



Florida Department of Environmental Protection
Office of Communications

Microbial Source Tracking: Interpretation of qPCR Results

03/30/2016





Overview

- General discussion of MST
- Available analyses at FDEP laboratory
- qPCR 101
- Human and gull markers
- LIMS final report – QC Decoded



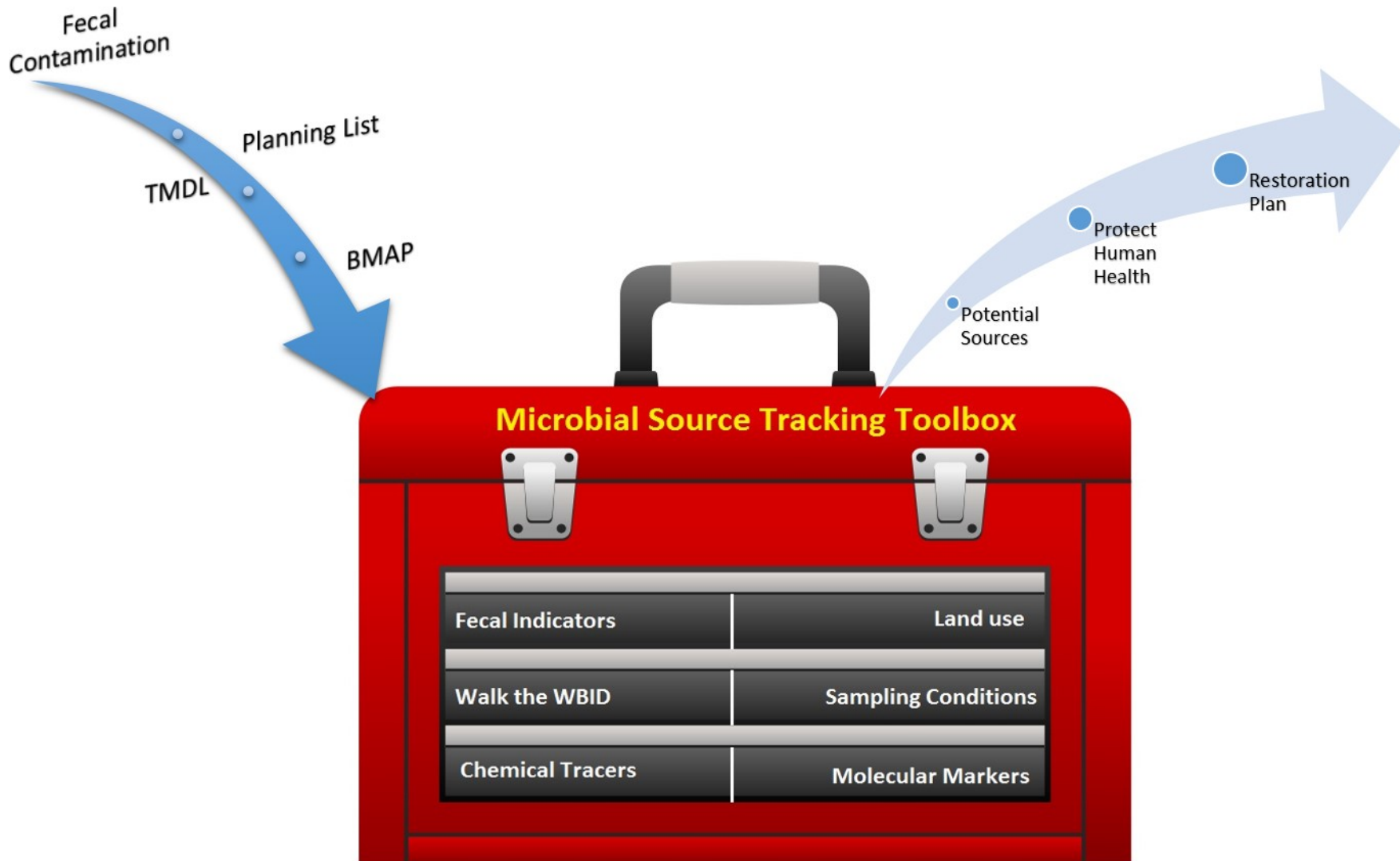
What is MST?



