# Biological Analysis Report

**CEN-DIST-2017-01-26-01**

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: **Spruce (F)/UnBranch 2641/Groover/Spruce (S)**
Request ID: **RQ-2017-01-16-28**
Customer: **CEN-DIST**
Project ID: **SMP**

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Date Certified: 16-MAR-2017 08:03

## 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: Chlorophyll/Grain Size/BOD, 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 $1.3 \times 10^3$ 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: Spruce Creek @ Spruce Creek Blvd** **Collection Date/Time: 01/25/2017 13:05**

**Field ID: G5CE0083** Matrix: W-SURF-FRH

| Sample ID | Ref. Method | Component | Result | Code | Units | Batch ID | QC Codes |
|---|---|---|---|---|---|---|---|
| 1866720 | SM 9223 Quanti-Tray | Escherichia Coli-Quanti-Tray | 238 | | MPN/100 mL | | |
| 1866738 | SM 10200 H (mod.) | Chlorophyll-a, Corrected | 1.3 | I | ug/L | P319243 | |
| | | Phaeophytin-a | 0.40 | U | ug/L | P319243 | |
| | | Chlorophyll/Phaeophytin Ratio | 1.6 | | | P319243 | |
| | | Chlorophyll-a, Uncorrected | 1.4 | | ug/L | P319243 | |
| 1866757 | SOP-PCR-4.0 | HF183 - Human Source Marker** | Below Threshold | | | | |

Ref. Method and Comment:
SM 10200 H (mod.): Precision data is not available for at least one component due to the small amount of analyte in the QC sample. Refer to QA report for available precision data.

**Sample Location: Groover Branch @ Airport Road** **Collection Date/Time: 01/25/2017 10:40**

**Field ID: G5CE0040** Matrix: W-SURF-FRH

| Sample ID | Ref. Method | Component | Result | Code | Units | Batch ID | QC Codes |
|---|---|---|---|---|---|---|---|
| 1866722 | SM 9223 Quanti-Tray | Escherichia Coli-Quanti-Tray | 364 | | MPN/100 mL | | |
| 1866740 | SM 10200 H (mod.) | Chlorophyll-a, Corrected | 2.6 | | ug/L | P319243 | |
| | | Phaeophytin-a | 0.40 | U | ug/L | P319243 | |
| | | Chlorophyll/Phaeophytin Ratio | 1.7 | | | P319243 | |
| | | Chlorophyll-a, Uncorrected | 2.7 | | ug/L | P319243 | |

Ref. Method and Comment:
SM 10200 H (mod.): Precision data is not available for at least one component due to the small amount of analyte in the QC sample. Refer to QA report for available precision data.

**Sample Location: Unnamed Branch upstream of Airport Rd** **Collection Date/Time: 01/25/2017 12:05**

**Field ID: G5CE0084** Matrix: W-SURF-FRH

| Sample ID | Ref. Method | Component | Result | Code | Units | Batch ID | QC Codes |
|---|---|---|---|---|---|---|---|
| 1866721 | SM 9223 Quanti-Tray | Escherichia Coli-Quanti-Tray | 478 | | MPN/100 mL | | |
| 1866739 | SM 10200 H (mod.) | Chlorophyll-a, Corrected | 0.55 | U | ug/L | P319243 | |
| | | Phaeophytin-a | 0.40 | U | ug/L | P319243 | |
| | | Chlorophyll/Phaeophytin Ratio | 1.9 | | | P319243 | |
| | | Chlorophyll-a, Uncorrected | 0.40 | U | ug/L | P319243 | |
| 1866758 | SOP-PCR-1.0 | HF183-qPCR** | 71 | U | GEU/100 mL | P319590 | |
| | SOP-PCR-1.2 | GULL2-qPCR** | 8.4E+03 | U | TSC/100 mL | P319590 | MS, RPD |
| | SOP-PCR-1.3 | GFD-purified-qPCR** | 40 | TJ | TSC/100 mL | P320062 | |
| | SOP-PCR-4.0 | HF183 - Human Source Marker** | Above Threshold | | | | |

Ref. Method and Comment:
SM 10200 H (mod.): Precision data is not available for at least one component due to the small amount of analyte in the QC sample. Refer to QA report for available precision data.
SOP-PCR-1.0: No amplification of HF183 marker.
SOP-PCR-1.2: No amplification of GULL2 marker.
SOP-PCR-1.3: Sample processed with an additional purification step to reduce inhibition, which can reduce overall DNA recovery.

