

Biological Analysis Report

CEN-DIST-2017-05-03-01

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

Overflow Analyses Performed By:
Flowers Chemical Laboratories Inc. - Altamonte
Springs
481 Newburyport Avenue
Altamonte Springs, Florida 32701
DOH Accreditation E83018

Event Description: **Smith/Six/Gee/Howell/ Soldier/Bear/ Wekiva Rvr**
Request ID: **RQ-2017-05-01-67**
Customer: **CEN-DIST**
Project ID: **SMP**

Send Reports to:
FL Dept. of Environmental Protection
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3319 Maguire Blvd., Suite 232
Orlando, FL 32803-3767
Attn: Mike Linger

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Certified by: Daisys Matthews,

Date Certified: 16-JUN-2017 07:35

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 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 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: Gee Creek @ SR Hwy 419

Collection Date/Time: 05/02/2017 12:22

Field ID: G2CE0075

Matrix: W-SURF-FRH

Sample ID	Ref. Method	Component	Result	Code	Units	Batch ID	QC Codes
1895701	SM 9223 Quanti-Tray	Escherichia Coli-Quanti-Tray	9800		MPN/100 mL		
1895704	SOP-PCR-1.0	HF183-qPCR**	310	I	GEU/100 mL	P325693	
	SOP-PCR-4.0	HF183 - Human Source Marker**	Above Threshold				

Sample Location: Soldier Creek @ 150m Upstream of SR Hwy 419 **Collection Date/Time: 05/02/2017 12:40**

Field ID: G2CE0071

Matrix: W-SURF-FRH

Sample ID	Ref. Method	Component	Result	Code	Units	Batch ID	QC Codes
1895703	SM 9223 Quanti-Tray	Escherichia Coli-Quanti-Tray	24200		MPN/100 mL		
1895706	SOP-PCR-1.0	HF183-qPCR**	1.07E+03		GEU/100 mL	P324915	LCS

Sample Location: Bear Crk just North of Winter Springs Blvd in Bear Crk Park **Collection Date/Time: 05/02/2017 13:23**

Field ID: G2CE0069

Matrix: W-SURF-FRH

Sample ID	Ref. Method	Component	Result	Code	Units	Batch ID	QC Codes
1895702	SM 9223 Quanti-Tray	Escherichia Coli-Quanti-Tray	563		MPN/100 mL		
1895705	SOP-PCR-1.0	HF183-qPCR**	94	U	GEU/100 mL	P325481	
	SOP-PCR-1.2	GULL2-qPCR**	5.6E+03	U	TSC/100 mL	P325481	MS
	SOP-PCR-1.3	GFD-purified-qPCR**	110	U	TSC/100 mL	P325960	LCS, MS, RPD
	SOP-PCR-1.4	BacR-qPCR**	1.0E+03	U	TSC/100 mL	P325481	LCS
	SOP-PCR-4.0	HF183 - Human Source Marker**	Above 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: No amplification of GFD marker.
 SOP-PCR-1.4: No amplification of BACR marker.

Quality Assurance Report Method Blank Results

Reference Method: SOP-PCR-1.0

Batch ID: P324915

Component	Result	Code	Units
HF183-qPCR	47	U	GEU/100 mL

Reference Method: SOP-PCR-1.0

Batch ID: P325481

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

Reference Method: SOP-PCR-1.0

Batch ID: P325693

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

Quality Assurance Report Method Blank Results

Reference Method: SOP-PCR-1.2
Batch ID: P325481

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

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

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

Reference Method: SOP-PCR-1.4
Batch ID: P325481

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

Quality Assurance Report Laboratory Control Sample Accuracy

Reference Method: SOP-PCR-1.0
Batch ID: P324915

Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
HF183-qPCR	87.5	20.9	P/F	50 - 175

Reference Method: SOP-PCR-1.0
Batch ID: P325481

Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
HF183-qPCR	66.9	91.8	P/P	50 - 175
HF183-qPCR	98.0	86.3	P/P	50 - 175

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-1.2
Batch ID: P325481

Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
GULL2-qPCR	92.7	107	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

Quality Assurance Report Laboratory Control Sample Accuracy

Reference Method: SOP-PCR-1.4
 Batch ID: P325481

Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
BacR-qPCR	101	38.6	P/F	50 - 175

Quality Assurance Report Matrix Spike Accuracy

Reference Method: SOP-PCR-1.0
 Batch ID: P324915

Spiked Sample	Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
1888809	HF183-qPCR	81.4		P	50 - 175
1893783	HF183-qPCR	74.5		P	50 - 175
1895471	HF183-qPCR	77.7	72.1	P/P	50 - 175
1895706	HF183-qPCR	82.7		P	50 - 175

Reference Method: SOP-PCR-1.0
 Batch ID: P325481

Spiked Sample	Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
1893555	HF183-qPCR	67.3		P	50 - 175
1893557	HF183-qPCR	79.4		P	50 - 175
1893558	HF183-qPCR	83.5		P	50 - 175
1894258	HF183-qPCR	73.7		P	50 - 175
1894305	HF183-qPCR	94.0		P	50 - 175
1894648	HF183-qPCR	66.5		P	50 - 175
1895705	HF183-qPCR	96.7		P	50 - 175
1896293	HF183-qPCR	94.3		P	50 - 175
1896294	HF183-qPCR	96.8		P	50 - 175
1897338	HF183-qPCR	86.0	88.9	P/P	50 - 175
1899957	HF183-qPCR	88.1		P	50 - 175
1899958	HF183-qPCR	84.6		P	50 - 175

