

## Appendix G – Fecal Contamination Source Assessment Plan

Brown County Regional Sewer District retained Lochmueller Group and Arion Consultants to evaluate *E. coli* levels in Brown County as part of efforts to develop a strategic wastewater management plan. They identified 14 sites in the Bean Blossom watershed and 21 sites in the Lake Monroe (East Fork White River) watershed for evaluation. After an initial round of sampling, 12 sites in the Bean Blossom watershed and 20 sites in the Lake Monroe watershed were sampled weekly over a five-week period (May 5, 2020 – June 2, 2020) and tested for *E. coli*. This data was used to calculate the geometric mean of the samples to compare to the state geometric mean standard of 125 CFU/100 ml.

Lochmueller Group and Arion Consultants then used a tiered decision-making approach to select sample sites for *E. coli* source assessment. The tiers are detailed below and note which samples meet the required decision and are thus included in the source assessment phase of the project. Table details the sites where source assessment sampling occurred.

Tier 1 Decision: The geometric mean calculated at that site using 2020 *E. coli* data exceeds the state geometric mean average for the basin. IDEM calculated average geometric mean values for *E. coli* samples collected during their most recent rotational basin sampling which occurred 2011 to 2019. The average geometric mean value for Beanblossom Creek Watershed is 466 col/100 ml, while the average geometric mean value for the Lake Monroe Watershed is 462 col/100 ml. Six sites in the Beanblossom Creek Watershed exceed the basin geometric mean average concentration, while no sites in the Lake Monroe Watershed exceed the basin geometric mean average concentration.

Sites which exceeded average basin geometric mean concentration include **BB01, BB06, BB07, BB08, BB10, and BB12.**

Tier 2 Decision: There is the potential for human impacts from non-compliant sewer systems or malfunctioning, not maintained or absent septic systems within sample site's subwatershed. The sample site's subwatershed is defined as the area draining to that sample point downstream of any upstream sample site. Lochmueller Group and Arion Consultants used three potential *E. coli* sources to determine if each immediate subwatershed possesses a potential for human impacts. Additional human sources could be present in upstream immediate watersheds and so may be observed in subwatershed where a source was not identified in the immediate drainage.

These sources include:

- There are several NPDES permitted facilities within Brown County. Subwatersheds which contain facilities with documented compliance issues were listed as basins which could have a potential human input.
- The Brown County Health Department residential septic data completed for Jackson Township – these data map residential addresses within Jackson County and detail septic system information for each residential point. The total number of residential facilities with documented septic systems was divided by the total number of residential facilities.

Lochmueller Group and Arion Consultants included any subwatershed where 40% or less of residential facilities possessed septic systems.

- The State of Indiana address point file provided point addresses for all properties within Brown County. For areas outside of Jackson Township, these points were used to simulate residential locations. A simple count was utilized to identify subwatersheds where a human impact could occur. Any subwatershed with more than 300 residences outside of Jackson Township were included.

Sites which have a potential human impact include **BB01, BB03, BB05, BB07, BB08, BB09, BB10, BB12, EF02, EF06, EF08, EF10, EF16, EF18, EF20.**

Tier 3 Decision: There is the potential for animal impacts from livestock on a confined feeding operation, hobby farm or with access to the stream or wildlife. The sample site’s subwatershed is defined as the area draining to that sample point downstream of any upstream sample site. Lochmueller Group and Arion Consultants used three potential *E. coli* sources to determine if each immediate subwatershed possesses a potential for animal impacts. Additional animal sources could be present in upstream immediate watersheds and so may be observed in subwatershed where a source was not identified in the immediate drainage.

These sources include:

- The presence of a confined feeding operation.
- The presence of a more than 50 observed livestock on hobby farms throughout the subwatershed.
- A high density of observed wildlife or the land use which would house high densities of wildlife.

Sites which have a potential animal impact include **BB04, BB07, BB08, BB10, BB11, EF18.**

**Table 1. Brown County Regional Sewer District Site Analysis and Selection**

Sample Site	Tier 1 (E. Coli Results)	Tier 2 (Human Source)	Tier 3 (Animal Source)
BB01	Yes	Yes	
BB03		Yes	
BB04			Yes
BB05		Yes	Yes
BB06	Yes		
BB07	Yes	Yes	Yes
BB08	Yes	Yes	Yes
BB09		Yes	
BB10	Yes	Yes	Yes
BB11			Yes
BB12	Yes	Yes	

Sample Site	Tier 1 (E. Coli Results)	Tier 2 (Human Source)	Tier 3 (Animal Source)
EF02		Yes	
EF06		Yes	
EF08		Yes	
EF10		Yes	
EF16		Yes	
EF18		Yes	Yes
EF20		Yes	

The Lake Monroe watershed coordinator also used a tiered decision-making approach to select sample sites for fecal contamination source assessment. The tiers are detailed below and note which samples meet the required decision and are thus included in the source assessment phase of the project.

