

Soil and Aquatic Microbiology Laboratory

Department of Soil and Crop Sciences

Texas A&M University

370 Olsen Blvd, 2474 TAMU

College Station, TX 77843-2474

Contact: Dr. Terry Gentry

Phone: (979) 845-5323

Email: tjgentry@tamu.edu



Test Report

Client Name: Nick Dornak

Client Address: Plum Creek Watershed Coordinator
ndornak@plumcreekwatershed.org
512-213-7389

Test Method: EPA Method 1603

Sample Information: See attached bacteriological data sheet.

Test Results:

SAML ID	Station ID	CFU/ 100mL
582571	12674 Down Stream RR 12	18,000
582572	12674 Up Stream RR 12	6,400

Signature of Technical Director Heidi Mjelde Date 9/15/2017
Technical Director: Heidi Mjelde

The above signature of the technical director certifies that the results are in compliance with TNI standards and that the above results relate only to the samples indicated.

This report shall not be reproduced except in full without written approval of the laboratory.

Report Identification
SAML-RPT-1122

SRF-v01

Date: **8/7/17**

Lot#	Chlorine residual (V/N)	Time Filtered	Time				Volume Filtered (mL) and Counts					
			IN Incubator#1 (35°C)	OUT Incubator#1 (35°C)	IN WtrBth#1 (44.5°C)	OUT WtrBth#1 (44.5°C)	Analyst Initials: HJM		Analyst Initials: HJM			
mTEC BioBall E. coli Lot# B 3628		1:24	1:27	3:47	3:52	1:54	25	10	1	0.1	0.01	24
mTEC BioBall E. fecalis Lot# B 3698		1:25					PASS (NO GROWTH)					
BHI BioBall E. fecalis Lot# B 3698		1:27					PASS (POSITIVE GROWTH)					
mTEC PBS Blank (50mls)		1:16					PASS (NO GROWTH)					
TSA PBS Blank (50mls)		1:17					PASS (NO GROWTH)					
TSA PBS Dilution Tube Blank (9mls)		1:18					PASS (NO GROWTH)					
582571 DOWN STREAM RR 12	NO	1:38	1:38	3:47	3:52	1:54	TNTC	TNTC	TNTC	18	0	18,000
582572 UP STREAM RR 12	NO	1:47	1:47				TNTC	TNTC	64	8	0	6400
582573 LAB DUP UP STREAM	NO	1:57	1:57				TNTC	TNTC	58	7	0	5800

Project:

SAML DATA LOG SHEET:
EPA METHOD 1603

Data Log Sheet v02
Revision 03
Effective Date 07/31/17

Date:

Consumable Information	Media Sterility
PBS: 17131	mTEC: PASS (NO GROWTH)
mTEC: AQP 6/18/17	TSA: PASS (NO GROWTH)
TSA: 17206	BHI: PASS (NO GROWTH)
BHI: 17136	Filter Sterility: PASS (NO GROWTH)
Specimen Container: 101399KS	
Filter Unit: 1170962	
0.45um Filter: F 5SA 56563	
Serological Pipette: 25ml 09215004 10ml 06516001	
Pipette Tip Lot:	
Dilution Tubes: 150827-188	
Chlorine Test Strips: 7159	

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Test Report

Client Name: Nick Dornak
Client Address: Plum Creek Watershed Coordinator
ndornak@plumcreekwatershed.org
512-213-7389
Test Method: EPA Method 1603
Sample Information: See attached bacteriological data sheet.
Test Results:

SAML ID	Station ID	CFU/ 100mL
582587	12674 Down Stream RR 12-A	276*
582589	12674 Up Stream RR 12-A	36

Signature of Technical Director Heidi Mjelde Date 9/15/2017
Technical Director: Heidi Mjelde

*This sample exceeded the six hour hold time by seventeen minutes.

The above signature of the technical director certifies that the results are in compliance with TNI standards and that the above results relate only to the samples indicated.

