

Biological Analysis Report

WQAP-2017-05-23-02

Florida Department of Environmental Protection
Central Laboratory
2600 Blair Stone Road
Tallahassee, FL 32399-2400
DOH Accreditation E31780

Event Description: **Pinellas LR5**
Request ID: **RQ-2017-05-22-20**
Customer: **WQAP**
Project ID: **SMP**

Send Reports to:
FL Dept. of Environmental Protection
13051 N. Telecom Parkway
Tampa, FL 33637
Attn: Judy Ashton

For additional information please contact
Cheryl Swanson - Administrator
J. Marshall Faircloth - Aquatic Toxicology
Jacqueline Savage - Bench Biology
Jacqueline Savage - Microbiology
Daisys Matthews - Molecular Biology
Jennifer Walters - Taxonomy (Invertebrate & Algal)
Thekkekalathil Chandrasekhar, Ph.D, QA Officer
Phone (850) 245-8177

Certified by: Daisys Matthews,

Date Certified: 26-JUN-2017 11:05

A handwritten signature in cursive script that reads "D Matthews". The signature is written in dark ink on a white background.

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 group are included in this report: Molecular.

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 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: Joes Creek

Collection Date/Time: 05/22/2017 08:59

Field ID: G5SW0139

Matrix: W-SURF-FRH

Sample ID	Ref. Method	Component	Result	Code	Units	Batch ID	QC Codes
1900676	SOP-PCR-1.0	HF183-qPCR**	14	T	GEU/100 mL	P325693	
	SOP-PCR-4.0	HF183 - Human Source Marker**	Below Threshold				

Sample Location: Joes Creek

Collection Date/Time: 05/22/2017 10:03

Field ID: G5SW0138

Matrix: W-SURF-FRH

Sample ID	Ref. Method	Component	Result	Code	Units	Batch ID	QC Codes
1900677	SOP-PCR-1.0	HF183-qPCR**	140	U	GEU/100 mL	P325693	
	SOP-PCR-1.2	GULL2-qPCR**	1.7E+04	U	TSC/100 mL	P325693	
	SOP-PCR-1.3	GFD-purified-qPCR**	44	TJ	TSC/100 mL	P325960	LCS, MS, RPD
	SOP-PCR-1.4	BacR-qPCR**	3.0E+03	U	TSC/100 mL	P325693	LCS
	SOP-PCR-4.0	HF183 - Human Source Marker**	Below Threshold				

Ref. Method and Comment:

SOP-PCR-1.0: No amplification of HF183 marker.

SOP-PCR-1.2: No amplification of GULL2 marker.

SOP-PCR-1.3: Duplicate was analyzed and result is 170 UJ. Quality control failure(s) observed. See QA report for details.

SOP-PCR-1.4: No amplification of BACR marker.

Sample Location: Joes Creek

Collection Date/Time: 05/22/2017 10:39

Field ID: G5SW0137

Matrix: W-SURF-FRH

Sample ID	Ref. Method	Component	Result	Code	Units	Batch ID	QC Codes
1900678	SOP-PCR-1.0	HF183-qPCR**	6.6	TJ	GEU/100 mL	P325960	LCS, SUR
	SOP-PCR-4.0	HF183 - Human Source Marker**	Below Threshold				

Ref. Method and Comment:

SOP-PCR-1.0: Sample processed with an additional purification step to reduce inhibition, which can reduce overall DNA recovery. See QA report.

Quality Assurance Report Method Blank Results

Reference Method: SOP-PCR-1.0

Batch ID: P325693

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

Reference Method: SOP-PCR-1.0

Batch ID: P325960

Component	Result	Code	Units
HF183-qPCR	15	T	GEU/100 mL

Reference Method: SOP-PCR-1.2

Batch ID: P325693

Component	Result	Code	Units
GULL2-qPCR	1.7E+04	U	TSC/100 mL

Quality Assurance Report Method Blank Results

Reference Method: SOP-PCR-1.3
 Batch ID: P325960

Component	Result	Code	Units
GFD-purified-qPCR	340	U	TSC/100 mL

Quality Assurance Report Laboratory Control Sample Accuracy

Reference Method: SOP-PCR-1.0
 Batch ID: P325693

Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
HF183-qPCR	88.1	134	P/P	50 - 175

