

Biological Analysis Report

CEN-DIST-2017-02-15-02

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 Cnl/6 Mile Crk/G Crk/Howell Crk**
Request ID: **RQ-2017-02-06-52**
Customer: **CEN-DIST**
Project ID: **SMP**

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

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, PhD, QA Officer
Phone (850) 245-8177

Certified by: Daisys Matthews,

Date Certified: 31-MAR-2017 15:04

A handwritten signature in black ink, appearing to read "D Matthews", with a stylized flourish at the end.

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: HOWELL CREEK @

Collection Date/Time: 02/14/2017 09:28

Field ID: G2CE0072

Matrix: W-SURF-FRH

Sample ID	Ref. Method	Component	Result	Code	Units	Batch ID	QC Codes
1872959	SM 9223 Quanti-Tray	Escherichia Coli-Quanti-Tray	771		MPN/100 mL		
1872963	SOP-PCR-1.0	HF183-qPCR**	140	U	GEU/100 mL	P320541	
	SOP-PCR-1.2	GULL2-qPCR**	1.09E+05	A	TSC/100 mL	P320541	
	SOP-PCR-1.3	GFD-purified-qPCR**	110	T	TSC/100 mL	P320888	LCS, MS
	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-1.3: Analyte detected at a level below the current nominal MDL. Duplicate was analyzed and result is 60 T.

Sample Location: GEE CREEK @

Collection Date/Time: 02/14/2017 10:05

Field ID: G2CE0075

Matrix: W-SURF-FRH

Sample ID	Ref. Method	Component	Result	Code	Units	Batch ID	QC Codes
1872960	SM 9223 Quanti-Tray	Escherichia Coli-Quanti-Tray	906		MPN/100 mL		
1872964	SOP-PCR-1.0	HF183-qPCR**	94	U	GEU/100 mL	P320541	
	SOP-PCR-1.2	GULL2-qPCR**	1.1E+04	U	TSC/100 mL	P320541	
	SOP-PCR-1.3	GFD-purified-qPCR**	1.3E+04	U	TSC/100 mL	P320888	LCS, MS
	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.

Sample Location: SIX MILE CREEK @ 400M US OF LAKE JESSUP **Collection Date/Time: 02/14/2017 10:41**

Field ID: G2CE0074

Matrix: W-SURF-FRH

Sample ID	Ref. Method	Component	Result	Code	Units	Batch ID	QC Codes
1872961	SM 9223 Quanti-Tray	Escherichia Coli-Quanti-Tray	605		MPN/100 mL		
1872965	SOP-PCR-1.0	HF183-qPCR**	94	U	GEU/100 mL	P320541	
	SOP-PCR-1.2	GULL2-qPCR**	1.1E+04	U	TSC/100 mL	P320541	
	SOP-PCR-1.3	GFD-purified-qPCR**	11	T	TSC/100 mL	P320888	LCS, MS
	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: Analyte detected at a level below the current nominal MDL.

Sample Location: SMITH CANAL @

Collection Date/Time: 02/14/2017 11:28

Field ID: G2CE0073

Matrix: W-SURF-FRH

Sample ID	Ref. Method	Component	Result	Code	Units	Batch ID	QC Codes
1872962	SM 9223 Quanti-Tray	Escherichia Coli-Quanti-Tray	187		MPN/100 mL		
1872966	SOP-PCR-4.0	HF183 - Human Source Marker**	Below Threshold				

Quality Assurance Report Method Blank Results

Quality Assurance Report Method Blank Results

Reference Method: SOP-PCR-1.0
Batch ID: P320541

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

Reference Method: SOP-PCR-1.2
Batch ID: P320541

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

Reference Method: SOP-PCR-1.3
Batch ID: P320888

Component	Result	Code	Units
GFD-purified-qPCR	4.0E+04	U	TSC/100 mL

Quality Assurance Report Laboratory Control Sample Accuracy

Reference Method: SOP-PCR-1.0
Batch ID: P320541

Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
HF183-qPCR	81.9	105	P/P	50 - 175
HF183-qPCR	97.1	82.5	P/P	50 - 175

Reference Method: SOP-PCR-1.2
Batch ID: P320541

Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
GULL2-qPCR	73.5	132	P/P	50 - 300

Reference Method: SOP-PCR-1.3
Batch ID: P320888

Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
GFD-purified-qPCR	103	17.1	P/F	50 - 175

Quality Assurance Report Matrix Spike Accuracy

Reference Method: SOP-PCR-1.0
Batch ID: P320541

Spiked Sample	Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
1871240	HF183-qPCR	73.4		P	50 - 175
1871241	HF183-qPCR	95.2		P	50 - 175
1871242	HF183-qPCR	93.4		P	50 - 175
1871243	HF183-qPCR	61.8		P	50 - 175
1871248	HF183-qPCR	65.9		P	50 - 175
1872963	HF183-qPCR	86.1	86.6	P/P	50 - 175
1872964	HF183-qPCR	85.5		P	50 - 175

Quality Assurance Report Matrix Spike Accuracy

Reference Method: SOP-PCR-1.0
Batch ID: P320541

Spiked Sample	Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
1872965	HF183-qPCR	90.8		P	50 - 175
1875946	HF183-qPCR	73.0		P	50 - 175
1875947	HF183-qPCR	84.6		P	50 - 175
1875948	HF183-qPCR	107		P	50 - 175
1875949	HF183-qPCR	81.9		P	50 - 175
1875950	HF183-qPCR	84.7		P	50 - 175