From microbe.com

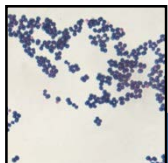


MST Toolbox Approach



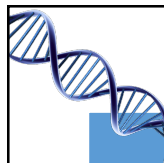


MST Tests in LIMS



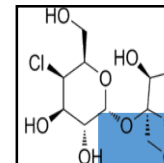
Microbiology

- ECOLI-18QT
- ENT-24-QT
- FCOLI-MF



Molecular Markers

- PCR-HF183
- PCR-GULL2



Chemical Tracers

- W-SUC-AA-R
 - Sucralose
- W-UHERB-AA
 - Acetaminophen
 - Carbamazepine
 - Fluridone
 - Primidone
 - Diuron
 - Fenuron
 - Imidacloprid
 - Linuron



Chemical Tracers

Artificial Sweetener (W-SUC-AA-R)

Sucralose

Pharmaceuticals (W-UHERB-AA)

Pain Reliever

- Acetaminophen

Anticonvulsant

- Carbamazepine
- Primidone

Pesticides (W-UHERB-AA)

Herbicide

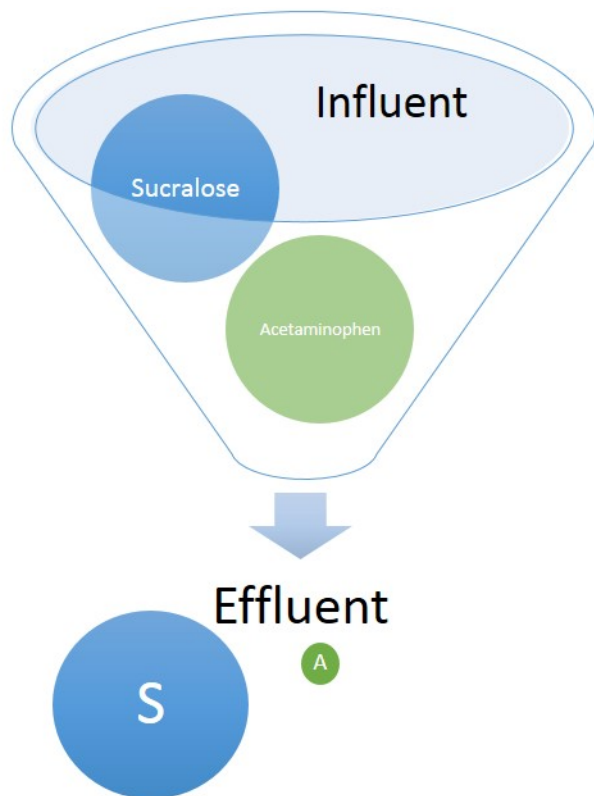
- Fluridone
- Diuron
- Fenuron
- Linuron