Reference Method: SOP-PCR-1.0
 Batch ID: P325960

Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
HF183-qPCR	83.6	16.5	P/F	50 - 175

Reference Method: SOP-PCR-1.2
 Batch ID: P325693

Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
GULL2-qPCR	92.7	158	P/P	50 - 300

Reference Method: SOP-PCR-1.3
 Batch ID: P325960

Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
GFD-purified-qPCR	118	30.9	P/F	50 - 175

Reference Method: SOP-PCR-1.4
 Batch ID: P325693

Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
BacR-qPCR	101	182	P/F	50 - 175

Quality Assurance Report Matrix Spike Accuracy

Reference Method: SOP-PCR-1.0
 Batch ID: P325693

Spiked Sample	Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
1895704	HF183-qPCR	114		P	50 - 175
1896803	HF183-qPCR	105		P	50 - 175
1897242	HF183-qPCR	107		P	50 - 175
1897244	HF183-qPCR	124		P	50 - 175
1898633	HF183-qPCR	130		P	50 - 175
1900676	HF183-qPCR	93.4		P	50 - 175
1900677	HF183-qPCR	93.1	84.8	P/P	50 - 175
1901185	HF183-qPCR	104		P	50 - 175
1901186	HF183-qPCR	95.6		P	50 - 175

Quality Assurance Report Matrix Spike Accuracy

Reference Method: SOP-PCR-1.0
Batch ID: P325960

Spiked Sample	Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
1894259	HF183-qPCR	94.2		P	50 - 175
1900678	HF183-qPCR	101		P	50 - 175

Reference Method: SOP-PCR-1.2
Batch ID: P325693

Spiked Sample	Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
1897242	GULL2-qPCR	100		P	50 - 300
1900677	GULL2-qPCR	103	84.4	P/P	50 - 300
1901185	GULL2-qPCR	74.6		P	50 - 300
1901186	GULL2-qPCR	52.7		P	50 - 300

Reference Method: SOP-PCR-1.3
Batch ID: P325960

Spiked Sample	Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
1893555	GFD-purified-qPCR	0.0		F	50 - 175
1893557	GFD-purified-qPCR	140		P	50 - 175
1893558	GFD-purified-qPCR	141		P	50 - 175
1894258	GFD-purified-qPCR	17.6		F	50 - 175
1895705	GFD-purified-qPCR	100		P	50 - 175
1897242	GFD-purified-qPCR	0.0		F	50 - 175
1900677	GFD-purified-qPCR	3.84	5.47	F/F	50 - 175
1901185	GFD-purified-qPCR	61.6		P	50 - 175
1901186	GFD-purified-qPCR	27.7		F	50 - 175

Reference Method: SOP-PCR-1.4
Batch ID: P325693

Spiked Sample	Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
1897242	BacR-qPCR	72.6		P	50 - 175
1900677	BacR-qPCR	61.1	68.0	P/P	50 - 175
1901185	BacR-qPCR	59.6		P	50 - 175
1901186	BacR-qPCR	51.8		P	50 - 175

Quality Assurance Report Precision

Reference Method: SOP-PCR-1.0
Batch ID: P325693

Replicated Lab Sample	Component	% RSD/RPD	Sample/Spike/LCS*	Pass/Fail	Control Limits
1900677	HF183-qPCR	9.24	Spike	P	0 - 25

Reference Method: SOP-PCR-1.2
Batch ID: P325693

Replicated Lab Sample	Component	% RSD/RPD	Sample/Spike/LCS*	Pass/Fail	Control Limits
1900677	GULL2-qPCR	19.7	Spike	P	0 - 25

Quality Assurance Report Precision

Reference Method: SOP-PCR-1.3
Batch ID: P325960

Replicated Lab Sample	Component	% RSD/RPD	Sample/Spike/LCS*	Pass/Fail	Control Limits
1900677	GFD-purified-qPCR	35.1	Spike	F	0 - 25

Reference Method: SOP-PCR-1.4
Batch ID: P325693

Replicated Lab Sample	Component	% RSD/RPD	Sample/Spike/LCS*	Pass/Fail	Control Limits
1900677	BacR-qPCR	10.8	Spike	P	0 - 25

* Sample, spike and/or laboratory control sample precision (LCS) is reported.
Replicate spike precision may be reported when sample results are below quantifiable levels.