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Report Identification
SAML-RPT-1126

SRF-v01

Project:

SAML DATA LOG SHEET:
EPA METHOD 1603

Data Log Sheet V02
Revision 03
Effective Date 07/31/17

Date:

Consumable Information	Media Sterility
PBS: 17131	mTEC: PASS (NO GROWTH)
mTEC: AOP 7/30/17	TSA: PASS (NO GROWTH)
TSA: 17206	BHI: PASS (NO GROWTH)
BHI: 17205	Filter Sterility: PASS (NO GROWTH)
Specimen Container: 101399 KS	
Filter Unit: 1170962	
0.45um Filter: FS SA 56563	
Serological Pipette: 25ml 09215004 10ml 06516001	
Pipette Tip Lot:	
Dilution Tubes: 150827-188	
Chlorine Test Strips: 7159	

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Test Report

Client Name: Nick Dornak

Client Address: Plum Creek Watershed Coordinator
ndornak@plumcreekwatershed.org
512-213-7389

Test Method: EPA Method 1603

Sample Information: See attached bacteriological data sheet.

Test Results:

SAML ID	Station ID	CFU/ 100mL
582596	12674 Down Stream RR 12-A	650
582597	12674 Up Stream RR 12-A	60

Signature of Assistant Technical Director _____
Date 10/18/2017

Maitreyee Mukherjee

The above signature of the technical director certifies that the results are in compliance with TNI standards and that the above results relate only to the samples indicated.

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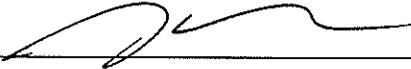
Report Identification
SAML-RPT-1128

SRF-v01

SAML Sample Report QA Checklist

Report #: SAML-RPT- 1128

Item	Meets Requirements		Notes
	Yes	No	
Hold time	✓		
Incubation time	✓		
Quality controls	✓		
Duplicates	✓		
Consumable information recorded	✓		
Media sterility	✓		

Signature of Quality Manager 

Date 10/18/17

Project: CYPRESS CREEK
 Date: OCTOBER 16, 2017

SAML DATA LOG SHEET:
 EPA METHOD 1603

Data Log Sheet v02
 Revision 03
 Effective Date 07/31/17

	Chlorine residual (V/N)	Time				Volume Filtered (mls) and Counts					
		Time Filtered	IN Incubator#1 (35°C)	OUT Incubator#1 (35°C)	MM WtrBth#1 (44.5°C)	MM WtrBth#1 (44.5°C)	MM WtrBth#1 (44.5°C)	MM Analyst Initials:	MM		
mTEC BioBall E. coli Lot# 3628		3:05	3:08	5:27 ¹⁶	5:34 ¹⁶	25	10	1	0.1	25	25
mTEC BioBall E. fecalis Lot# 3943		3:06				25	(NO GROWTH)				
BHI BioBall E. fecalis Lot# 3943		3:07					PASS (POSITIVE GROWTH)				
mTEC PBS Blank (50mls)		3:01					PASS (NO GROWTH)				
TSA PBS Blank (50mls)		3:02					PASS (NO GROWTH)				
TSA PBS Dilution Tube Blank (9mls)		3:03					PASS (NO GROWTH)				
582596 - RR12 - DOWN	N	3:19	3:20			25	TNTC	65	7	1	650
582597 - RR12 - UP	N	3:29	3:30			15	4	1	0	0	60
582598 - RR12 - DOWN (LAB DUP)		3:38	3:39			TNTC	59	6	1		590

Consumable Information	Media Sterility
PBS: 17131	mTEC: PASS (NO GROWTH)
mTEC: AQP 10/1/17	TSA: PASS (NO GROWTH)
TSA: 17257B	BHI: PASS (NO GROWTH)
BHI: 17205	Filter Sterility: PASS (NO GROWTH)
Specimen Container: 101399KS	
Filter Unit: 1184490	
0.45µm Filter: F55A56563	
Serological Pipette: 25ml 06516001	
10ml 02016018	
Pipette Tip Lot:	
Dilution Tubes: 150827-188	
Chlorine Test Strips: 7159	

September 12, 2017

Nick Dornak
Plum Creek Watershed Coordinator
ndornak@plumcreekwatershed.org

Dear Nick:

Attached please find the bacterial source tracking (BST) results for the two Cypress Creek water samples collected on 08/07/2017. For BST, we DNA fingerprinted four isolates from each sample using enterobacterial repetitive intergenic consensus sequence-PCR and RiboPrinting. The fingerprints were compared against the Texas *E. coli* BST Library (ver. 5-15) for identification of source categories using both 3-way and 7-way source splits.

The following pages detail: 1) the composition and performance of the current Texas *E. coli* BST library that was used to categorize your isolates and 2) a summary of the BST results for your isolates.

Please do not hesitate to contact me if you have any questions or would like to discuss the results further. We look forward to the continuation of this project and assisting you further with the characterization of your watershed.

