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QUALITY CONTROL/QUALITY ASSURANCE DOCUMENTATION

Title: Processing *E. coli* Bacteria Water Quality Data
 Type: Standard Operation Procedure #7.29
 Version: 2
 Author: Emily Joynt

VERSION HISTORY

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1.0 SCOPE AND APPLICABILITY

This document presents the North Dakota Department of Environmental Quality (NDDEQ), Division of Water Quality, Watershed Management Program's (WMP) Standard Operating Procedure (SOP) for processing *Escherichia coli* (*E. coli*) bacteria water quality data. This SOP applies to all WMP staff and should be followed to ensure appropriate comparison of *E. coli* lab results.

2.0 BACKGROUND

The North Dakota Public Health Laboratory (NDPHL) analyzes surface water samples for *E. coli* bacteria. In 2018 NDPHL methods changed from membrane filtration and dilution to multi-well distribution. Membrane filtration and dilution measures *E. coli* as Colony Forming Units (CFU) per 100 mL of solution. Samples may be diluted in order to count large numbers of organisms. Multi-well distribution (Quanti-Tray) measures *E. coli* as Most Probable Number (MPN). MPN and CFU represent units specific to analytical techniques but are considered equivalent measures of bacteria concentration (EPA, 2001). As a result, NDPHL displays all *E. coli* sample results, regardless of method, as units of CFU/100mL.

Although CFU and MPN are considered equivalent measures of *E. coli*, each laboratory method has different upper limits. Membrane filtration and dilution measures up to 800 or 8,000 CFU/100mL (depending on the number of dilutions performed) and Quanti-Tray measures up to 24,000 CFU/100mL (MPN). All results are reported in bacteria counts/100 mL (CFU/100mL).

To compare data analyzed with membrane filtration and dilution the appropriate dilution result needs to be selected. To compare data from both membrane filtration and dilution and Quanti-Tray methods, which have different measurement limits, data must first be processed to reflect the same scale. The following methods outlines steps for processing *E. coli* data from each method, and from both methods together.

3.0 SUMMARY OF METHODS

Steps for processing *E. coli* data fall into three scenarios:

- 1) Processing *E. coli* data from (only) the membrane filtration laboratory method.
- 2) Processing *E. coli* data from (only) the Quanti-Tray laboratory method.
- 3) Processing *E. coli* data from BOTH membrane filtration and Quanti-Tray methods.

Selecting an *E. coli* dataset (period of time) may be specific to the project or analysis. When possible and representative (for example, considering sample size and frequency), compare data from the same analytical laboratory method rather than comparing data across different methods. A one-page reference sheet of all three methods is included in Appendix A.

3.1 Scenario 1: Processing *E. coli* Data from the Membrane Filtration and Dilution Method

The membrane filtration and dilution laboratory method may include multiple dilutions, and as a result may report more than one result for the same sample (1st dilution, 2nd dilution, etc.). If multiple dilutions are performed, all dilution results will be reported. The 1st result is based on analyzing 10 mL of stream water and the second 1 mL, or a ten-fold dilution. Larger sample volumes (1st dilution) yield more consistency in results, but are more difficult to count and as a result may need to be diluted.

If your *E. coli* dataset consists of results ONLY from the membrane filtration and dilution laboratory method (pre-2018), use the following steps to process:

- 1) For samples with only one dilution (1st dilution, only one result for the sample) -
 - a. If result is “too numerous to count” update to “800” (1st dilution upper limit)
 - b. If result is “non-detect” update to “5” (half detection level)
- 2) For samples with multiple dilutions -
 - a. If 1st dilution < 800, select 1st dilution result
 - i. If result is “non-detect” update to “5” (half detection level)
 - b. If 1st dilution ≥ 800 or “too numerous to count” select 2nd dilution result
 - i. If 2nd dilution result is “too numerous to count” update to 8,000 (2nd dilution upper limit)
 - c. Always default to the higher result
 - i. *For example*, if the 1st dilution result is “TNTC” but the 2nd dilution result is less than 800, select the 1st dilution limit of “TNTC” and update to 800

Note: Other (uncommon) but possible variations of a sample with a very high count include:

- Confluent (or, Confluent Growth)
- Present > QL
- Present Above Quantification Limit

For bacteria, these can be interpreted the same as “too numerous to count.”

