

Biological Analysis Report

NW-DIST-2023-04-05-01

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-2023-04-03-45**
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: 05-MAY-2023 12:32

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: Little Black Creek upstream of Black Creek Collection Date/Time: 04/03/2023 11:00

Field ID: G3NW0200					Matrix: W-SURF-FRH		
Sample ID	Ref. Method	Component	Result	Code	Units	Batch ID	QC Codes
2398309	SM 9223 B Quanti-Tray	Escherichia Coli-Quanti-Tray	122		MPN/100 mL		
2398313	SOP-PCR-1.0	HF183-qPCR**	3.3E+03	U	TSC/100 mL	P427755	
	SOP-PCR-1.2	GULL2-qPCR**	3.6E+03	U	TSC/100 mL	P427755	
	SOP-PCR-1.3	GFD-qPCR**	720	U	TSC/100 mL	P427755	
	SOP-PCR-1.4	BacR-qPCR**	3.0E+03	U	TSC/100 mL	P427755	
	SOP-PCR-1.5	DG3-qPCR**	2.6E+03	U	TSC/100 mL	P427755	

Sample Location: Open Branch above Eglin 374 Collection Date/Time: 04/03/2023 12:25

Field ID: G3NW0105					Matrix: W-SURF-FRH		
Sample ID	Ref. Method	Component	Result	Code	Units	Batch ID	QC Codes
2398310	SM 9223 B Quanti-Tray	Escherichia Coli-Quanti-Tray	52		MPN/100 mL		

Sample Location: Seven Runs Upstream of Hwy 81 Collection Date/Time: 04/03/2023 10:30

Field ID: G3NW0116					Matrix: W-SURF-FRH		
Sample ID	Ref. Method	Component	Result	Code	Units	Batch ID	QC Codes
2398311	SM 9223 B Quanti-Tray	Escherichia Coli-Quanti-Tray	369		MPN/100 mL		

Sample Location: Mouth of Mullet Creek at Hwy 20 Collection Date/Time: 04/03/2023 11:40

Field ID: G3NW0137					Matrix: W-SURF-FRH		
Sample ID	Ref. Method	Component	Result	Code	Units	Batch ID	QC Codes
2398312	SM 9223 B Quanti-Tray	Escherichia Coli-Quanti-Tray	301		MPN/100 mL		

Quality Assurance Report
Method Blank Results

Reference Method: SOP-PCR-1.0
Batch ID: P427755

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

Reference Method: SOP-PCR-1.2
Batch ID: P427755

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

Reference Method: SOP-PCR-1.3
Batch ID: P427755

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: P427755

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

Reference Method: SOP-PCR-1.5
Batch ID: P427755

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: P427755

Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
HF183-qPCR	89.1	100	P/P	50 - 175

Reference Method: SOP-PCR-1.2
Batch ID: P427755

Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
GULL2-qPCR	94.8	87.7	P/P	50 - 300

Reference Method: SOP-PCR-1.3
Batch ID: P427755

Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
GFD-qPCR	112	95.5	P/P	50 - 175

Reference Method: SOP-PCR-1.4
Batch ID: P427755

Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
BacR-qPCR	96.5	87.5	P/P	50 - 175

Reference Method: SOP-PCR-1.5
Batch ID: P427755

Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
DG3-qPCR	102	95.5	P/P	50 - 175

Quality Assurance Report Matrix Spike Accuracy

Reference Method: SOP-PCR-1.0
Batch ID: P427755

Spiked Sample	Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
2396582	HF183-qPCR	76.2	83.8	P/P	50 - 175
2396696	HF183-qPCR	79.9		P	50 - 175
2397523	HF183-qPCR	91.2		P	50 - 175

Quality Assurance Report Matrix Spike Accuracy

Reference Method: SOP-PCR-1.0
Batch ID: P427755

Spiked Sample	Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
2397524	HF183-qPCR	95.7		P	50 - 175
2397894	HF183-qPCR	81.3		P	50 - 175
2398313	HF183-qPCR	79.7		P	50 - 175
2398618	HF183-qPCR	84.6		P	50 - 175
2398619	HF183-qPCR	85.8		P	50 - 175
2398964	HF183-qPCR	85.9		P	50 - 175
2398965	HF183-qPCR	85.9		P	50 - 175
2399288	HF183-qPCR	80.6		P	50 - 175
2399389	HF183-qPCR	85.9		P	50 - 175