**Sample Location: G5CE0080** **Collection Date/Time: 01/25/2017 13:07**

**Field ID: G5CE0080** Matrix: W-SURF-FRH

| Sample ID | Ref. Method | Component | Result | Code | Units | Batch ID | QC Codes |
|---|---|---|---|---|---|---|---|
| 1867986 | Enterolert/QT | Enterococci-Quanti-Tray | 145 | | MPN/100 mL | | |

# Quality Assurance Report
# Method Blank Results

**Reference Method: SM 10200 H (mod.)**
**Batch ID: P319243**

| Component | Result | Code | Units |
|---|---|---|---|
| Chlorophyll-a, Corrected | 0.55 | U | ug/L |
| Chlorophyll-a, Uncorrected | 0.40 | U | ug/L |
| Phaeophytin-a | 0.40 | U | ug/L |

**Reference Method: SOP-PCR-1.0**
**Batch ID: P319590**

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

**Reference Method: SOP-PCR-1.2**
**Batch ID: P319590**

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

# Quality Assurance Report
# Laboratory Control Sample Accuracy

**Reference Method: SM 10200 H (mod.)**
**Batch ID: P319243**

| Component | % Rec.1 | % Rec.2 | Pass/Fail | Control Limits |
|---|---|---|---|---|
| Chlorophyll-a, Corrected | 88.8 | | P | 84 - 116 |

**Reference Method: SOP-PCR-1.0**
**Batch ID: P319590**

| Component | % Rec.1 | % Rec.2 | Pass/Fail | Control Limits |
|---|---|---|---|---|
| HF183-qPCR | 93.3 | 76.8 | P/P | 50 - 175 |

**Reference Method: SOP-PCR-1.2**
**Batch ID: P319590**

| Component | % Rec.1 | % Rec.2 | Pass/Fail | Control Limits |
|---|---|---|---|---|
| GULL2-qPCR | 77.9 | | P | 50 - 300 |

# Quality Assurance Report
# Matrix Spike Accuracy

**Reference Method: SOP-PCR-1.0**
**Batch ID: P319590**

| Spiked Sample | Component | % Rec.1 | % Rec.2 | Pass/Fail | Control Limits |
|---|---|---|---|---|---|
| 1864645 | HF183-qPCR | 74.4 | | P | 50 - 175 |
| 1866758 | HF183-qPCR | 89.3 | | P | 50 - 175 |
| 1866855 | HF183-qPCR | 143 | | P | 50 - 175 |
| 1868574 | HF183-qPCR | 132 | | P | 50 - 175 |
| 1868575 | HF183-qPCR | 116 | | P | 50 - 175 |
| 1868829 | HF183-qPCR | 110 | | P | 50 - 175 |
| 1871159 | HF183-qPCR | 72.5 | | P | 50 - 175 |

# Quality Assurance Report
# Matrix Spike Accuracy

**Reference Method: SOP-PCR-1.0**
**Batch ID: P319590**

| Spiked Sample | Component | % Rec.1 | % Rec.2 | Pass/Fail | Control Limits |
|---|---|---|---|---|---|
| 1871160 | HF183-qPCR | 108 | 136 | P/P | 50 - 175 |
| 1871161 | HF183-qPCR | 132 | | P | 50 - 175 |
| 1871162 | HF183-qPCR | 85.8 | | P | 50 - 175 |
| 1871755 | HF183-qPCR | 117 | | P | 50 - 175 |
| 1871756 | HF183-qPCR | 87.9 | | P | 50 - 175 |

**Reference Method: SOP-PCR-1.2**
**Batch ID: P319590**

| Spiked Sample | Component | % Rec.1 | % Rec.2 | Pass/Fail | Control Limits |
|---|---|---|---|---|---|
| 1866758 | GULL2-qPCR | 125 | | P | 50 - 300 |
| 1866855 | GULL2-qPCR | 83.8 | | P | 50 - 300 |
| 1871160 | GULL2-qPCR | 87.7 | 302 | P/F | 50 - 300 |

# Quality Assurance Report
# Precision

**Reference Method: SM 10200 H (mod.)**
**Batch ID: P319243**

| Replicated Lab Sample | Component | % RSD/RPD | Sample/Spike/LCS* | Pass/Fail | Control Limits |
|---|---|---|---|---|---|
| 1866696 | Chlorophyll/Phaeophytin Ratio | 0.0 | Sample | P | 0 - 25 |
| 1866696 | Chlorophyll-a, Corrected | 4.96 | Sample | P | 0 - 25 |
| 1866696 | Chlorophyll-a, Uncorrected | 4.42 | Sample | P | 0 - 25 |