The best accuracy for a 1st dilution is between 200 and 600. The best accuracy for a 2nd dilution is between 2,000 and 6,000. TNTC (“too numerous to count”) on a 1st dilution is ≥ 800; TNTC on a 2nd dilution is ≥ 8,000 (upper limits of dilutions).

3.2 Scenario 2: Processing Quanti-tray *E. coli* Data

In 2018 the NDPHL stopped analyzing *E. coli* samples using membrane filtration and dilution and began using the Quanti-Tray/2000 method. Quanti-Tray/2000 yields a count of 1-2,419. For surface waters a 10 mL sample is tested, giving a 1:10 dilution so that the range is < 10 to 24,000. If all Quanti-Tray wells are positive, the result is reported as > 24,000 (“too numerous to count” or “TNTC”).

If your *E. coli* dataset consists of results ONLY from the Quanti-Tray laboratory method (2018-present), use the following steps to process:

- 1) Use the single result given
 - a. If the result is “too numerous to count” update to “24,000” (upper limit)
 - b. If the result is “non-detect” update to “5” (half detection level)

Appendix B includes an example and illustration of the Quanti-Tray/2000 laboratory method.

3.3 Scenario 3: Processing *E. coli* Data from BOTH Membrane Filtration and Quanti-Tray

If your *E. coli* dataset consists of results from BOTH the membrane filtration and Quanti-Tray methods, use the following steps to process:

- 1) Follow the steps detailed in section 3.1 to process membrane filtration *E. coli* data to select the appropriate dilution for each sample
 - a. If ALL of the membrane filtration data selected are 1st dilution results only, cap all results (including Quanti-Tray) at 800
 - b. If any of the membrane filtration data being used consist of 2nd dilution results, cap all results (including Quanti-Tray) at 8,000
 - c. Update any “non-detect” results to “5” (half detection level)

4.0 RESPONSIBILITIES

Staff should keep note of the method(s) they use to process data. When analyzing *E. coli* data that has been processed, include a footnote or similar in your data table or description that clarifies the steps you have taken to refine the data.

5.0 QUALITY ASSURANCE AND QUALITY CONTROL

This SOP exclusively outlines methods for processing surface water *E. coli* results from samples analyzed at NDPHL. Proper data processing procedures ensure consistent and appropriate comparison of results from lab methods with different measurement limits.

6.0 REFERENCES

EPA. (2001). Protocol for Developing Pathogen TMDLs. EPA 841-R-00-002. U.S. Environmental Protection Agency, Office of Water, Washington, D.C.
<https://nepis.epa.gov/Exe/ZyPDF.cgi/20004QSZ.PDF?Dockkey=20004QSZ.PDF>

NDDEQ & NDPHL email communications (multiple).

APPENDIX A – Quick Reference Sheet for Processing *E. coli* Data

- In 2018 the lab switched from membrane filtration and dilution to Quanti-Tray
- When possible, compare data from the same lab method
- All results are reported in bacteria counts/100 mL (CFU/100 mL)

Scenario 1 – Processing *E. coli* data from membrane filtration and dilution method

- 1) For samples with only one dilution (1st dilution, only one result for the sample) -
 - a. If result is “too numerous to count” update to “800” (1st dilution upper limit)
 - b. If result is “non-detect” update to “5” (half detection level)
- 2) For samples with multiple dilutions -
 - a. If 1st dilution < 800, select 1st dilution result
 - i. If result is “non-detect” update to “5” (half detection level)
 - b. If 1st dilution ≥ 800 or “too numerous to count” select 2nd dilution result
 - i. If 2nd dilution result is “too numerous to count” update to 8,000 (2nd dilution upper limit)
 - c. Always default to the higher result
 - i. *For example*, if the 1st dilution result is “TNTC” but the 2nd dilution result is less than 800, select the 1st dilution limit of “TNTC” and update to 800

Scenario 2 – Processing *E. coli* data from Quanti-Tray method

- 1) Use the single result given
 - a. If the result is “too numerous to count” update to “24,000” (upper limit)
 - b. If the result is “non-detect” update to “5” (half detection level)

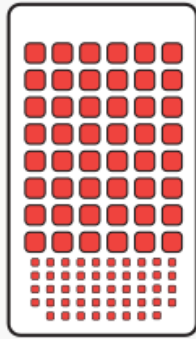
Scenario 3 – Processing *E. coli* data from BOTH membrane filtration and Quanti-Tray methods