Sincerely,



Terry J. Gentry
Associate Professor & Laboratory Director

Heep Center, 370 Olsen Boulevard
2474 TAMU
College Station, TX 77843-2474

Tel. 979.845.5323 Fax 979.845.0456
tjgentry@tamu.edu
<http://microbe.tamu.edu/>

Bacterial Source Tracking Methods

From each water sample collected on 08/07/2017, a total of four isolates each were DNA fingerprinted using enterobacterial repetitive intergenic consensus sequence-PCR and RiboPrinting (ERIC-RP). The DNA fingerprints were compared to known-source fingerprints in the Texas *E. coli* BST Library (ver. 5-15) for identification of source categories (Table 1). The current library contains 1,765 *E. coli* isolates from 1,554 different fecal samples representing over 50 animal subclasses. This is the result of collecting over 3,500 domestic sewage, wildlife, livestock, and pet fecal samples from 13 watersheds across Texas and screening over 6,000 isolates for clones and host specificity.

Table 1. Texas *E. coli* BST Library (ver. 5-15, cross-library validation) composition and rates of correct classification (RCCs) by Jackknife analysis of ERIC-RP composite data sets using an 80% similarity cutoff and 3- and 7-way splits.

Source Class	Number of Isolates	Number of Samples	Library Composition and Expected Random Rate of Correct Classification	Calculated Rate of Correct Classification (RCC)	RCC to Random Ratio ^{***}	Left Unidentified (unique patterns)
HUMAN	384	330	22%	100	4.5	6
DOMESTIC ANIMALS	532	495	30%	100	3.3	19
Pets	83	74	5%	84	16.8	41
Cattle	232	216	13%	93	7.2	11
Avian Livestock	95	88	5%	89	17.8	26
Other Non-Avian Livestock	122	117	7%	94	13.4	15
WILDLIFE	849	729	48%	100	2.1	16
Avian Wildlife	273	250	15%	79	5.3	19
Non-Avian Wildlife	576	479	33%	91	2.8	15
Overall	1765	1554		ARCC^{**} = 100% (3-way) 91% (7-way)		18%

^{**}ARCC = average rate of correct classification: the proportion of all identification attempts which were correctly identified to source class for the entire library, which is similar to the mean of the RCCs for all source classes when the number of isolates in each source class is similar.

^{***}A RCC/Random Ratio greater than 1.0 indicates that the rate of correct classification is better than random. For example, the rate of correct classification for human is 4.5-fold greater than random chance.

Source Categories

With currently available BST methods, it is not possible to discriminate between all possible species of animal sources simultaneously, since each added category tends to decrease the accuracy of source classification. This is illustrated in Table 1 where the 7-way split has an overall lower average rate of correct classification (91%) than the 3-way split does (100%). Therefore, we currently combine most species into groups based upon similarity of their physiology and/or potential management. Below are the 3- and 7-way split categories that were used for categorizing your *E. coli* isolates and which we have most frequently used for characterizing watersheds:

3-way split

1. Domesticated animals and livestock (livestock and pets)
2. Wildlife (including feral hogs)
3. Humans

7-way split

1. Cattle
2. Other livestock, non-avian (non-avian livestock other than cattle; sheep, etc.)
3. Other livestock, avian (chickens, etc.)
4. Pets (dogs, cats)
5. Avian wildlife (ducks, geese, sparrows, etc.)
6. Non-avian wildlife (deer, feral hogs, coyotes, etc.)
7. Humans

For any *E. coli* isolate that could not be matched to a group in the Texas *E. coli* BST Library, its source category was designated as being “unidentified.”

In Tables 2 & 3, the far right column lists the ID of the closest library match for each tested *E. coli* isolate. The ID of the closest library match for each isolate should be used for informational purposes only and not be interpreted as species-level source classification of the isolates since our current methods are not capable of doing this (e.g., they cannot distinguish between isolates from different species of non-avian wildlife, such as deer and feral hogs).

Bacterial Source Tracking Results

Results for samples 582571 and 582572 collected on 08/07/17 are shown in Tables 2 & 3. Overall out of the 8 isolates, 3 were classified as originating from livestock and domesticated animals, 3 from wildlife, and 1 isolate was identified to be from a human source, using a 3-way source split. Using the more detailed 7-way split, 3 of the isolates were classified as originating from cattle, 3 from non-avian wildlife, and 1 was identified as originating from a human source. The source could not be identified for 1 of the isolates.

