

Biological Analysis Report

CEN-DIST-2017-03-15-01

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**
Request ID: **RQ-2017-03-13-45**
Customer: **CEN-DIST**
Project ID: **SMP**

Send Reports to:
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3319 Maguire Blvd., Suite 232
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Certified by: Daisys Matthews,

Date Certified: 17-APR-2017 08:02

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: Smith Canal @ Upstream of Nebraska Ave

Collection Date/Time: 03/14/2017 09:54

Field ID: G2CE0073

Matrix: W-SURF-FRH

Sample ID	Ref. Method	Component	Result	Code	Units	Batch ID	QC Codes
1880165	SM 9223 Quanti-Tray	Escherichia Coli-Quanti-Tray	422		MPN/100 mL		
1880174	SOP-PCR-1.0	HF183-qPCR**	94	U	GEU/100 mL	P322137	
	SOP-PCR-1.2	GULL2-qPCR**	1.0E+03	TJ	TSC/100 mL	P322137	LCS, MS, RPD
	SOP-PCR-1.3	GFD-purified-qPCR**	17	TJ	TSC/100 mL	P321737	LCS, MS, RPD
	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: Quality control failure(s) observed. See QA report for details.

SOP-PCR-1.3: Analyte detected at a level below the current nominal MDL. Quality control failure(s) observed. See QA report for details.

Sample Location: Six Mile Creek @ 400m US of Lake Jessup

Collection Date/Time: 03/14/2017 10:20

Field ID: G2CE0074

Matrix: W-SURF-FRH

Sample ID	Ref. Method	Component	Result	Code	Units	Batch ID	QC Codes
1880166	SM 9223 Quanti-Tray	Escherichia Coli-Quanti-Tray	521		MPN/100 mL		
1880175	SOP-PCR-1.0	HF183-qPCR**	94	U	GEU/100 mL	P322137	
	SOP-PCR-1.2	GULL2-qPCR**	5.6E+03	UJ	TSC/100 mL	P322137	LCS, MS, RPD
	SOP-PCR-1.3	GFD-purified-qPCR**	20	T	TSC/100 mL	P321737	LCS, MS, RPD
	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. Quality control failure(s) observed. See QA report for details.

SOP-PCR-1.3: Analyte detected at a level below the current nominal MDL.

Sample Location: Soldier Creek @ 150m Upstream of SR Hwy 419

Collection Date/Time: 03/14/2017 10:40

Field ID: G2CE0071

Matrix: W-SURF-FRH

Sample ID	Ref. Method	Component	Result	Code	Units	Batch ID	QC Codes
1880167	SM 9223 Quanti-Tray	Escherichia Coli-Quanti-Tray	578		MPN/100 mL		
1880176	SOP-PCR-1.0	HF183-qPCR**	17	T	GEU/100 mL	P322137	
	SOP-PCR-4.0	HF183 - Human Source Marker**	Above Threshold				

Sample Location: Gee Creek @ SR Hwy 419

Collection Date/Time: 03/14/2017 11:03

Field ID: G2CE0075

Matrix: W-SURF-FRH

Sample ID	Ref. Method	Component	Result	Code	Units	Batch ID	QC Codes
1880168	SM 9223 Quanti-Tray	Escherichia Coli-Quanti-Tray	504		MPN/100 mL		
1880177	SOP-PCR-1.0	HF183-qPCR**	94	U	GEU/100 mL	P322137	
	SOP-PCR-1.2	GULL2-qPCR**	5.6E+03	U	TSC/100 mL	P322137	LCS, MS, RPD
	SOP-PCR-1.3	GFD-purified-qPCR**	1.3E+04	UJ	TSC/100 mL	P321737	LCS, MS, RPD
	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. Quality control failure(s) observed. See QA report for details.