- 1) Follow the steps detailed in section 3.1 to process membrane filtration *E. coli* data to select the appropriate dilution for each sample
 - a. If ALL of the membrane filtration data selected are 1st dilution results only, cap all results (including Quanti-Tray) at 800
 - b. If any of the membrane filtration data being used consist of 2nd dilution results, cap all results (including Quanti-Tray) at 8,000
 - c. Update any “non-detect” results to “5” (half detection level)

APPENDIX B - Quanti-Tray Laboratory Method Example

As an example, if there are 49 positive large wells and 44 positive small wells the MPN (most probable number) value is 1553.1, multiplied by the dilution factor of 10 (1553.1 x 10 = 15,530). Results are reported to two significant figures so it would be rounded to 16,000.

Quanti-Tray/2000



Simplify with Quanti-Tray/2000



IDEXX Quanti-Tray/2000 MPN Table

# Large Wells Positive	# Small Wells Positive																							
	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48
0	25.3	26.4	27.4	28.4	29.5	30.5	31.5	32.6	33.6	34.7	35.7	36.8	37.8	38.9	40.0	41.0	42.1	43.1	44.2	45.3	46.3	47.4	48.5	49.5
1	26.6	27.7	28.7	29.8	30.8	31.9	32.9	34.0	35.0	36.1	37.2	38.2	39.3	40.4	41.4	42.5	43.6	44.7	45.7	46.8	47.9	49.0	50.1	51.2
2	27.9	29.0	30.0	31.1	32.2	33.2	34.3	35.4	36.5	37.5	38.6	39.7	40.8	41.9	43.0	44.0	45.1	46.2	47.3	48.4	49.5	50.6	51.7	52.8
3	29.3	30.4	31.4	32.5	33.6	34.7	35.8	36.8	37.9	39.0	40.1	41.2	42.3	43.4	44.5	45.6	46.7	47.8	48.9	50.0	51.2	52.3	53.4	54.5
4	30.7	31.8	32.8	33.9	35.0	36.1	37.2	38.3	39.4	40.5	41.6	42.8	43.9	45.0	46.1	47.2	48.3	49.5	50.6	51.7	52.9	54.0	55.1	56.3
5	32.1	33.2	34.3	35.4	36.5	37.6	38.7	39.9	41.0	42.1	43.2	44.4	45.5	46.6	47.7	48.9	50.0	51.2	52.3	53.5	54.6	55.8	56.9	58.1
6	33.5	34.7	35.8	36.9	38.0	39.2	40.3	41.4	42.6	43.7	44.8	46.0	47.1	48.3	49.4	50.6	51.7	52.9	54.1	55.2	56.4	57.6	58.7	59.9
7	35.0	36.2	37.3	38.4	39.6	40.7	41.9	43.0	44.2	45.3	46.5	47.7	48.8	50.0	51.2	52.3	53.5	54.7	55.9	57.1	58.3	59.4	60.6	61.8
8	36.6	37.7	38.9	40.0	41.2	42.3	43.5	44.7	45.9	47.0	48.2	49.4	50.6	51.8	53.0	54.1	55.3	56.5	57.7	59.0	60.2	61.4	62.6	63.8
9	38.1	39.3	40.5	41.6	42.8	44.0	45.2	46.4	47.6	48.8	50.0	51.2	52.4	53.6	54.8	56.0	57.2	58.4	59.7	60.9	62.1	63.4	64.6	65.8
10	39.7	40.9	42.1	43.3	44.5	45.7	46.9	48.1	49.3	50.6	51.8	53.0	54.2	55.5	56.7	57.9	59.2	60.4	61.7	62.9	64.2	65.4	66.7	67.9
11	41.4	42.6	43.8	45.0	46.3	47.5	48.7	49.9	51.2	52.4	53.7	54.9	56.1	57.4	58.6	59.9	61.2	62.4	63.7	65.0	66.3	67.5	68.8	70.1