Insecticide

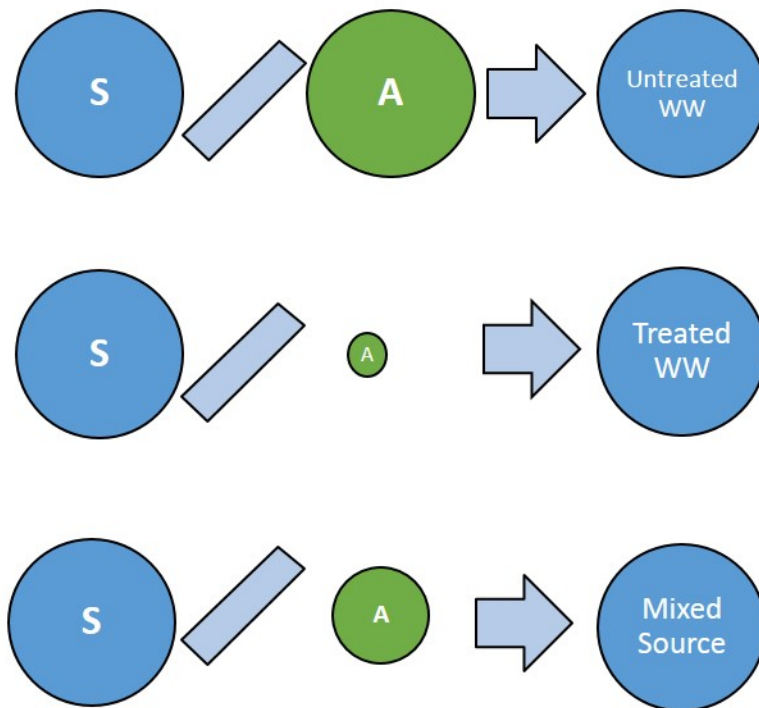
- Imidacloprid



Sucralose/Acetaminophen



Sucralose to Acetaminophen Ratio





What is qPCR?

- quantitative Polymerase Chain Reaction
 - Copy specific DNA sequence and quantify original DNA in sample
- How it works:
 - Extract DNA from the sample
 - Double amount of target DNA sequence every cycle
 - Fluorescent signal increases when target DNA increases
 - Determine amount of DNA in sample based on signal



qPCR Sample Preparation

Filtration



- Filter water sample
- Concentrates the sample onto a filter

Extraction



- Mechanical cell lysis (bead beater)
- DNA is released
- Contaminants not removed