Reference Method: SOP-PCR-1.2
Batch ID: P320541

Spiked Sample	Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
1872963	GULL2-qPCR	91.4	77.0	P/P	50 - 300
1872964	GULL2-qPCR	94.6		P	50 - 300
1872965	GULL2-qPCR	99.4		P	50 - 300

Reference Method: SOP-PCR-1.3
Batch ID: P320888

Spiked Sample	Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
1872963	GFD-purified-qPCR	91.9	109	P/P	50 - 175
1872964	GFD-purified-qPCR	124		P	50 - 175
1872965	GFD-purified-qPCR	112		P	50 - 175
1873034	GFD-purified-qPCR	83.5		P	50 - 175
1873071	GFD-purified-qPCR	72.2		P	50 - 175
1874753	GFD-purified-qPCR	110		P	50 - 175
1874864	GFD-purified-qPCR	19.8		F	50 - 175
1876739	GFD-purified-qPCR	84.2		P	50 - 175
1878468	GFD-purified-qPCR	121		P	50 - 175

Quality Assurance Report Precision

Reference Method: SOP-PCR-1.0
Batch ID: P320541

Replicated Lab Sample	Component	% RSD/RPD	Sample/Spike/LCS*	Pass/Fail	Control Limits
1872963	HF183-qPCR	0.524	Spike	P	0 - 25

Reference Method: SOP-PCR-1.2
Batch ID: P320541

Replicated Lab Sample	Component	% RSD/RPD	Sample/Spike/LCS*	Pass/Fail	Control Limits
1872963	GULL2-qPCR	23.8	Sample	P	0 - 25
1872963	GULL2-qPCR	17.2	Spike	P	0 - 25

Quality Assurance Report Precision

Reference Method: SOP-PCR-1.3
Batch ID: P320888

Replicated Lab Sample	Component	% RSD/RPD	Sample/Spike/LCS*	Pass/Fail	Control Limits
1872963	GFD-purified-qPCR	16.9	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: 1872963
Field Sample ID: G2CE0072

Reference Method	Surrogate	% Rec.	Pass/Fail	Control Limits
SOP-PCR-1.0	SKETA-qPCR	86.2	P	50 - 300
SOP-PCR-1.2	SKETA-qPCR	98.4	P	50 - 300

Lab Sample ID: 1872964
Field Sample ID: G2CE0075

Reference Method	Surrogate	% Rec.	Pass/Fail	Control Limits
SOP-PCR-1.0	SKETA-qPCR	94.1	P	50 - 300
SOP-PCR-1.2	SKETA-qPCR	106	P	50 - 300

Lab Sample ID: 1872965
Field Sample ID: G2CE0074

Reference Method	Surrogate	% Rec.	Pass/Fail	Control Limits
SOP-PCR-1.0	SKETA-qPCR	79.8	P	50 - 300
SOP-PCR-1.2	SKETA-qPCR	98.5	P	50 - 300

Quality Assurance Report Summary

Ref. Method	Analyte	LCS % Recovery			MS % Recovery			LCS	Precision	
									SMP	MS
SOP-PCR-1.0	HF183-qPCR	81.9	82.5	97.1	73.4	95.2	93.4			0.524
		105			61.8					
SOP-PCR-1.2	GULL2-qPCR	132	73.5		91.4	77.0	94.6		23.8	17.2
					99.4					
SOP-PCR-1.3	GFD-purified-qPCR	17.1	103		91.9	109	124			16.9
					112					

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	1872959, 1872960, 1872961, 1872962
SOP-PCR-1.0 / E31780	Detection and quantification of human-specific HF183 Bacteroides genetic marker with quantitative polymerase chain reaction	1872963, 1872964, 1872965
SOP-PCR-1.2 / E31780	Detection and quantification of coastal bird specific Catellicoccus marimammalum genetic marker with quantitative polymerase chain reaction	1872963, 1872964, 1872965

Reference Method Descriptions

Method / DoH Cert #	Description	<u>Associated Samples</u>
SOP-PCR-1.3 / E31780	Detection and quantification of bird specific Helicobacter genetic marker with quantitative polymerase chain reaction	1872963, 1872964, 1872965
SOP-PCR-4.0 / E31780	Preparation of Samples by Filtration and held for qPCR Analysis	1872963, 1872964, 1872965, 1872966

Preparation and Analysis Log

Ref. Method	Received Date	Prep Date/Time	Prepared By	Analysis Date/Time	Analyzed By	Associated Samples
SM 9223 Quanti-Tray	02/14/2017			02/14/2017 15:03	Flowers - Altamonte Springs	1872959, 1872960, 1872961, 1872962
SOP-PCR-1.0	02/15/2017	02/15/2017 13:15	Daisys Matthews	03/02/2017	Sarah Menz	1872963, 1872964, 1872965
SOP-PCR-1.2	02/15/2017	02/15/2017 13:15	Daisys Matthews	03/13/2017	Sarah Menz	1872963, 1872964, 1872965
SOP-PCR-1.3	02/15/2017	02/15/2017 13:15	Sarah Menz	03/22/2017	Sarah Menz	1872963, 1872964, 1872965
SOP-PCR-4.0	02/15/2017	02/15/2017 13:15	Avril Wood	02/24/2017	Daisys Matthews	1872963, 1872964, 1872965, 1872966