12	43.1	44.3	45.6	46.8	48.1	49.3	50.6	51.8	53.1	54.3	55.6	56.8	58.1	59.4	60.7	62.0	63.2	64.5	65.8	67.1	68.4	69.7	71.0	72.4
13	44.9	46.1	47.4	48.6	49.9	51.2	52.5	53.7	55.0	56.3	57.6	58.9	60.2	61.5	62.8	64.1	65.4	66.7	68.0	69.3	70.7	72.0	73.3	74.7
14	46.7	48.0	49.3	50.5	51.8	53.1	54.4	55.7	57.0	58.3	59.6	60.9	62.3	63.6	64.9	66.3	67.6	68.9	70.3	71.6	73.0	74.4	75.7	77.1
15	48.6	49.9	51.2	52.5	53.8	55.1	56.4	57.8	59.1	60.4	61.8	63.1	64.5	65.8	67.2	68.5	69.9	71.3	72.6	74.0	75.4	76.8	78.2	79.6
16	50.5	51.8	53.2	54.5	55.8	57.2	58.5	59.9	61.2	62.6	64.0	65.3	66.7	68.1	69.5	70.9	72.3	73.7	75.1	76.5	77.9	79.3	80.8	82.2
17	52.5	53.9	55.2	56.6	58.0	59.3	60.7	62.1	63.5	64.9	66.3	67.7	69.1	70.5	71.9	73.3	74.8	76.2	77.6	79.1	80.5	82.0	83.5	84.9
18	54.6	56.0	57.4	58.8	60.2	61.6	63.0	64.4	65.8	67.2	68.6	70.1	71.5	73.0	74.4	75.9	77.3	78.8	80.3	81.8	83.3	84.8	86.3	87.8
19	56.8	58.2	59.6	61.0	62.4	63.9	65.3	66.8	68.2	69.7	71.1	72.6	74.1	75.5	77.0	78.5	80.0	81.5	83.1	84.6	86.1	87.6	89.2	90.7
20	59.0	60.4	61.9	63.3	64.8	66.3	67.7	69.2	70.7	72.2	73.7	75.2	76.7	78.2	79.8	81.3	82.8	84.4	85.9	87.5	89.1	90.7	92.2	93.8
21	61.3	62.8	64.3	65.8	67.3	68.8	70.3	71.8	73.3	74.9	76.4	77.9	79.5	81.1	82.6	84.2	85.8	87.4	89.0	90.6	92.2	93.8	95.4	97.1
22	63.8	65.3	66.8	68.3	69.8	71.4	72.9	74.5	76.1	77.6	79.2	80.8	82.4	84.0	85.6	87.2	88.9	90.5	92.1	93.8	95.5	97.1	98.8	100.5
23	66.3	67.8	69.4	71.0	72.5	74.1	75.7	77.3	78.9	80.5	82.2	83.8	85.4	87.1	88.7	90.4	92.1	93.8	95.5	97.2	98.9	100.6	102.4	104.1
24	68.9	70.5	72.1	73.7	75.3	77.0	78.6	80.3	81.9	83.6	85.2	86.9	88.6	90.3	92.0	93.8	95.5	97.2	99.0	100.7	102.5	104.3	106.1	107.9
25	71.7	73.3	75.0	76.6	78.3	80.0	81.7	83.3	85.1	86.8	88.5	90.2	92.0	93.7	95.5	97.3	99.1	100.9	102.7	104.5	106.3	108.2	110.0	111.9
26	74.6	76.3	78.0	79.7	81.4	83.1	84.8	86.6	88.4	90.1	91.9	93.7	95.5	97.3	99.2	101.0	102.9	104.7	106.6	108.5	110.4	112.3	114.2	116.2
27	77.6	79.4	81.1	82.9	84.6	86.4	88.2	90.0	91.9	93.7	95.5	97.4	99.3	101.2	103.1	105.0	106.9	108.8	110.8	112.7	114.7	116.7	118.7	120.7
28	80.8	82.6	84.4	86.3	88.1	89.9	91.8	93.7	95.6	97.5	99.4	101.3	103.3	105.2	107.2	109.2	111.2	113.2	115.2	117.3	119.3	121.4	123.5	125.6
29	84.2	86.1	87.9	89.8	91.7	93.7	95.6	97.5	99.5	101.5	103.5	105.5	107.5	109.5	111.6	113.7	115.7	117.8	120.0	122.1	124.2	126.4	128.6	130.8
30	87.8	89.7	91.7	93.6	95.6	97.6	99.6	101.6	103.7	105.7	107.8	109.9	112.0	114.2	116.3	118.5	120.6	122.8	125.1	127.3	129.5	131.8	134.1	136.4
31	91.6	93.6	95.6	97.7	99.7	101.8	103.9	106.0	108.2	110.3	112.5	114.7	116.9	119.1	121.4	123.6	125.9	128.2	130.5	132.9	135.3	137.7	140.1	142.5