Quality Assurance Report Surrogates

Lab Sample ID: 1900676
Field Sample ID: G5SW0139

Reference Method	Surrogate	% Rec.	Pass/Fail	Control Limits
SOP-PCR-1.0	SKETA-qPCR	65.6	P	50 - 300

Lab Sample ID: 1900677
Field Sample ID: G5SW0138

Reference Method	Surrogate	% Rec.	Pass/Fail	Control Limits
SOP-PCR-1.0	SKETA-qPCR	69.7	P	50 - 300
SOP-PCR-1.2	SKETA-qPCR	85.0	P	50 - 300
SOP-PCR-1.4	SKETA-qPCR	98.7	P	50 - 300

Lab Sample ID: 1900678
Field Sample ID: G5SW0137

Reference Method	Surrogate	% Rec.	Pass/Fail	Control Limits
SOP-PCR-1.0	SKETA-qPCR	14.4	F	30 - 300

Quality Assurance Report Summary

Ref. Method	Analyte	LCS % Recovery		MS % Recovery			LCS	Precision SMP	MS
SOP-PCR-1.0	HF183-qPCR	134	88.1	114	93.4	93.1			9.24
				84.8					
	HF183-qPCR	16.5	83.6	94.2	101				
SOP-PCR-1.2	GULL2-qPCR	158	92.7	103	84.4	74.6			19.7
				52.7					
SOP-PCR-1.3	GFD-purified-qPCR	30.9	118	0.0	140	141			35.1
				17.6					
SOP-PCR-1.4	BacR-qPCR	182	101	61.1	68.0	59.6			10.8
				51.8					

Reference Method Descriptions

Method / DoH Cert #	Description	<u>Associated Samples</u>
SOP-PCR-1.0 / E31780	Detection and quantification of human-specific HF183 Bacteroides genetic marker with quantitative polymerase chain reaction	1900676, 1900677, 1900678
SOP-PCR-1.2 / E31780	Detection and quantification of coastal bird specific Catellicoccus marimammalium genetic marker with quantitative polymerase chain reaction	1900677
SOP-PCR-1.3 / E31780	Detection and quantification of bird specific Helicobacter genetic marker with quantitative polymerase chain reaction	1900677
SOP-PCR-1.4 / E31780	Detection and quantification of Ruminant specific Bacteroidetes genetic marker with quantitative polymerase chain reaction	1900677
SOP-PCR-4.0 / E31780	Preparation of Samples by Filtration and held for qPCR Analysis	1900676, 1900677, 1900678

Preparation and Analysis Log

Ref. Method	Received Date	Prep Date/Time	Prepared By	Analysis Date/Time	Analyzed By	Associated Samples
SOP-PCR-1.0	05/23/2017	05/23/2017 13:45	Puja Jasrotia	05/25/2017	Puja Jasrotia	1900676, 1900677
	05/23/2017	05/23/2017 13:45	Puja Jasrotia	06/05/2017	Puja Jasrotia	1900678
SOP-PCR-1.2	05/23/2017	05/23/2017 13:45	Puja Jasrotia	05/26/2017	Sarah Menz	1900677
SOP-PCR-1.3	05/23/2017	05/23/2017 13:45	Sarah Menz	06/05/2017	Sarah Menz	1900677
SOP-PCR-1.4	05/23/2017	05/23/2017 13:45	Puja Jasrotia	05/26/2017	Sarah Menz	1900677
SOP-PCR-4.0	05/23/2017	05/23/2017 13:45	Avril Wood	06/06/2017	Puja Jasrotia	1900676, 1900677, 1900678