Purification



- Remove contaminants
- Reduces inhibition
- Reduces overall DNA recovery

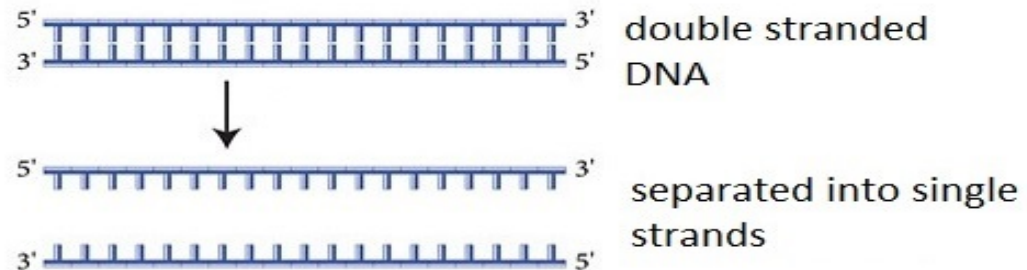


PCR Cycle

PCR CYCLE STEPS

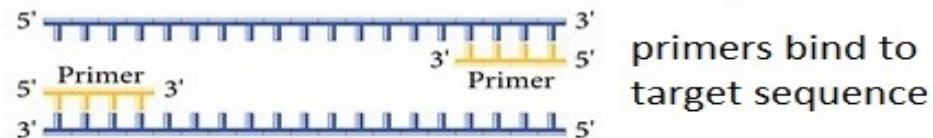
DENATURATION

95°C
15 sec

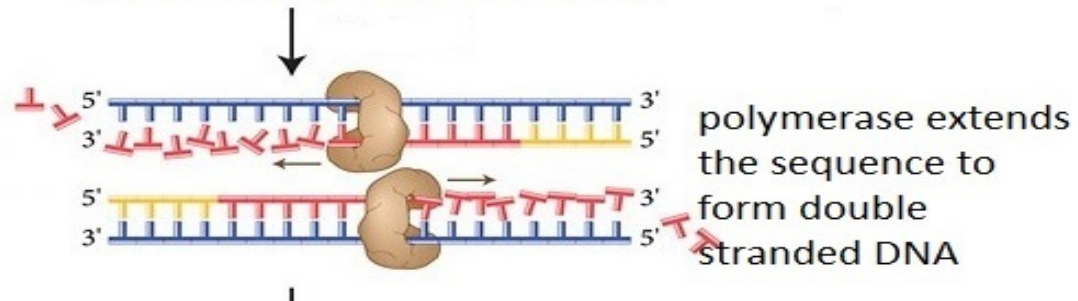


ANNEALING