**Reference Method: SOP-PCR-1.0**
**Batch ID: P319590**

| Replicated Lab Sample | Component | % RSD/RPD | Sample/Spike/LCS* | Pass/Fail | Control Limits |
|---|---|---|---|---|---|
| 1871160 | HF183-qPCR | 6.13 | Sample | P | 0 - 25 |
| 1871160 | HF183-qPCR | 22.6 | Spike | P | 0 - 25 |

**Reference Method: SOP-PCR-1.2**
**Batch ID: P319590**

| Replicated Lab Sample | Component | % RSD/RPD | Sample/Spike/LCS* | Pass/Fail | Control Limits |
|---|---|---|---|---|---|
| 1871160 | GULL2-qPCR | 110 | 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: 1866758**
**Field Sample ID: G5CE0084**

| Reference Method | Surrogate | % Rec. | Pass/Fail | Control Limits |
|---|---|---|---|---|

# Quality Assurance Report
# Surrogates

**Lab Sample ID: 1866758**
**Field Sample ID: G5CE0084**

| Reference Method | Surrogate | % Rec. | Pass/Fail | Control Limits |
|---|---|---|---|---|
| SOP-PCR-1.0 | SKETA-qPCR | 113 | P | 50 - 300 |
| SOP-PCR-1.2 | SKETA-qPCR | 105 | P | 50 - 300 |

# Quality Assurance Report
# Summary

| Ref. Method | Analyte | LCS % Recovery | MS % Recovery | Precision | | |
|---|---|---|---|---|---|---|
| | | | | LCS | SMP | MS |
| SM 10200 H (mod.) | Chlorophyll/Phaeophytin Ratio | | | | 0.0 | |
| | Chlorophyll-a, Corrected | 88.8 | | | 4.96 | |
| | Chlorophyll-a, Uncorrected | | | | 4.42 | |
| SOP-PCR-1.0 | HF183-qPCR | 76.8 93.3 | 87.9 72.5 108 136 | | 6.13 | 22.6 |
| SOP-PCR-1.2 | GULL2-qPCR | 77.9 | 125 83.8 87.7 302 | | | 110 |

# Reference Method Descriptions

| Method / DoH Cert # | Description | Associated Samples |
|---|---|---|
| Enterolert/QT / E83018 | Enterococci by Enterolert Quanti-Tray method by Flowers Overflow Laboratory for Central District | 1867986 |
| SM 10200 H (mod.) / E31780 | Phytoplankton chlorophyll-a corrected, uncorrected, phaeophytin and ratio by spectrophotometry | 1866738, 1866739, 1866740 |
| SM 9223 Quanti-Tray / E83018 | Escherichia coli by Colilert 18 Quanti-Tray method by FlowersOverflow Laboratory for Central District | 1866720, 1866721, 1866722 |
| SOP-PCR-1.0 / E31780 | Detection and quantification of human-specific HF183 Bacteroides genetic marker with quantitative polymerase chain reaction | 1866758 |
| SOP-PCR-1.2 / E31780 | Detection and quantification of coastal bird specific Catellicoccus marimammalium genetic marker with quantitative polymerase chain reaction | 1866758 |
| SOP-PCR-1.3 / E31780 | Detection and quantification of bird specific Helicobacter genetic marker with quantitative polymerase chain reaction | 1866758 |
| SOP-PCR-4.0 / E31780 | Preparation of Samples by Filtration and held for qPCR Analysis | 1866757, 1866758 |

# Preparation and Analysis Log

| Ref. Method | Received Date | Prep Date/Time | Prepared By | Analysis Date/Time | Analyzed By | Associated Samples |
|---|---|---|---|---|---|---|
| Enterolert/QT | 01/25/2017 | | | 01/25/2017 16:03 | Flowers - Altamonte Springs | 1867986 |
| SM 10200 H (mod.) | 01/26/2017 | 01/26/2017 13:05 | Morgan Lane | 02/01/2017 | Brian Cumbie | 1866738, 1866739, 1866740 |
| SM 9223 Quanti-Tray | 01/25/2017 | | | 01/25/2017 16:55 | Flowers - Altamonte Springs | 1866720, 1866721, 1866722 |
| SOP-PCR-1.0 | 01/26/2017 | 01/26/2017 13:20 | Sarah Menz | 02/15/2017 | Sarah Menz | 1866758 |
| SOP-PCR-1.2 | 01/26/2017 | 01/26/2017 13:20 | Daisys Matthews | 02/28/2017 | Sarah Menz | 1866758 |
| SOP-PCR-1.3 | 01/26/2017 | 01/26/2017 13:20 | Sarah Menz | 02/28/2017 | Sarah Menz | 1866758 |
| SOP-PCR-4.0 | 01/26/2017 | 01/26/2017 13:20 | Puja Jasrotia | 02/14/2017 | Daisys Matthews | 1866757, 1866758 |