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

Reference Method: SOP-PCR-1.2
 Batch ID: P325481

Spiked Sample	Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
1893555	GULL2-qPCR	47.0		F	50 - 300
1893557	GULL2-qPCR	91.3		P	50 - 300

Quality Assurance Report Matrix Spike Accuracy

Reference Method: SOP-PCR-1.2

Batch ID: P325481

Spiked Sample	Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
1893558	GULL2-qPCR	64.6		P	50 - 300
1894258	GULL2-qPCR	88.0		P	50 - 300
1894305	GULL2-qPCR	103		P	50 - 300
1895705	GULL2-qPCR	76.9		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: P325481

Spiked Sample	Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
1893555	BacR-qPCR	51.0		P	50 - 175
1893557	BacR-qPCR	57.6		P	50 - 175
1893558	BacR-qPCR	58.4		P	50 - 175
1894258	BacR-qPCR	61.0		P	50 - 175
1894305	BacR-qPCR	66.5		P	50 - 175
1895705	BacR-qPCR	58.8		P	50 - 175
1896294	BacR-qPCR	58.4		P	50 - 175

Quality Assurance Report Precision

Reference Method: SOP-PCR-1.0

Batch ID: P324915

Replicated

Lab Sample	Component	% RSD/RPD	Sample/Spike/LCS*	Pass/Fail	Control Limits
1895471	HF183-qPCR	7.43	Spike	P	0 - 25

Reference Method: SOP-PCR-1.0

Batch ID: P325481

Replicated

Lab Sample	Component	% RSD/RPD	Sample/Spike/LCS*	Pass/Fail	Control Limits
1897338	HF183-qPCR	3.30	Spike	P	0 - 25

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: P325481

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

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: P325481

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: 1895704
Field Sample ID: G2CE0075

Reference Method	Surrogate	% Rec.	Pass/Fail	Control Limits
SOP-PCR-1.0	SKETA-qPCR	79.2	P	50 - 300

Lab Sample ID: 1895705
Field Sample ID: G2CE0069

Reference Method	Surrogate	% Rec.	Pass/Fail	Control Limits
SOP-PCR-1.0	SKETA-qPCR	109	P	50 - 300
SOP-PCR-1.2	SKETA-qPCR	105	P	50 - 300
SOP-PCR-1.4	SKETA-qPCR	106	P	50 - 300

Lab Sample ID: 1895706
Field Sample ID: G2CE0071

Reference Method	Surrogate	% Rec.	Pass/Fail	Control Limits
SOP-PCR-1.0	SKETA-qPCR	35.0	P	30 - 300

Quality Assurance Report Summary

Ref. Method	Analyte	LCS % Recovery			MS % Recovery			LCS	Precision SMP	MS
SOP-PCR-1.0	HF183-qPCR	20.9	87.5		74.5	81.4	77.7			7.43
					72.1					
	HF183-qPCR	66.9	86.3	98.0	84.6	67.3	79.4			3.30
		91.8			83.5					
	HF183-qPCR	134	88.1		114	93.4	93.1			9.24
					84.8					
SOP-PCR-1.2	GULL2-qPCR	107	92.7		91.3	64.6	88.0			19.7
					103					
SOP-PCR-1.3	GFD-purified-qPCR	30.9	118		0.0	140	141			35.1
					17.6					
SOP-PCR-1.4	BacR-qPCR	38.6	101		58.8	58.4	51.0			10.8
					57.6					

Reference Method Descriptions

Method / DoH Cert #	Description	Associated Samples
SM 9223 Quanti-Tray / E83018	Escherichia coli by Colilert 18 Quanti-Tray method by FlowersOverflow Laboratory for Central District	1895701, 1895702, 1895703
SOP-PCR-1.0 / E31780	Detection and quantification of human-specific HF183 Bacteroides genetic marker with quantitative polymerase chain reaction	1895704, 1895705, 1895706
SOP-PCR-1.2 / E31780	Detection and quantification of coastal bird specific Catellicoccus marimammalium genetic marker with quantitative polymerase chain reaction	1895705
SOP-PCR-1.3 / E31780	Detection and quantification of bird specific Helicobacter genetic marker with quantitative polymerase chain reaction	1895705
SOP-PCR-1.4 / E31780	Detection and quantification of Ruminant specific Bacteroidetes genetic marker with quantitative polymerase chain reaction	1895705
SOP-PCR-4.0 / E31780	Preparation of Samples by Filtration and held for qPCR Analysis	1895704, 1895705

Preparation and Analysis Log

Ref. Method	Received Date	Prep Date/Time	Prepared By	Analysis Date/Time	Analyzed By	Associated Samples
SM 9223 Quanti-Tray	05/02/2017			05/02/2017 17:25	Flowers - Altamonte Springs	1895701, 1895702, 1895703
SOP-PCR-1.0	05/03/2017	05/03/2017 13:12	Puja Jasrotia	05/17/2017	Puja Jasrotia	1895706
	05/03/2017	05/03/2017 13:31	Puja Jasrotia	05/23/2017	Puja Jasrotia	1895705
	05/03/2017	05/03/2017 13:31	Puja Jasrotia	05/25/2017	Puja Jasrotia	1895704
SOP-PCR-1.2	05/03/2017	05/03/2017 13:31	Puja Jasrotia	05/26/2017	Sarah Menz	1895705
SOP-PCR-1.3	05/03/2017	05/03/2017 13:31	Sarah Menz	06/05/2017	Sarah Menz	1895705
SOP-PCR-1.4	05/03/2017	05/03/2017 13:31	Puja Jasrotia	05/26/2017	Sarah Menz	1895705
SOP-PCR-4.0	05/03/2017	05/03/2017 13:31	Avril Wood	05/19/2017	Puja Jasrotia	1895704, 1895705