**Tier 1 Decision:** Samples collected during the Spring Blitz and Fall Blitz were compared to the state E. coli standard of 235 cfu/100 mL. All sites with a sample exceeding the state standard were selected for further investigation. Note that only one sample from the Spring Blitz exceeded the state standard, Site 881.

Sites with at least one sample result which exceeded the state standard are: 277, 317, 338, 341, 398, 404, 425, 440, 644, 685, 692, 697, 816, 855, 881, 915, and 930.

Two additional sites were added based on the monthly stream monitoring results from Middle Fork Salt Creek (668) and the CBU storm team monitoring of South Fork Salt Creek at Kurtz (853).

**Tier 2 Decision:** There is the potential for human impacts from non-compliant sewer systems or malfunctioning, not maintained or absent septic systems within a sample site’s sub-watershed. The sample site’s sub-watershed is defined as the area draining to that sample point downstream of any upstream sample site. The watershed coordinator used two potential *E. coli* sources to determine if each immediate sub-watershed possesses a potential for human impacts. One was identifying sub-watersheds which contain NPDES permitted facilities with documented compliance issues (Nashville WWTP and Gnaw Bone WWTP) which could have a potential human input. Only site 398 was identified. The other criterion was estimating the number of unsewered homes per acre. The number of unsewered homes was estimated by counting the number of buildings per sub-watershed shown in the “Building Footprints” 2019 shapefile from Indiana MAP and approximate acreage was measured in GIS. Additional human sources could be present that were not identified by these methods.

Sites identified as having a density of unsewered homes greater than or equal to 0.05 are: 317, 338, 341, 398, 404, 425, 440, 644, 685, 692, 697, 816, and 881.

**Tier 3 Decision:** There is the potential for animal impacts from livestock within the subwatershed. The watershed coordinator classified sites into three categories based on site observations of livestock and land cover maps. Sites with livestock or fenced pasture observed at the site or immediately upstream were categorized as “high.” Sites with no livestock observed but with pasture identified upstream on land cover maps were categorized as “medium.” Sites with no livestock observed and no pasture shown on land cover maps upstream were categorized as “low.” Additional animal sources could be present that were not identified by these methods.

Sites which have a high or medium potential animal impact are: 277, 338, 341, 398, 425, 440, 685, 692, 697, 816, 855, 881, 915, and 930.

**Table 2. Lake Monroe Watershed Coordinator Site Analysis and Selection**

Sample Site	Tier 1 (E. Coli Results)	Tier 2 (Human Source)	Tier 3 (Animal Source)	Source Assessment?	Reason	Stream
277	Yes	0.02	High	No	Low human source	Lick Creek
317	Yes	0.40	Low	No	Low animal source	East Branch Sweetwater
338	Yes	0.10	Medium	No	Redundant; sample 425 in same stream	Stephens Creek
341	Yes	0.10	High	Yes	Both sources probable	Kerr Creek
398	Yes	0.05	Medium	No	Redundant. BCRSD will sample upstream EF10 and downstream EF08	North Fork Salt (below Gnaw Bone Creek)
404	Yes	0.06	Low	No	Low animal source	Henderson Creek
425	Yes	0.13	Medium	Yes	Both sources probable	Stephens Creek
440	Yes	0.05	Medium	Yes	Relatively low concentration (298.7)	Owl Creek
644	Yes	0.05	Low	No	Low animal source	Unnamed Tributary to South Branch
685	Yes	0.07	Medium	No	Redundant; BCRSD will sample nearby EF18	Middle Fork Salt Creek
692	Yes	0.08	Medium	Yes	Both sources probable	Unnamed Tributary to Hamilton Creek
697	Yes	0.06	High	Yes	Both sources probable	South Branch Salt Creek
816	Yes	0.05	High	Yes	Both sources probable	Little Salt Creek

Sample Site	Tier 1 (E. Coli Results)	Tier 2 (Human Source)	Tier 3 (Animal Source)	Source Assessment?	Reason	Stream
855	Yes	0.03	Medium	Yes	Low human source but concentration was very high	Unnamed Tributary to Little Salt Creek
881	Yes	0.05	High	Yes	Both sources probable	Kiper Creek
915	Yes	0.01	Medium	No	Low human source	Unnamed Tributary to Little Salt Creek
930	Yes	0.03	High	No	Redundant; sample 881	Kiper Creek
853	Yes (storm team)	0.04	High	Yes	CBU Storm team sampling had many exceedances; both sources probable	South Fork Salt Creek at Kurtz
668/E15	Yes (monthly samples)	0.05	Medium	Yes	Monthly stream sampling and BCRSD samples had several exceedances; both sources probable	Middle Fork Salt Creek at SR-135

**Final Selection:** Ten sites were chosen for source analysis through the Lake Monroe Watershed Management Plan project (LMWMP) in addition to the seven sites previously selected by the Brown County Regional Sewer District (BCRSD). The ten LMWMP sites were: 440, 425, and 341 in the North Fork sub-watershed; 697, 668, and 692 in the Middle Fork sub-watershed; and 816, 853, 855, and 881 in the South Fork sub-watershed.