Table 2. Classification of *E. coli* isolates from sample 582571 collected on 08/07/17

Isolate	3 way id	7 way id	Closest Match*
Cypress Creek-582571-8/7-A	Human	Human	Raw Sewage
Cypress Creek-582571-8/7-B	Unidentified	Unidentified	Cattle
Cypress Creek-582571-8/7-C	Livestock and Domesticated Animals	Cattle	Cattle
Cypress Creek-582571-8/7-E	Wildlife	Wildlife, Non-Avian	Feral Hog

Table 3. Classification of *E. coli* isolates from sample 572572 collected on 08/07/17

Isolate	3 way id	7 way id	Closest Match*
Cypress Creek-582572-8/7-A	Livestock and Domesticated Animals	Cattle	Cattle
Cypress Creek-582572-8/7-C	Wildlife	Wildlife, Non-Avian	Mouse
Cypress Creek-582572-8/7-D	Livestock and Domesticated Animals	Cattle	Cattle
Cypress Creek-582572-8/7-E	Wildlife	Wildlife, Non-Avian	Feral Hog

* The ID of the closest library match for each isolate is provided for informational purposes only.

Notes

The BST results in this report should be interpreted cautiously since they represent only 4 *E. coli* isolates from each sample and the samples were collected at only a single time-point. Analysis of additional *E. coli* isolates from multiple sampling events may strengthen and further validate these initial results.

End of Report

December 1, 2017

Nick Dornak
Plum Creek Watershed Coordinator
ndornak@plumcreekwatershed.org

Dear Nick:

Attached please find the bacterial source tracking (BST) results for the Cypress Creek water samples collected on 09/12/2017 and 10/16/2017. For BST, we DNA fingerprinted three isolates from each sample using enterobacterial repetitive intergenic consensus sequence-PCR and RiboPrinting. The fingerprints were compared against the Texas *E. coli* BST Library (ver. 5-15) for identification of source categories using both 3-way and 7-way source splits.

The following pages detail: 1) the composition and performance of the current Texas *E. coli* BST library that was used to categorize your isolates and 2) a summary of the BST results for your isolates.

Please do not hesitate to contact me if you have any questions or would like to discuss the results further. We appreciate the opportunity to assist you with characterization of this watershed.

Sincerely,



Terry J. Gentry
Professor & Laboratory Director

Heep Center, 370 Olsen Boulevard
2474 TAMU
College Station, TX 77843-2474

Tel. 979.845.5323 Fax 979.845.0456
tjgentry@tamu.edu
<http://microbe.tamu.edu/>

Bacterial Source Tracking Methods

From each water sample collected on 09/12/2017 and 10/16/2017, a total of three isolates each were DNA fingerprinted using enterobacterial repetitive intergenic consensus sequence-PCR and RiboPrinting (ERIC-RP). The DNA fingerprints were compared to known-source fingerprints in the Texas *E. coli* BST Library (ver. 5-15) for identification of source categories (Table 1). The current library contains 1,765 *E. coli* isolates from 1,554 different fecal samples representing over 50 animal subclasses. This is the result of collecting over 3,500 domestic sewage, wildlife, livestock, and pet fecal samples from 13 watersheds across Texas and screening over 6,000 isolates for clones and host specificity.

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Source Class	Number of Isolates	Number of Samples	Library Composition and Expected Random Rate of Correct Classification	Calculated Rate of Correct Classification (RCC)	RCC to Random Ratio ^{***}	Left Unidentified (unique patterns)
HUMAN	384	330	22%	100	4.5	6
DOMESTIC ANIMALS	532	495	30%	100	3.3	19
Pets	83	74	5%	84	16.8	41
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Avian Livestock	95	88	5%	89	17.8	26
Other Non-Avian Livestock	122	117	7%	94	13.4	15
WILDLIFE	849	729	48%	100	2.1	16
Avian Wildlife	273	250	15%	79	5.3	19
Non-Avian Wildlife	576	479	33%	91	2.8	15
Overall	1765	1554		ARCC^{**} = 100% (3-way) 91% (7-way)		18%

^{**}ARCC = average rate of correct classification: the proportion of all identification attempts which were correctly identified to source class for the entire library, which is similar to the mean of the RCCs for all source classes when the number of isolates in each source class is similar.

^{***}A RCC/Random Ratio greater than 1.0 indicates that the rate of correct classification is better than random. For example, the rate of correct classification for human is 4.5-fold greater than random chance.