Sample Location: Bear Crk just north of Winter Springs Blvd in Bear Crk Park

Collection Date/Time: 03/14/2017 11:26

Field ID: G2CE0069

Matrix: W-SURF-FRH

Sample ID	Ref. Method	Component	Result	Code	Units	Batch ID	QC Codes
1880169	SM 9223 Quanti-Tray	Escherichia Coli-Quanti-Tray	120		MPN/100 mL		
1880178	SOP-PCR-4.0	HF183 - Human Source Marker**	Below Threshold				

Sample Location: Howell Creek @ 150m US of Lake Howell

Collection Date/Time: 03/14/2017 12:00

Field ID: G2CE0072

Matrix: W-SURF-FRH

Sample ID	Ref. Method	Component	Result	Code	Units	Batch ID	QC Codes
1880170	SM 9223 Quanti-Tray	Escherichia Coli-Quanti-Tray	435		MPN/100 mL		
1880179	SOP-PCR-1.0	HF183-qPCR**	94	U	GEU/100 mL	P322475	
	SOP-PCR-1.2	GULL2-qPCR**	5.6E+03	UJ	TSC/100 mL	P322475	MS, RPD
	SOP-PCR-1.3	GFD-purified-qPCR**	22	T	TSC/100 mL	P321737	LCS, MS, RPD
	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. Quality control failure(s) observed. See QA report for details.

SOP-PCR-1.3: Analyte detected at a level below the current nominal MDL.

Quality Assurance Report Method Blank Results

Reference Method: SOP-PCR-1.0

Batch ID: P322137

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

Reference Method: SOP-PCR-1.0

Batch ID: P322475

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

Reference Method: SOP-PCR-1.2

Batch ID: P322137

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

Reference Method: SOP-PCR-1.2

Batch ID: P322475

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

Reference Method: SOP-PCR-1.3

Batch ID: P321737

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: P322137

Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
HF183-qPCR	76.2	83.3	P/P	50 - 175

Reference Method: SOP-PCR-1.0
Batch ID: P322475

Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
HF183-qPCR	78.1	56.1	P/P	50 - 175

Reference Method: SOP-PCR-1.2
Batch ID: P322137

Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
GULL2-qPCR	50.8	306	P/F	50 - 300

Reference Method: SOP-PCR-1.2
Batch ID: P322475

Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
GULL2-qPCR	50.8	123	P/P	50 - 300

Reference Method: SOP-PCR-1.3
Batch ID: P321737

Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
GFD-purified-qPCR	54.5	32.1	P/F	50 - 175

Quality Assurance Report Matrix Spike Accuracy

Reference Method: SOP-PCR-1.0
Batch ID: P322137

Spiked Sample	Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
1879807	HF183-qPCR	80.5		P	50 - 175
1880174	HF183-qPCR	125		P	50 - 175
1880175	HF183-qPCR	111		P	50 - 175
1880176	HF183-qPCR	90.7		P	50 - 175
1880177	HF183-qPCR	100		P	50 - 175
1883270	HF183-qPCR	106	118	P/P	50 - 175
1883305	HF183-qPCR	99.7		P	50 - 175
1883306	HF183-qPCR	82.3		P	50 - 175
1883307	HF183-qPCR	84.0		P	50 - 175
1883308	HF183-qPCR	88.6		P	50 - 175
1883309	HF183-qPCR	84.6		P	50 - 175
1883310	HF183-qPCR	95.6		P	50 - 175
1884037	HF183-qPCR	146		P	50 - 175

Reference Method: SOP-PCR-1.0
Batch ID: P322475

Spiked Sample	Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
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Quality Assurance Report Matrix Spike Accuracy

Reference Method: SOP-PCR-1.0
 Batch ID: P322475

Spiked Sample	Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
1879553	HF183-qPCR	88.3		P	50 - 175
1879779	HF183-qPCR	84.1		P	50 - 175
1880179	HF183-qPCR	92.3		P	50 - 175
1880471	HF183-qPCR	109		P	50 - 175
1884446	HF183-qPCR	113		P	50 - 175
1884448	HF183-qPCR	92.9	110	P/P	50 - 175
1884449	HF183-qPCR	95.0		P	50 - 175
1885629	HF183-qPCR	100		P	50 - 175

