

Biological Analysis Report

NW-DIST-2021-02-15-01

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

Eurofins TestAmerica Pensacola
3355 McLemore Drive
Pensacola, FL 32514
DOH Accreditation E81010

Event Description: **Twin Lakes Run**
Request ID: **RQ-2021-02-08-42**
Customer: **NW-DIST**
Project ID: **SMP**

Send Reports to:
FL Dept. of Environmental Protection
160 Government Center
Suite 208
Pensacola, FL 32502-5794
Attn: Cheryl Bunch

For additional information please contact
Cheryl Swanson - Administrator
Jacqueline Savage - Bench Biology & Aquatic
Toxicity
Puja Jasrotia, Ph.D. - Molecular Biology & Taxonomy
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Phone (850) 245-8177

Certified by: Jacqueline Savage, Environmental Administrator

Date Certified: 30-MAR-2021 07:39



NON-CONFORMANCE REPORT INCLUDED

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: Twin Lake

Collection Date/Time: 02/10/2021 10:45

Field ID: G3NW0215

Matrix: W-SURF-FRH

Sample ID	Ref. Method	Component	Result	Code	Units	Batch ID	QC Codes
2225323	SM 9222 D	Fecal Coliforms-Membrane Filter	209		cfu/100 mL		
2225328	Enterolert/QT	Enterococci-Quanti-Tray	199		MPN/100 mL		
	SM 9223 B Quanti-Tray	Escherichia Coli-Quanti-Tray	301		MPN/100 mL		

Sample Location: Allen Lake Input

Collection Date/Time: 02/10/2021 10:05

Field ID: G3NW0177

Matrix: W-SURF-FRH

Sample ID	Ref. Method	Component	Result	Code	Units	Batch ID	QC Codes
2225333	SM 9223 B Quanti-Tray	Escherichia Coli-Quanti-Tray	4611		MPN/100 mL		
2225334	SOP-PCR-1.0	HF183-qPCR**	280	UQ	GEU/100 mL	P393711	
	SOP-PCR-1.2	GULL2-qPCR**	3.6E+03	UQJ	TSC/100 mL	P393711	MS
	SOP-PCR-1.3	GFD-qPCR**	720	UQ	TSC/100 mL	P393711	
	SOP-PCR-1.4	BacR-qPCR**	3.0E+03	UQ	TSC/100 mL	P393711	MS
	SOP-PCR-1.5	DG3-qPCR**	2.6E+03	UQ	TSC/100 mL	P393711	

Ref. Method and Comment:
 SOP-PCR-1.2: Refer to the Lab Analysis Report for an explanation of QC Codes.

Sample Location: Twin Lake South Lobe

Collection Date/Time: 02/10/2021 11:00

Field ID: G3NW0448

Matrix: W-SURF-FRH

Sample ID	Ref. Method	Component	Result	Code	Units	Batch ID	QC Codes
2225324	SM 9222 D	Fecal Coliforms-Membrane Filter	88.0		cfu/100 mL		
2225329	Enterolert/QT	Enterococci-Quanti-Tray	20		MPN/100 mL		
	SM 9223 B Quanti-Tray	Escherichia Coli-Quanti-Tray	86		MPN/100 mL		

Sample Location: Coleman Lake

Collection Date/Time: 02/10/2021 11:55

Field ID: G3NW0216

Matrix: W-SURF-FRH

Sample ID	Ref. Method	Component	Result	Code	Units	Batch ID	QC Codes
2225325	SM 9222 D	Fecal Coliforms-Membrane Filter	510		cfu/100 mL		
2225330	Enterolert/QT	Enterococci-Quanti-Tray	473		MPN/100 mL		
	SM 9223 B Quanti-Tray	Escherichia Coli-Quanti-Tray	563		MPN/100 mL		

Sample Location: Kell-aire Lake

Collection Date/Time: 02/10/2021 12:10

Field ID: G3NW0217

Matrix: W-SURF-FRH

Sample ID	Ref. Method	Component	Result	Code	Units	Batch ID	QC Codes
2225326	SM 9222 D	Fecal Coliforms-Membrane Filter	80.0		cfu/100 mL		
2225331	Enterolert/QT	Enterococci-Quanti-Tray	72		MPN/100 mL		
	SM 9223 B Quanti-Tray	Escherichia Coli-Quanti-Tray	110		MPN/100 mL		

Sample Location: Twin Lakes Eastern Lake

Collection Date/Time: 02/10/2021 11:25

Field ID: G3NW0449

Matrix: W-SURF-FRH

Sample ID	Ref. Method	Component	Result	Code	Units	Batch ID	QC Codes
2225327	SM 9222 D	Fecal Coliforms-Membrane Filter	100		cfu/100 mL		

Field ID: G3NW0449

Matrix: W-SURF-FRH

Sample ID	Ref. Method	Component	Result	Code	Units	Batch ID	QC Codes
2225332	Enterolert/QT	Enterococci-Quanti-Tray	62		MPN/100 mL		
	SM 9223 B Quanti-Tray	Escherichia Coli-Quanti-Tray	354		MPN/100 mL		

Non-Conformance Report

NCR ID: 8658

Event(s)

Job(s)

Sample(s)

Test(s)

NW-DIST-2021-02-15-01

NCR Type: SHIPPING/RECEIVING

NCR Category: Samples arrived late

Observation: Samples arrived one or more days late by FedEx, samples received within the acceptable temperature range.