This meant a total of seventeen sites were evaluated using source analysis at Scientific Methods in Granger, Indiana. The complete list of sites is presented in Table 3.

**Table 3. Source Sampling Sites in Lake Monroe Watershed**

<b>BC_ID</b>	<b>LM_ID</b>	<b>Sub</b>	<b>Stream</b>	<b>Sampler</b>
EF02	332	NF	North Fork Salt (above Sweetwater Creek)	BCRSD
EF10		NF	North Fork Salt (above Gnow Bone Creek)	BCRSD
EF08		NF	North Fork Salt (SR 46 below Salt Creek Trail)	BCRSD
EF06	256	NF	North Fork Salt (Yellowwood Rd)	BCRSD
	440	NF	Owl Creek (above West Branch Owl Creek)	LMWMP
EF20		NF	Greasy Creek (downstream @ SR-46)	BCRSD
	425	NF	Stephens Creek (upstream)	LMWMP
	341	NF	Kerr Creek	LMWMP
	697	MF	South Branch Salt Creek (upstream of EF18/685)	LMWMP
EF18		MF	Middle Fork Salt Creek (upstream near Christianburg Rd)	BCRSD
EF15	668	MF	Middle Fork Salt Creek (SR-135, above Hamilton Creek)	LMWMP
	692	MF	unnamed tributary to Hamilton Creek	LMWMP
	816	SF	Little Salt Creek (down from EF21 at SR-135)	LMWMP
	853	SF	South Fork Salt Creek (midstream at Kurtz)	LMWMP
	855	SF	unnamed trib to South Fork (enters below 853)	LMWMP
	881	SF	Kiper Creek (downstream)	LMWMP



## Laboratory Report

Client: Kyle Myers  
Lochmueller Group  
6200 Vogel Rd  
Evansville, IN 47715  
kmyers@lochgroup.com

Report no.: 31427

**Sample collection date:** April 27, 2021

**Received date:** April 27, 2021

**Analysis date and time:** April 27, 2021 @ 17:59

Samples submitted: 13

### Serotyping

<u>Sample ID</u>	<u>Sample Location</u>	<u>pfu/100mL</u>	<u># of Isolates</u>	<u>I</u>	<u>I/II</u>	<u>II</u>	<u>II/III</u>	<u>III</u>	<u>IV</u>	<u>% Human</u>	<u>% Animal</u>
31427	Stephens Creek (upstream 0425)	<1	0	NA	NA	NA	NA	NA	NA	NA	NA
31428	Kerr Creek 0341	<1	0	NA	NA	NA	NA	NA	NA	NA	NA
31429	Owl Creek	<1	0	NA	NA	NA	NA	NA	NA	NA	NA
31430	Middle Fork Salt Creek 0668	<1	0	NA	NA	NA	NA	NA	NA	NA	NA
31431	South Fork Salt Creek (mid kurts) 0853	0.1	1	0	1	0	0	0	0	50.5	49.5
31432	Unnamed Trib to south fork 0855	<1	0	NA	NA	NA	NA	NA	NA	NA	NA
31433	Kiper Creek 0881	0.1	1	0	1	0	0	0	0	50.5	49.5
31434	Little Salt Creek 0816	<1	0	NA	NA	NA	NA	NA	NA	NA	NA
31435	Trib to Hamilton Creek 0692	<1	0	NA	NA	NA	NA	NA	NA	NA	NA
31436	South Branch Salt Creek 0697	<1	0	NA	NA	NA	NA	NA	NA	NA	NA
31437	Reed Point-Lemon	<1	0	NA	NA	NA	NA	NA	NA	NA	NA
31438	Riddle Point-Lemon	<1	0	NA	NA	NA	NA	NA	NA	NA	NA
31439	Chitwood-Lemon	<1	0	NA	NA	NA	NA	NA	NA	NA	NA

**Note:** Not all samples produced plaques. *E.coli* may be present without coliphages in any given sample

Scientific Methods appreciates the opportunity to provide you with this analysis. Please feel free to contact us (574-277-4078) if you have any questions regarding this report.