Reference Method: SOP-PCR-1.2
 Batch ID: P322137

Spiked Sample	Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
1880174	GULL2-qPCR	35.0		F	50 - 300
1880175	GULL2-qPCR	36.3		F	50 - 300
1880177	GULL2-qPCR	58.4		P	50 - 300
1884037	GULL2-qPCR	65.5		P	50 - 300

Reference Method: SOP-PCR-1.2
 Batch ID: P322475

Spiked Sample	Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
1880179	GULL2-qPCR	47.7		F	50 - 300

Reference Method: SOP-PCR-1.3
 Batch ID: P321737

Spiked Sample	Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
1879036	GFD-purified-qPCR	57.7		P	50 - 175
1879937	GFD-purified-qPCR	86.9		P	50 - 175
1879938	GFD-purified-qPCR	68.6		P	50 - 175
1880174	GFD-purified-qPCR	32.2		F	50 - 175
1880175	GFD-purified-qPCR	60.6		P	50 - 175
1880177	GFD-purified-qPCR	44.5		F	50 - 175
1880179	GFD-purified-qPCR	57.9		P	50 - 175
1880223	GFD-purified-qPCR	91.1		P	50 - 175
1881136	GFD-purified-qPCR	47.5		F	50 - 175
1881137	GFD-purified-qPCR	8.76		F	50 - 175
1881138	GFD-purified-qPCR	82.1	32.3	P/F	50 - 175
1881139	GFD-purified-qPCR	32.0		F	50 - 175
1881140	GFD-purified-qPCR	45.4		F	50 - 175
1881227	GFD-purified-qPCR	29.8		F	50 - 175
1881228	GFD-purified-qPCR	28.5		F	50 - 175
1881229	GFD-purified-qPCR	47.6		F	50 - 175
1881230	GFD-purified-qPCR	53.9		P	50 - 175
1884037	GFD-purified-qPCR	110		P	50 - 175

Quality Assurance Report Precision

Quality Assurance Report Precision

Reference Method: SOP-PCR-1.0
Batch ID: P322137

Replicated Lab Sample	Component	% RSD/RPD	Sample/Spike/LCS*	Pass/Fail	Control Limits
1883270	HF183-qPCR	10.7	Spike	P	0 - 25

Reference Method: SOP-PCR-1.0
Batch ID: P322475

Replicated Lab Sample	Component	% RSD/RPD	Sample/Spike/LCS*	Pass/Fail	Control Limits
1884448	HF183-qPCR	16.9	Spike	P	0 - 25

Reference Method: SOP-PCR-1.2
Batch ID: P322137

Replicated Lab Sample	Component	% RSD/RPD	Sample/Spike/LCS*	Pass/Fail	Control Limits
1879960	GULL2-qPCR	37.5	Spike	F	0 - 25

Reference Method: SOP-PCR-1.2
Batch ID: P322475

Replicated Lab Sample	Component	% RSD/RPD	Sample/Spike/LCS*	Pass/Fail	Control Limits
1879960	GULL2-qPCR	37.5	Spike	F	0 - 25

Reference Method: SOP-PCR-1.3
Batch ID: P321737

Replicated Lab Sample	Component	% RSD/RPD	Sample/Spike/LCS*	Pass/Fail	Control Limits
1881138	GFD-purified-qPCR	87.0	Spike	F	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: 1880174
Field Sample ID: G2CE0073

Reference Method	Surrogate	% Rec.	Pass/Fail	Control Limits
SOP-PCR-1.0	SKETA-qPCR	96.6	P	50 - 300
SOP-PCR-1.2	SKETA-qPCR	84.4	P	50 - 300