Reference Method: SOP-PCR-1.2
Batch ID: P427755

Spiked Sample	Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
2396582	GULL2-qPCR	65.7	73.2	P/P	50 - 300
2396696	GULL2-qPCR	73.6		P	50 - 300
2397523	GULL2-qPCR	90.9		P	50 - 300
2397894	GULL2-qPCR	77.9		P	50 - 300
2398313	GULL2-qPCR	79.2		P	50 - 300
2398618	GULL2-qPCR	76.7		P	50 - 300
2398619	GULL2-qPCR	69.7		P	50 - 300
2398964	GULL2-qPCR	51.3		P	50 - 300
2398965	GULL2-qPCR	75.7		P	50 - 300
2399389	GULL2-qPCR	73.0		P	50 - 300

Reference Method: SOP-PCR-1.3
Batch ID: P427755

Spiked Sample	Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
2396582	GFD-qPCR	93.1	103	P/P	50 - 175
2396696	GFD-qPCR	106		P	50 - 175
2397523	GFD-qPCR	104		P	50 - 175
2397894	GFD-qPCR	98.4		P	50 - 175
2398313	GFD-qPCR	99.5		P	50 - 175
2398618	GFD-qPCR	105		P	50 - 175
2398619	GFD-qPCR	97.4		P	50 - 175
2398964	GFD-qPCR	106		P	50 - 175
2398965	GFD-qPCR	88.2		P	50 - 175
2399389	GFD-qPCR	90.5		P	50 - 175

Reference Method: SOP-PCR-1.4
Batch ID: P427755

Spiked Sample	Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
2396582	BacR-qPCR	78.1	79.7	P/P	50 - 175
2396696	BacR-qPCR	87.9		P	50 - 175
2397523	BacR-qPCR	94.2		P	50 - 175
2397524	BacR-qPCR	88.4		P	50 - 175
2397894	BacR-qPCR	80.9		P	50 - 175
2398313	BacR-qPCR	80.2		P	50 - 175
2398618	BacR-qPCR	84.2		P	50 - 175
2398619	BacR-qPCR	83.2		P	50 - 175

Quality Assurance Report Matrix Spike Accuracy

Reference Method: SOP-PCR-1.4
Batch ID: P427755

Spiked Sample	Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
2398964	BacR-qPCR	79.2		P	50 - 175
2398965	BacR-qPCR	82.8		P	50 - 175
2399288	BacR-qPCR	66.8		P	50 - 175
2399389	BacR-qPCR	81.0		P	50 - 175

Reference Method: SOP-PCR-1.5
Batch ID: P427755

Spiked Sample	Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
2396582	DG3-qPCR	92.7	88.0	P/P	50 - 175
2396696	DG3-qPCR	94.2		P	50 - 175
2397523	DG3-qPCR	105		P	50 - 175
2397524	DG3-qPCR	102		P	50 - 175
2397894	DG3-qPCR	96.7		P	50 - 175
2398313	DG3-qPCR	95.7		P	50 - 175
2398618	DG3-qPCR	97.2		P	50 - 175
2398619	DG3-qPCR	98.7		P	50 - 175
2398964	DG3-qPCR	86.6		P	50 - 175
2398965	DG3-qPCR	95.9		P	50 - 175
2399288	DG3-qPCR	90.7		P	50 - 175
2399389	DG3-qPCR	108		P	50 - 175

Quality Assurance Report Precision

Reference Method: SOP-PCR-1.0
Batch ID: P427755

Replicated Lab Sample	Component	% RSD/RPD	Sample/Spike/LCS*	Pass/Fail	Control Limits
2396582	HF183-qPCR	9.55	Spike	P	0 - 25

Reference Method: SOP-PCR-1.2
Batch ID: P427755

Replicated Lab Sample	Component	% RSD/RPD	Sample/Spike/LCS*	Pass/Fail	Control Limits
2396582	GULL2-qPCR	10.7	Spike	P	0 - 25