60°C
1 min



EXTENSION

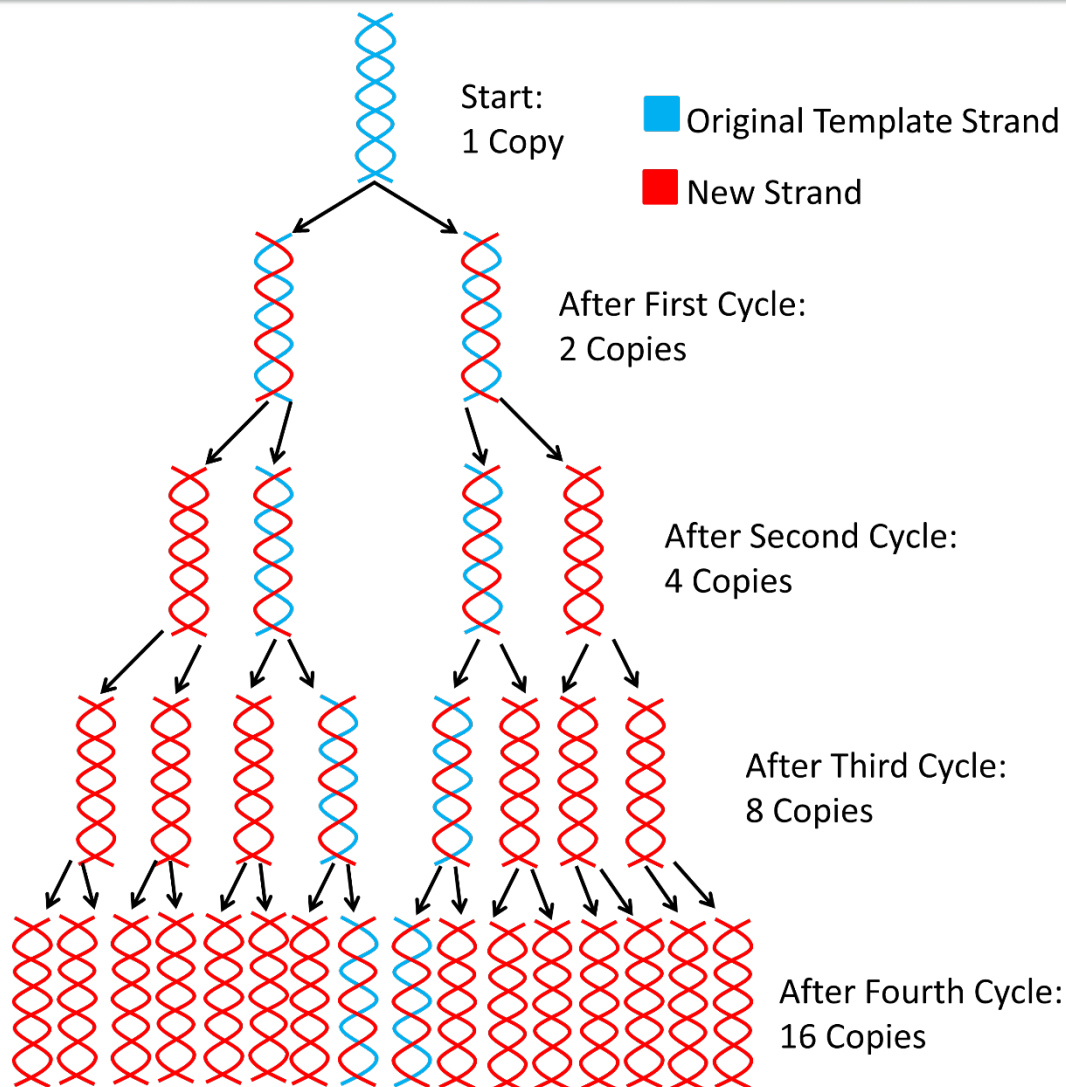




PCR Efficiency

At 100% Efficiency

After 35 cycles –
34 billion copies

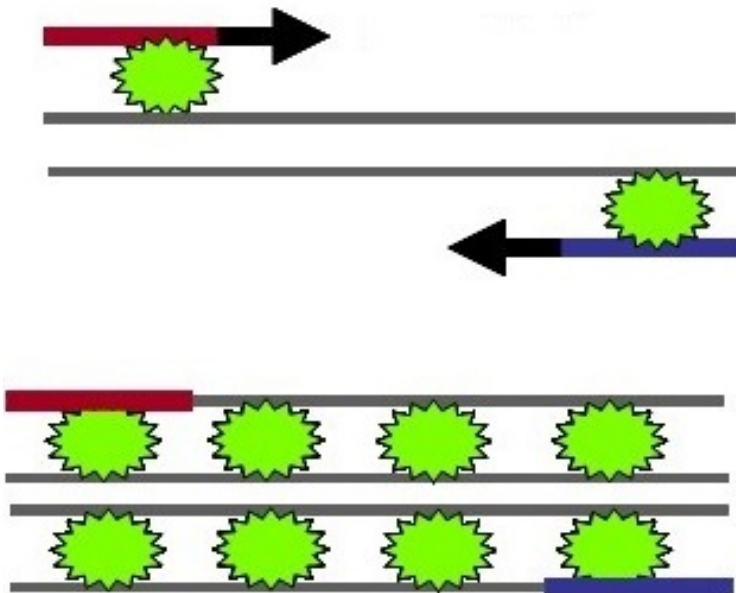


Source: <https://antisensescienceblog.files.wordpress.com/2013/12/exponential-inc2.png>