Lab Sample ID: 1880175
Field Sample ID: G2CE0074

Reference Method	Surrogate	% Rec.	Pass/Fail	Control Limits
SOP-PCR-1.0	SKETA-qPCR	105	P	50 - 300
SOP-PCR-1.2	SKETA-qPCR	80.4	P	50 - 300

Quality Assurance Report Surrogates

Lab Sample ID: 1880176
Field Sample ID: G2CE0071

Reference Method	Surrogate	% Rec.	Pass/Fail	Control Limits
SOP-PCR-1.0	SKETA-qPCR	101	P	50 - 300

Lab Sample ID: 1880177
Field Sample ID: G2CE0075

Reference Method	Surrogate	% Rec.	Pass/Fail	Control Limits
SOP-PCR-1.0	SKETA-qPCR	91.7	P	50 - 300
SOP-PCR-1.2	SKETA-qPCR	92.1	P	50 - 300

Lab Sample ID: 1880179
Field Sample ID: G2CE0072

Reference Method	Surrogate	% Rec.	Pass/Fail	Control Limits
SOP-PCR-1.0	SKETA-qPCR	95.0	P	50 - 300
SOP-PCR-1.2	SKETA-qPCR	91.2	P	50 - 300

Quality Assurance Report Summary

Ref. Method	Analyte	LCS % Recovery		MS % Recovery			LCS	Precision SMP	MS
SOP-PCR-1.0	HF183-qPCR	83.3	76.2	106	118	99.7			10.7
	HF183-qPCR	56.1	78.1	92.3	109	113			16.9
SOP-PCR-1.2	GULL2-qPCR	306	50.8	65.5	35.0	36.3			37.5
	GULL2-qPCR	123	50.8	47.7					37.5
SOP-PCR-1.3	GFD-purified-qPCR	32.1	54.5	47.5	8.76	82.1			87.0
				32.3					

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	1880165, 1880166, 1880167, 1880168, 1880169, 1880170
SOP-PCR-1.0 / E31780	Detection and quantification of human-specific HF183 Bacteroides genetic marker with quantitative polymerase chain reaction	1880174, 1880175, 1880176, 1880177, 1880179
SOP-PCR-1.2 / E31780	Detection and quantification of coastal bird specific Catellicoccus marimammalium genetic marker with quantitative polymerase chain reaction	1880174, 1880175, 1880177, 1880179
SOP-PCR-1.3 / E31780	Detection and quantification of bird specific Helicobacter genetic marker with quantitative polymerase chain reaction	1880174, 1880175, 1880177, 1880179
SOP-PCR-4.0 / E31780	Preparation of Samples by Filtration and held for qPCR Analysis	1880174, 1880175, 1880176, 1880177, 1880178, 1880179

Preparation and Analysis Log

Ref. Method	Received Date	Prep Date/Time	Prepared By	Analysis Date/Time	Analyzed By	Associated Samples
SM 9223 Quanti-Tray	03/14/2017			03/14/2017 16:22	Flowers - Altamonte Springs	1880165, 1880166, 1880167, 1880168, 1880169, 1880170
SOP-PCR-1.0	03/15/2017	03/15/2017 13:00	Avril Wood	03/31/2017	Sarah Menz	1880174, 1880175, 1880176, 1880177
	03/15/2017	03/15/2017 13:00	Daisys Matthews	04/05/2017	Sarah Menz	1880179
SOP-PCR-1.2	03/15/2017	03/15/2017 13:00	Avril Wood	04/10/2017	Sarah Menz	1880174, 1880175, 1880177
	03/15/2017	03/15/2017 13:00	Daisys Matthews	04/10/2017	Sarah Menz	1880179
SOP-PCR-1.3	03/15/2017	03/15/2017 13:00	Sarah Menz	04/13/2017	Sarah Menz	1880174, 1880175, 1880177, 1880179
SOP-PCR-4.0	03/15/2017	03/15/2017 13:00	Sarah Menz	03/30/2017	Daisys Matthews	1880174, 1880175, 1880176, 1880177, 1880178, 1880179