Reference Method: SOP-PCR-1.3
Batch ID: P427755

Replicated Lab Sample	Component	% RSD/RPD	Sample/Spike/LCS*	Pass/Fail	Control Limits
2396582	GFD-qPCR	10.1	Spike	P	0 - 25

Reference Method: SOP-PCR-1.4
Batch ID: P427755

Replicated Lab Sample	Component	% RSD/RPD	Sample/Spike/LCS*	Pass/Fail	Control Limits
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Quality Assurance Report Precision

Reference Method: SOP-PCR-1.4
 Batch ID: P427755

Replicated Lab Sample	Component	% RSD/RPD	Sample/Spike/LCS*	Pass/Fail	Control Limits
2396582	BacR-qPCR	1.99	Spike	P	0 - 25

Reference Method: SOP-PCR-1.5
 Batch ID: P427755

Replicated Lab Sample	Component	% RSD/RPD	Sample/Spike/LCS*	Pass/Fail	Control Limits
2396582	DG3-qPCR	5.17	Spike	P	0 - 25

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

Quality Assurance Report Surrogates

Lab Sample ID: 2398313
 Field Sample ID: G3NW0200

Reference Method	Surrogate	% Rec.	Pass/Fail	Control Limits
SOP-PCR-1.0	SKETA-qPCR	123	P	50 - 300
SOP-PCR-1.2	SKETA-qPCR	116	P	50 - 300
SOP-PCR-1.3	SKETA-qPCR	125	P	50 - 300
SOP-PCR-1.4	SKETA-qPCR	114	P	50 - 300
SOP-PCR-1.5	SKETA-qPCR	124	P	50 - 300

Quality Assurance Report Summary

Ref. Method	Analyte	LCS % Recovery		MS % Recovery			Precision SMP	MS
SOP-PCR-1.0	HF183-qPCR	100	89.1	76.2	83.8	79.9		9.55
SOP-PCR-1.2	GULL2-qPCR	87.7	94.8	73.2	73.6	90.9		10.7
SOP-PCR-1.3	GFD-qPCR	95.5	112	93.1	103	106		10.1
SOP-PCR-1.4	BacR-qPCR	87.5	96.5	78.1	79.7	87.9		1.99
SOP-PCR-1.5	DG3-qPCR	95.5	102	92.7	88.0	94.2		5.17
				105				

Reference Method Descriptions

Method	Description	Associated Samples
SM 9223 B Quanti-Tray	E. coli by Colilert-18/QT by UWF in Pensacola	2398309, 2398310, 2398311, 2398312
SOP-PCR-1.0	Detection and quantification of human-specific HF183 Bacteroides genetic marker with quantitative polymerase chain reaction	2398313
SOP-PCR-1.2	Detection and quantification of coastal bird specific Catellicoccus marimammalium Gull2 genetic marker with quantitative polymerase chain reaction	2398313
SOP-PCR-1.3	Detection and quantification of bird specific Helicobacter GFD genetic marker with quantitative polymerase chain reaction	2398313

Reference Method Descriptions

Method	Description	<u>Associated Samples</u>
SOP-PCR-1.4	Detection and quantification of Ruminant specific Bacteroidetes BacR genetic marker with quantitative polymerase chain reaction	2398313
SOP-PCR-1.5	Detection and quantification of canine-specific DG3 Bacteroides genetic marker with quantitative polymerase chain reaction	2398313

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	04/03/2023	04/03/2023 16:10		04/04/2023 12:24	UWF Lab	2398309, 2398310, 2398311, 2398312
SOP-PCR-1.0	04/05/2023	04/05/2023 09:50	Claudia Castro	04/14/2023 10:06	Roberta Tatti	2398313
SOP-PCR-1.2	04/05/2023	04/05/2023 09:50	Claudia Castro	04/20/2023 16:21	Jennifer Mihalic	2398313
SOP-PCR-1.3	04/05/2023	04/05/2023 09:50	Claudia Castro	04/19/2023 13:12	Jennifer Mihalic	2398313
SOP-PCR-1.4	04/05/2023	04/05/2023 09:50	Claudia Castro	04/14/2023 15:28	Roberta Tatti	2398313
SOP-PCR-1.5	04/05/2023	04/05/2023 09:50	Claudia Castro	04/17/2023 11:02	Roberta Tatti	2398313