Replicated Lab Sample	Component	% RSD/RPD	Sample/Spike/LCS*	Pass/Fail	Control Limits
2434239	GULL2-qPCR	9.65	Spike	P	0 - 25

Reference Method: SOP-PCR-1.3
 Batch ID: P434608

Replicated Lab Sample	Component	% RSD/RPD	Sample/Spike/LCS*	Pass/Fail	Control Limits
2434239	GFD-qPCR	0.278	Spike	P	0 - 25

Reference Method: SOP-PCR-1.4
 Batch ID: P434608

Replicated Lab Sample	Component	% RSD/RPD	Sample/Spike/LCS*	Pass/Fail	Control Limits
2434239	BacR-qPCR	6.94	Spike	P	0 - 25

Reference Method: SOP-PCR-1.5
 Batch ID: P434608

Replicated Lab Sample	Component	% RSD/RPD	Sample/Spike/LCS*	Pass/Fail	Control Limits
2434239	DG3-qPCR	3.51	Spike	P	0 - 25

Quality Assurance Report Precision

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

Quality Assurance Report Surrogates

Lab Sample ID: 2434239
Field Sample ID: G3NW0200

Reference Method	Surrogate	% Rec.	Pass/Fail	Control Limits
SOP-PCR-1.0	SKETA-qPCR	96.7	P	50 - 300
SOP-PCR-1.0	SKETA-qPCR	88.7	P	50 - 300
SOP-PCR-1.2	SKETA-qPCR	101	P	50 - 300
SOP-PCR-1.2	SKETA-qPCR	104	P	50 - 300
SOP-PCR-1.3	SKETA-qPCR	101	P	50 - 300
SOP-PCR-1.3	SKETA-qPCR	102	P	50 - 300
SOP-PCR-1.4	SKETA-qPCR	104	P	50 - 300
SOP-PCR-1.4	SKETA-qPCR	105	P	50 - 300
SOP-PCR-1.5	SKETA-qPCR	95.3	P	50 - 300
SOP-PCR-1.5	SKETA-qPCR	92.6	P	50 - 300

Quality Assurance Report Summary

Ref. Method	Analyte	LCS % Recovery		MS % Recovery			Precision SMP	MS
SOP-PCR-1.0	HF183-qPCR	76.4	87.0	101	88.0	81.4		6.69
				74.6				
SOP-PCR-1.2	GULL2-qPCR	59.0	84.9	60.1	66.1	93.5		9.65
				90.3				
SOP-PCR-1.3	GFD-qPCR	56.3	83.5	87.7	87.9	88.8		0.278
				85.7				
SOP-PCR-1.4	BacR-qPCR	69.2	108	62.8	58.5	90.9		6.94
				89.9				
SOP-PCR-1.5	DG3-qPCR	66.1	48.1	83.2	80.3	91.1		3.51
				87.9				

Reference Method Descriptions

Method	Description	Associated Samples
SM 9223 B Quanti-Tray	E. coli by Colilert-18/QT by UWF in Pensacola	2434231, 2434232, 2434233, 2434234
SOP-PCR-1.0	Detection and quantification of human-specific HF183 Bacteroides genetic marker with quantitative polymerase chain reaction	2434239
SOP-PCR-1.2	Detection and quantification of coastal bird specific Catellicoccus marimammalium Gull2 genetic marker with quantitative polymerase chain reaction	2434239
SOP-PCR-1.3	Detection and quantification of bird specific Helicobacter GFD genetic marker with quantitative polymerase chain reaction	2434239
SOP-PCR-1.4	Detection and quantification of Ruminant specific Bacteroidetes BacR genetic marker with quantitative polymerase chain reaction	2434239
SOP-PCR-1.5	Detection and quantification of canine-specific DG3 Bacteroides genetic marker with quantitative polymerase chain reaction	2434239

Preparation and Analysis Log

Ref. Method	Received Date	Prep Date/Time	Prepared By	Analysis Date/Time	Analyzed By	Associated Samples
SM 9223 B Quanti-Tray	09/05/2023	09/05/2023 15:50		09/06/2023 12:06	UWF Lab	2434231, 2434232, 2434233, 2434234
SOP-PCR-1.0	09/06/2023	09/06/2023 13:50	Claudia Castro	09/22/2023 16:51	Madeline Gruver	2434239
SOP-PCR-1.2	09/06/2023	09/06/2023 13:50	Claudia Castro	09/28/2023 09:09	Roberta Tatti	2434239
SOP-PCR-1.3	09/06/2023	09/06/2023 13:50	Claudia Castro	10/02/2023 11:38	Roberta Tatti	2434239
SOP-PCR-1.4	09/06/2023	09/06/2023 13:50	Claudia Castro	09/26/2023 17:29	Madeline Gruver	2434239
SOP-PCR-1.5	09/06/2023	09/06/2023 13:50	Claudia Castro	09/26/2023 18:37	Madeline Gruver	2434239