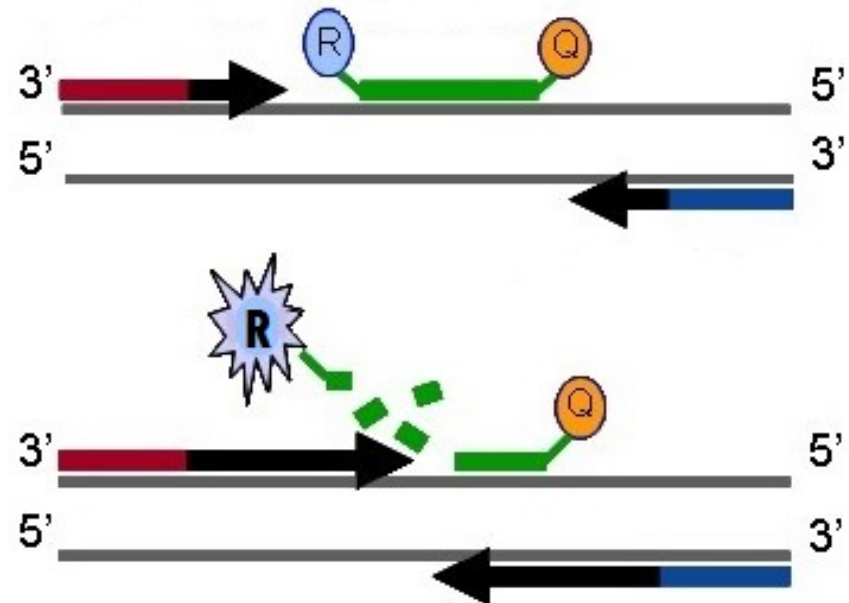


qPCR Chemistry

SYBR® Green I dye



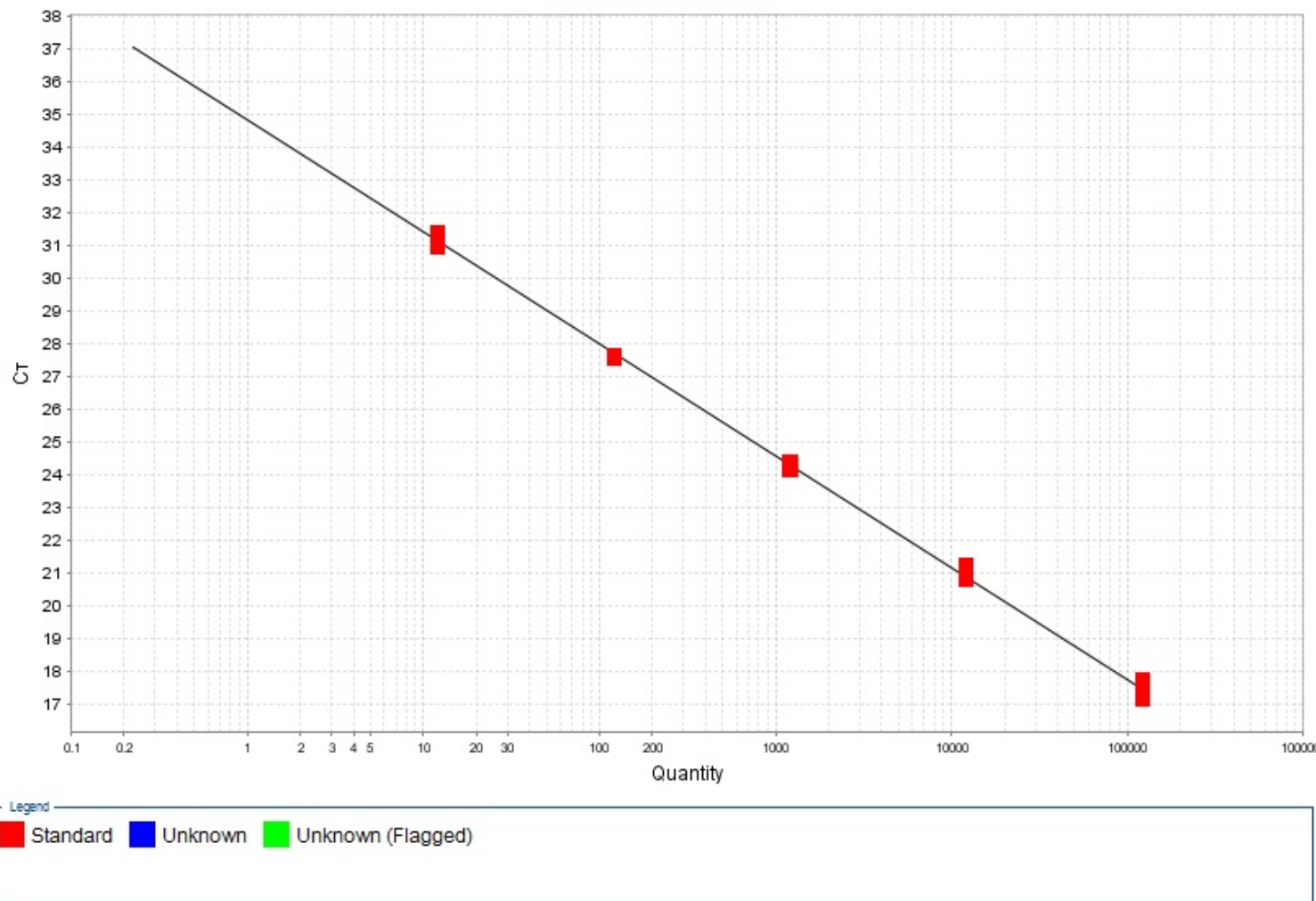
TaqMan® Probe





qPCR Standard Curves

Standard Curve





qPCR Limitations

- Sensitivity
 - May not be abundant in the environment or wastewater
- Specificity
 - Cross-reactivity with bacterial DNA from other sources
- Inhibition
 - Sample matrix can interfere with DNA amplification
 - humic acids, tannic acids, ethanol
- Standardization
 - No standardized method
 - No certified standards
- Live vs. Dead
 - Amplifies all DNA with target sequence