Resolution: Samples will be qualified as appropriate.

Authorized by/Date: Joshua Ayres 2/16/2021

The Non-Conformance Report details exceptions or problems encountered with the events/jobs/samples/test.
Please address questions to:

Chemistry Colin Wright (850) 245-8085
Biology Cheryl Swanson (850) 245-8177

Quality Assurance Report Method Blank Results

Reference Method: SOP-PCR-1.0

Batch ID: P393711

Component	Result	Code	Units
HF183-qPCR	280	U	GEU/100 mL

Reference Method: SOP-PCR-1.2

Batch ID: P393711

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

Reference Method: SOP-PCR-1.3

Batch ID: P393711

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

Reference Method: SOP-PCR-1.4

Batch ID: P393711

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

Reference Method: SOP-PCR-1.5

Batch ID: P393711

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: P393711

Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
HF183-qPCR	88.9	96.8	P/P	50 - 175

Reference Method: SOP-PCR-1.2
Batch ID: P393711

Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
GULL2-qPCR	89.0	93.7	P/P	50 - 300

Reference Method: SOP-PCR-1.3
Batch ID: P393711

Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
GFD-qPCR	66.6	74.9	P/P	50 - 175

Reference Method: SOP-PCR-1.4
Batch ID: P393711

Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
BacR-qPCR	90.5	85.1	P/P	50 - 175

Reference Method: SOP-PCR-1.5
Batch ID: P393711

Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
DG3-qPCR	87.4	85.0	P/P	50 - 175

Quality Assurance Report Matrix Spike Accuracy

Reference Method: SOP-PCR-1.0
Batch ID: P393711

Spiked Sample	Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
2225179	HF183-qPCR	85.4		P	50 - 175
2225334	HF183-qPCR	89.7		P	50 - 175
2225550	HF183-qPCR	60.9	52.0	P/P	50 - 175
2226117	HF183-qPCR	80.9		P	50 - 175
2226118	HF183-qPCR	76.2		P	50 - 175
2226119	HF183-qPCR	64.2		P	50 - 175
2226120	HF183-qPCR	78.2		P	50 - 175
2226121	HF183-qPCR	83.5		P	50 - 175
2226122	HF183-qPCR	81.2		P	50 - 175

Reference Method: SOP-PCR-1.2
Batch ID: P393711

Spiked Sample	Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
2225179	GULL2-qPCR	70.7		P	50 - 300
2225334	GULL2-qPCR	43.3		F	50 - 300
2225550	GULL2-qPCR	12.2	9.76	F/F	50 - 300

Quality Assurance Report Matrix Spike Accuracy

Reference Method: SOP-PCR-1.3
Batch ID: P393711

Spiked Sample	Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
2225179	GFD-qPCR	71.1		P	50 - 175
2225334	GFD-qPCR	77.4		P	50 - 175
2225550	GFD-qPCR	62.2	59.4	P/P	50 - 175

Reference Method: SOP-PCR-1.4
Batch ID: P393711

Spiked Sample	Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
2225179	BacR-qPCR	74.6		P	50 - 175
2225334	BacR-qPCR	67.5		P	50 - 175
2225550	BacR-qPCR	44.1	43.1	F/F	50 - 175

Reference Method: SOP-PCR-1.5
Batch ID: P393711

Spiked Sample	Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
2225179	DG3-qPCR	81.7		P	50 - 175
2225334	DG3-qPCR	72.5		P	50 - 175
2225550	DG3-qPCR	67.7	67.5	P/P	50 - 175

Quality Assurance Report Precision

Reference Method: SOP-PCR-1.0
Batch ID: P393711

Replicated Lab Sample	Component	% RSD/RPD	Sample/Spike/LCS*	Pass/Fail	Control Limits
2225550	HF183-qPCR	15.8	Spike	P	0 - 25

Reference Method: SOP-PCR-1.2
Batch ID: P393711

Replicated Lab Sample	Component	% RSD/RPD	Sample/Spike/LCS*	Pass/Fail	Control Limits
2225550	GULL2-qPCR	22.4	Spike	P	0 - 25

Reference Method: SOP-PCR-1.3
Batch ID: P393711

Replicated Lab Sample	Component	% RSD/RPD	Sample/Spike/LCS*	Pass/Fail	Control Limits
2225550	GFD-qPCR	4.60	Spike	P	0 - 25