Source Categories

With currently available BST methods, it is not possible to discriminate between all possible species of animal sources simultaneously, since each added category tends to decrease the accuracy of source classification. This is illustrated in Table 1 where the 7-way split has an overall lower average rate of correct classification (91%) than the 3-way split does (100%). Therefore, we currently combine most species into groups based upon similarity of their physiology and/or potential management. Below are the 3- and 7-way split categories that were used for categorizing your *E. coli* isolates and which we have most frequently used for characterizing watersheds:

3-way split

1. Domesticated animals and livestock (livestock and pets)
2. Wildlife (including feral hogs)
3. Humans

7-way split

1. Cattle
2. Other livestock, non-avian (non-avian livestock other than cattle; sheep, etc.)
3. Other livestock, avian (chickens, etc.)
4. Pets (dogs, cats)
5. Avian wildlife (ducks, geese, sparrows, etc.)
6. Non-avian wildlife (deer, feral hogs, coyotes, etc.)
7. Humans

For any *E. coli* isolate that could not be matched to a group in the Texas *E. coli* BST Library, its source category was designated as being “unidentified.”

In Tables 2-5, the far right column lists the ID of the closest library match for each tested *E. coli* isolate. The ID of the closest library match for each isolate should be used for informational purposes only and not be interpreted as species-level source classification of the isolates since our current methods are not capable of doing this (e.g., they cannot distinguish between isolates from different species of non-avian wildlife, such as deer and feral hogs).

Bacterial Source Tracking Results

Results for samples Downstream and Upstream RR12 collected on 09/12/2017 are shown in Tables 2 & 3. Overall, all of the 6 isolates were classified as originating from wildlife using a 3-way source split. Using the more detailed 7-way split, 5 of the isolates were classified as originating from non-avian wildlife, and 1 isolate was identified as originating from avian wildlife.

Table 2. Classification of *E. coli* isolates from sample Downstream RR12 (582587) collected on 09/12/17

Isolate	3 way id	7 way id	Closest Match*
Cypress Creek-9/12-582587-C	Wildlife	Wildlife, Non-Avian	Deer
Cypress Creek-9/12-582587-D	Wildlife	Wildlife, Non-Avian	Feral Hog
Cypress Creek-9/12-582587-E	Wildlife	Wildlife, Non-Avian	Coyote

Table 3. Classification of *E. coli* isolates from sample Upstream RR12 (572589) collected on 09/12/17

Isolate	3 way id	7 way id	Closest Match*
Cypress Creek-9/12-582589-B	Wildlife	Wildlife, Non-Avian	Coyote
Cypress Creek-9/12-582589-D	Wildlife	Wildlife, Non-Avian	Opossum
Cypress Creek-9/12-582589-E	Wildlife	Wildlife, Avian	Vulture

Results for samples Downstream and Upstream RR12 collected on 10/16/2017 are shown in Tables 4 & 5. Overall, 4 of the isolates were classified as originating from wildlife and 2 from domesticated animals using a 3-way source split. Using the more detailed 7-way split, 3 of the isolates were classified as originating from non-avian wildlife, 1 from avian wildlife, and 2 from avian livestock.

Table 4. Classification of *E. coli* isolates from sample Downstream RR12 (585596) collected on 10/16/17

Isolate	3 way id	7 way id	Closest Match*
Cypress Creek-10/16-582596-A	Wildlife	Wildlife, Avian	Duck
Cypress Creek-10/16-582596-B	Wildlife	Wildlife, Non-Avian	Feral Hog
Cypress Creek-10/16-582596-C	Wildlife	Wildlife, Non-Avian	Raccoon

Table 5. Classification of *E. coli* isolates from sample Upstream RR12 (582597) collected on 10/16/17

Isolate	3 way id	7 way id	Closest Match*
Cypress Creek-10/16-582597-A	Wildlife	Wildlife, Non-Avian	Skunk
Cypress Creek-10/16-582597-B	Domesticated Animals	Livestock, Avian	Chicken
Cypress Creek-10/16-582597-C	Domesticated Animals	Livestock, Avian	Chicken

* The ID of the closest library match for each isolate is provided for informational purposes only.

Notes

The BST results in this report should be interpreted cautiously since they represent only 3 *E. coli* isolates from each sample. Analysis of additional *E. coli* isolates from multiple sampling events may strengthen and further validate these initial results.

End of Report