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Reviewed by: *Miriam Svoboda*  
Miriam Svoboda, Project Manager

Date: May 11, 2021

Finalized by: *Jordyn Andre*  
Jordyn Andre, Laboratory Analyst

Date: May 11, 2021





## References and definitions

### References:

Easyphage SPL, based on:

**EPA Method 1602:** Male-specific (F<sup>+</sup>) and Somatic Coliphage in Water by Single Agar Layer Procedure (821-R-01-029)

**Genotyping Male-specific RNA Coliphages by Hybridization with Oligonucleotide Probes.** HSU, F.-C., Y.-S. CAROL SHIEH, J. VAN DUIN, M. J. BEEKWILDER, and M. D. SOBSEY. *Appl. Environ. Microbiol.* 61:3960-3966.

**Applying meta-analysis of male-specific RNA coliphages to determine the probable sources of fecal contamination during poultry processing.** F.-C. Hsu, Y. C. Shieh, J. Larkin, and M. D. Sobsey. 2004. The Annual Meeting of the American Society for Microbiology, New Orleans, LA.

Definitions:

MRL: Minimum reporting limit

< = “less than.” It indicates the lowest reportable value by the procedure used for analysis.

pfu/100mL: plaque forming units per 100 mL

**Coliphages** belong to the group of bacterial viruses that infect and replicate exclusively within the coliform bacteria group.

**Coliphages** can be further classified as belonging to the “male-specific” or “somatic” groups depending upon their method of attachment to host cells.

**Male-specific coliphages**, also known as F<sup>+</sup>coliphages, specifically infect the coliform bacteria that express physical appendages called sex pili. They are classified into F<sup>+</sup>DNA and F<sup>+</sup>RNA coliphages. Only F<sup>+</sup>RNA coliphages can be serotyped or genotyped.

**Meta-analysis** is based on a database, compiled of all data available on the published literature.



## Laboratory Report

Client: Kyle Myers  
Lochmueller Group  
6200 Vogel Rd  
Evansville, IN 47715  
kmyers@lochgroup.com

Report no.: 31409

**Sample collection date:** April 27, 2021

**Received date:** April 27, 2021

**Analysis date and time:** April 27, 2021 @ 17:00

Samples submitted: 18

### Serotyping

<u>Sample ID</u>	<u>Sample Location</u>	<u>pfu/100mL</u>	<u># of Isolates</u>	<u>I</u>	<u>I/II</u>	<u>II</u>	<u>II/III</u>	<u>III</u>	<u>IV</u>	<u>% Human</u>	<u>% Animal</u>
31409*	EF02	0.1	1	0	0	0	0	0	0	NA	NA
31410	BB09	< 1	0	NA	NA	NA	NA	NA	NA	NA	NA
<b>31411</b>	<b>BB10</b>	<b>23</b>	<b>20</b>	<b>0</b>	<b>0</b>	<b>20</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>94</b>	<b>6</b>
31412	BB11	< 1	0	NA	NA	NA	NA	NA	NA	NA	NA
<b>31413</b>	<b>BB12</b>	<b>15</b>	<b>20</b>	<b>0</b>	<b>6</b>	<b>14</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>74</b>	<b>26</b>
31414	BB08	< 1	0	NA	NA	NA	NA	NA	NA	NA	NA
31415	BB07	< 1	0	NA	NA	NA	NA	NA	NA	NA	NA
31416	BB06	< 1	0	NA	NA	NA	NA	NA	NA	NA	NA
31417	BB05	< 1	0	NA	NA	NA	NA	NA	NA	NA	NA
31418	BB04	< 1	0	NA	NA	NA	NA	NA	NA	NA	NA
31419	BB01	< 1	0	NA	NA	NA	NA	NA	NA	NA	NA
31420	BB03	< 1	0	NA	NA	NA	NA	NA	NA	NA	NA
<b>31421</b>	<b>EF06</b>	<b>0.6</b>	<b>6</b>	<b>0</b>	<b>5</b>	<b>1</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>54</b>	<b>46</b>
31422	EF08	< 1	0	NA	NA	NA	NA	NA	NA	NA	NA
<b>31423*</b>	<b>EF20</b>	<b>0.4</b>	<b>4</b>	<b>0</b>	<b>0</b>	<b>1</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>94</b>	<b>6</b>
31424	EF10	< 1	0	NA	NA	NA	NA	NA	NA	NA	NA
<b>31425*</b>	<b>EF18</b>	<b>0.4</b>	<b>4</b>	<b>0</b>	<b>1</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>50.5</b>	<b>49.5</b>
31426*	EF16	0.3	3	0	0	0	0	0	0	NA	NA

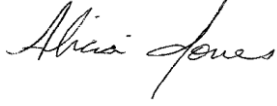
**Note:** Not all samples produced plaques. *E.coli* may be present without coliphages in any given sample

\*These samples contained plaques that did not produce any lysis during the spot testing.




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Reviewed by:   
Alicia Jones, Senior Laboratory Analyst

Date: May 10, 2021

Finalized by:   
Miriam Svoboda, Project Manager

Date: May 10, 2021



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