Reference Method: SOP-PCR-1.4
Batch ID: P393711

Replicated Lab Sample	Component	% RSD/RPD	Sample/Spike/LCS*	Pass/Fail	Control Limits
2225550	BacR-qPCR	2.19	Spike	P	0 - 25

Quality Assurance Report Precision

Reference Method: SOP-PCR-1.5
Batch ID: P393711

Replicated Lab Sample	Component	% RSD/RPD	Sample/Spike/LCS*	Pass/Fail	Control Limits
2225550	DG3-qPCR	0.309	Spike	P	0 - 25

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

Quality Assurance Report Surrogates

Lab Sample ID: 2225334
Field Sample ID: G3NW0177

Reference Method	Surrogate	% Rec.	Pass/Fail	Control Limits
SOP-PCR-1.0	SKETA-qPCR	98.3	P	50 - 300
SOP-PCR-1.2	SKETA-qPCR	92.8	P	50 - 300
SOP-PCR-1.3	SKETA-qPCR	93.8	P	50 - 300
SOP-PCR-1.4	SKETA-qPCR	92.1	P	50 - 300
SOP-PCR-1.5	SKETA-qPCR	76.6	P	50 - 300

Quality Assurance Report Summary

Ref. Method	Analyte	LCS % Recovery		MS % Recovery			LCS	Precision SMP	MS
SOP-PCR-1.0	HF183-qPCR	96.8	88.9	85.4	89.7	60.9			15.8
SOP-PCR-1.2	GULL2-qPCR	93.7	89.0	70.7	43.3	12.2			22.4
SOP-PCR-1.3	GFD-qPCR	74.9	66.6	71.1	77.4	62.2			4.60
SOP-PCR-1.4	BacR-qPCR	90.5	85.1	67.5	74.6	44.1			2.19
SOP-PCR-1.5	DG3-qPCR	85.0	87.4	81.7	72.5	67.7			0.309

Reference Method Descriptions

Method	Description	Associated Samples
Enterolert/QT	Enterococci by Enterolert/QT by UWF in Pensacola	2225328, 2225329, 2225330, 2225331, 2225332
SM 9222 D	Fecal coliforms by MF by Test America in Pensacola	2225323, 2225324, 2225325, 2225326, 2225327
SM 9223 B Quanti-Tray	E. coli by Colilert-18/QT by UWF in Pensacola	2225328, 2225329, 2225330, 2225331, 2225332, 2225333
SOP-PCR-1.0	Detection and quantification of human-specific HF183 Bacteroides genetic marker with quantitative polymerase chain reaction	2225334
SOP-PCR-1.2	Detection and quantification of coastal bird specific Catellicoccus marimammalium Gull2 genetic marker with quantitative polymerase chain reaction	2225334
SOP-PCR-1.3	Detection and quantification of bird specific Helicobacter GFD genetic marker with quantitative polymerase chain reaction	2225334
SOP-PCR-1.4	Detection and quantification of Ruminant specific Bacteroidetes BacR genetic marker with quantitative polymerase chain reaction	2225334

Reference Method Descriptions

Method	Description	<u>Associated Samples</u>
SOP-PCR-1.5	Detection and quantification of canine-specific DG3 Bacteroides genetic marker with quantitative polymerase chain reaction	2225334

Preparation and Analysis Log

Ref. Method	Received Date	Prep Date/Time	Prepared By	Analysis Date/Time	Analyzed By	Associated Samples
Enterolert/QT	02/10/2021	02/10/2021 15:15		02/11/2021 15:40	UWF Lab	2225328, 2225329, 2225330, 2225331, 2225332
SM 9222 D	02/10/2021	02/10/2021 15:50		02/11/2021 15:19	TestAmerica Pensacola	2225323, 2225324, 2225325, 2225326, 2225327
SM 9223 B Quanti-Tray	02/10/2021	02/10/2021 15:35		02/11/2021 13:13	UWF Lab	2225328, 2225329, 2225330, 2225331, 2225332, 2225333
SOP-PCR-1.0	02/15/2021	02/15/2021 12:15	DeAsia Armster	02/23/2021 11:28	Mailin Lopez	2225334
SOP-PCR-1.2	02/15/2021	02/15/2021 12:15	DeAsia Armster	03/10/2021 08:43	Mailin Lopez	2225334
SOP-PCR-1.3	02/15/2021	02/15/2021 12:15	DeAsia Armster	03/11/2021 08:24	Mailin Lopez	2225334
SOP-PCR-1.4	02/15/2021	02/15/2021 12:15	DeAsia Armster	03/08/2021 10:11	Mailin Lopez	2225334
SOP-PCR-1.5	02/15/2021	02/15/2021 12:15	DeAsia Armster	03/08/2021 11:03	Mailin Lopez	2225334