32	95.7	97.8	99.9	102.0	104.2	106.3	108.5	110.7	113.0	115.2	117.5	119.8	122.1	124.5	126.8	129.2	131.6	134.0	136.5	139.0	141.5	144.0	146.6	149.1
33	100.0	102.2	104.4	106.6	108.9	111.2	113.5	115.8	118.2	120.5	122.9	125.4	127.8	130.3	132.8	135.3	137.8	140.4	143.0	145.6	148.3	150.9	153.7	156.4
34	104.7	107.0	109.3	111.7	114.0	116.4	118.9	121.3	123.8	126.3	128.8	131.4	134.0	136.6	139.2	141.9	144.6	147.4	150.1	152.9	155.7	158.6	161.5	164.4
35	109.7	112.2	114.6	117.1	119.6	122.2	124.7	127.3	129.9	132.6	135.3	138.0	140.8	143.6	146.4	149.2	152.1	155.0	158.0	161.0	164.0	167.1	170.2	173.3
36	115.2	117.8	120.4	123.0	125.7	128.4	131.1	133.9	136.7	139.5	142.4	145.3	148.3	151.3	154.3	157.3	160.5	163.6	166.8	170.0	173.3	176.6	179.9	183.3
37	121.3	124.0	126.8	129.6	132.4	135.3	138.2	141.2	144.2	147.3	150.3	153.5	156.7	159.9	163.1	166.5	169.8	173.2	176.7	180.2	183.7	187.3	191.0	194.7
38	127.9	130.8	133.8	136.8	139.9	143.0	146.2	149.4	152.6	155.9	159.2	162.6	166.1	169.6	173.2	176.8	180.4	184.2	188.0	191.8	195.7	199.7	203.7	207.7
39	135.3	138.5	141.7	145.0	148.3	151.7	155.1	158.6	162.1	165.7	169.4	173.1	176.9	180.7	184.7	188.7	192.7	196.8	201.0	205.3	209.6	214.0	218.5	223.0
40	143.7	147.1	150.6	154.2	157.8	161.5	165.3	169.1	173.0	177.0	181.1	185.2	189.4	193.7	198.1	202.5	207.1	211.7	216.4	221.1	226.0	231.0	236.0	241.0
41	153.2	157.0	160.9	164.8	168.9	173.0	177.2	181.5	185.8	190.3	194.8	199.5	204.2	209.1	214.0	219.1	224.2	229.4	234.8	240.2	245.8	251.5	257.2	263.1
42	164.3	168.6	172.9	177.3	181.9	186.5	191.3	196.1	201.1	206.2	211.4	216.7	222.2	227.7	233.4	239.2	245.2	251.3	257.5	263.8	270.3	276.9	283.6	290.5
43	177.5	182.3	187.3	192.4	197.6	202.9	208.4	214.0	219.8	225.8	231.8	238.1	244.5	251.0	257.7	264.6	271.7	278.9	286.3	293.8	301.5	309.4	317.4	325.7
44	193.6	199.3	205.1	211.0	217.2	223.5	230.0	236.7	243.6	250.8	258.1	265.6	273.3	281.2	289.4	297.8	306.3	315.1	324.1	333.3	342.8	352.4	362.3	372.4
45	214.1	220.9	227.9	235.2	242.7	250.4	258.4	266.7	275.3	284.1	293.3	302.6	312.3	322.3	332.5	343.0	353.8	364.9	376.2	387.9	399.8	412.0	424.5	437.4
46	241.5	250.0	258.9	268.2	277.8	287.8	298.1	308.8	319.9	331.4	343.3	355.5	368.1	381.1	394.5	408.3	422.5	437.1	452.0	467.4	483.3	499.6	516.3	533.5
47	280.9	292.4	304.4	316.9	330.0	343.6	357.8	372.5	387.7	403.4	419.8	436.6	454.1	472.1	490.7	509.9	529.8	550.4	571.7	593.8	616.7	640.5	665.3	691.0
48	344.1	360.9	378.4	396.8	416.0	436.0	456.9	478.6	501.2	524.7	549.3	574.8	601.5	629.4	658.6	68								