HF183 qPCR Marker

- LIMS: PCR-HF183
- Target
 - Human-associated bacteria of the *Bacteroides* genus
- Obligate anaerobe
 - Does not survive well in oxygenated environment
- Standard – *Bacteroides dorei* – Bacterial culture
 - GEU/100 mL – Genomic Equivalent Units
- Limited cross reactivity with deer



GULL2 MARKER

- LIMS: PCR-GULL2
- Target
 - Seagull associated bacteria species *Catelliboccus marimammalium*
 - Also found in other coastal birds
- Facultative anaerobic
- Standard – plasmid with target sequence
 - TSC/100 mL – Target Sequence Copies



Upcoming Markers

- Next Marker
 - GFD – General Bird Marker
 - Includes gull, goose, duck, chicken, pigeon, cormorant, egret, pelican, crow, and others
- After that....
 - Ruminants (Cow, deer, sheep, goats)
 - Additional Human Markers
 - Dog marker
 - Pigs
 - Horses
- Can we differentiate live vs. dead cells?
- Can we differentiate environmental vs. enteric?



LIMS Final Report

NW-DIST-2015-11-25-01
Serial Number: 0080083
Biological Analysis Report
Page 1 of 7

Biological Analysis Report

NW-DIST-2015-11-25-01

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

Overflow Analyses Performed By:
University of West Florida
11000 University Parkway
Pensacola, FL 32514
DOH Accreditation E71969

Event Description: **Reference Streams**
Request ID: **RQ-2015-02-09-44**
Customer: **NW-DIST**
Project ID: **BIOCRIT**

Send Reports to:
FL Dept. of Environmental Protection
160 W Government St
Suite 208A
Pensacola, FL 32502-5794
Attn: Rick Abad

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

Certified by: Daisys Matthews,

Date Certified: 18-FEB-2016 10:35

A handwritten signature in black ink that reads "D Matthews".

NON-CONFORMANCE REPORT INCLUDED



Final Report: Sample Results

Sample Location: Coffee Cr N of Jamesville Rd

Collection Date/Time: 11/24/2015 10:40

Field ID: ST-1

Matrix: W-SURF-FRH

Sample ID	Ref. Method	Component	Result	Code	Units	Batch ID	QC Codes
1753240	SM 5210 B	Biochemical Oxygen Demand-5 Day	0.20	U	mg/L	P295190	
1753242	SM 10200 H (mod.)	Chlorophyll-a, Corrected	0.55	U	ug/L	P295335	
		Phaeophytin-a	0.40	U	ug/L	P295335	
		Chlorophyll/Phaeophytin Ratio	1.1			P295335	
		Chlorophyll-a, Uncorrected	0.40	U	ug/L	P295335	
1753244	SM 9223 Quanti-Tray	Escherichia Coli-Quanti-Tray**	No Result	OQ	MPN/100 mL		
1753248	SM 9222 D	Fecal Coliforms-Membrane Filter	31	B	cfu/100 mL		
1753250	SOP-PCR01	HF183-qPCR**	9.5E+03	U	GEU/100 mL	P294959	

Ref. Method and Comment:

SM 5210 B: Precision data is unavailable due to the small amount of analyte in the QC sample.

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.

SM 9223 Quanti-Tray: See NCR #5616

SOP-PCR01: No amplification of HF183 marker.

- Typical Comments:
 - No amplification of HF183 marker.
 - Analyte detected at a level below the current nominal MDL.
 - Sample processed with an additional purification step to reduce inhibition, which can reduce overall DNA recovery. See QA report.
 - Duplicate was analyzed and result is 1.60E+03 U.
 - Quality control failure observed. See QA Report.



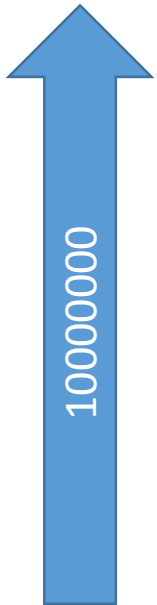
HF183 Results – GEU/100 mL

MDL – 1010 GEU/100 mL

Raw Sewage

10s of millions

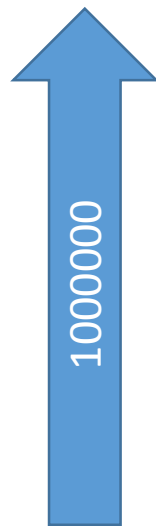
1×10^7



High Signal

Millions

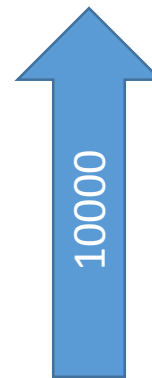
$\geq 1 \times 10^6$



Moderate Signal

10 thousands

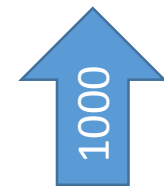
1×10^4



Low Signal

< 10 thousands

$< 1 \times 10^4$





Final Report: QC Codes

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;

- QC Codes list quality control failures
 - Results likely “J” qualified
 - Comment will say:
 - Quality control failure(s) observed. See QA report for details.



QA Report: Method Blanks

Quality Assurance Report

Method Blank Results

Reference Method: SM 10200 H (mod.)

Batch ID: P295335

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: SM 5210 B

Batch ID: P295190

Component	Result	Code	Units
Biochemical Oxygen Demand-5 Day	0.20	U	mg/L

Reference Method: SM 5210 B

Batch ID: P295191

Component	Result	Code	Units
Biochemical Oxygen Demand-5 Day	0.20	U	mg/L

Reference Method: SOP-PCR01

Batch ID: P294959

Component	Result	Code	Units
HF183-qPCR	1.9E+04	U	GEU/100 mL



QA Report: LCS

Quality Assurance Report Laboratory Control Sample Accuracy

Reference Method: SM 5210 B

Batch ID: P295190

Component

Biochemical Oxygen Demand-5 Day

% Rec.1

112

% Rec.2

Pass/Fail

P

Control Limits

84 - 116

Reference Method: SM 5210 B

Batch ID: P295191

Component

Biochemical Oxygen Demand-5 Day

% Rec.1

106

% Rec.2

Pass/Fail

P

Control Limits

84 - 116

Reference Method: SOP-PCR01

Batch ID: P294959

Component

HF183-qPCR

% Rec.1

100

% Rec.2

87.0

Pass/Fail

P/P

Control Limits

50 - 175



QA Report: MS

Quality Assurance Report Matrix Spike Accuracy

Reference Method: SOP-PCR01

Batch ID: P294959

Spiked Sample	Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
1753144	HF183-qPCR	109	96.5	P/P	50 - 175
1753250	HF183-qPCR	109		P	50 - 175
1753251	HF183-qPCR	99.9		P	50 - 175
1753634	HF183-qPCR	101		P	50 - 175
1755758	HF183-qPCR	89.7		P	50 - 175
1757294	HF183-qPCR	79.6		P	50 - 175
1757580	HF183-qPCR	95.5		P	50 - 175
1758254	HF183-qPCR	86.9		P	50 - 175
1758571	HF183-qPCR	111		P	50 - 175
1758572	HF183-qPCR	100		P	50 - 175
1758722	HF183-qPCR	109		P	50 - 175
1759166	HF183-qPCR	91.2		P	50 - 175



QA Report: RPD

Quality Assurance Report Precision

Reference Method: SOP-PCR01

Batch ID: P294959

Replicated

Lab Sample	Component	% RSD/RPD	Sample/Spike/LCS*	Pass/Fail	Control Limits
1753144	HF183-qPCR	11.9	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.



QA Report: SUR

Quality Assurance Report Surrogates

Lab Sample ID: 1753250

Field Sample ID: ST-1

Reference Method
SOP-PCR01

Surrogate
SKETA-qPCR

% Rec.
147

Pass/Fail
P

Control Limits
50 - 300

Lab Sample ID: 1753251

Field Sample ID: ST-3

Reference Method
SOP-PCR01

Surrogate
SKETA-qPCR

% Rec.
163

Pass/Fail
P

Control Limits
50 - 300



Questions?

Contact Information

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