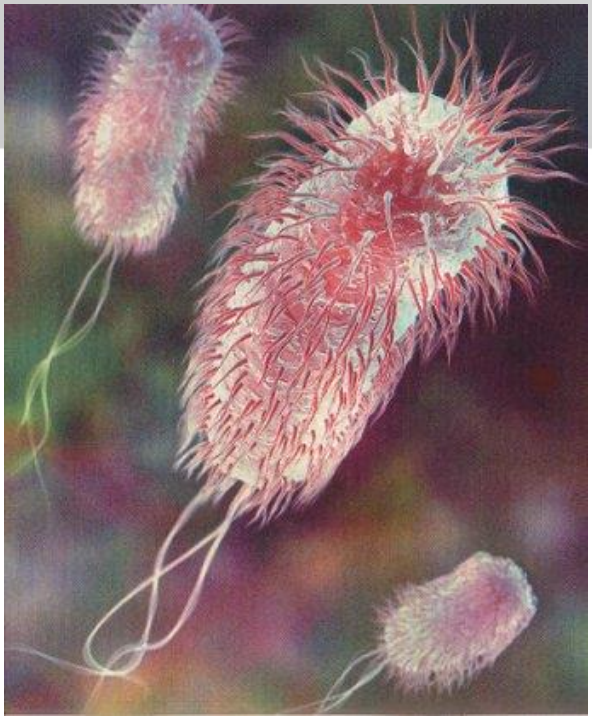
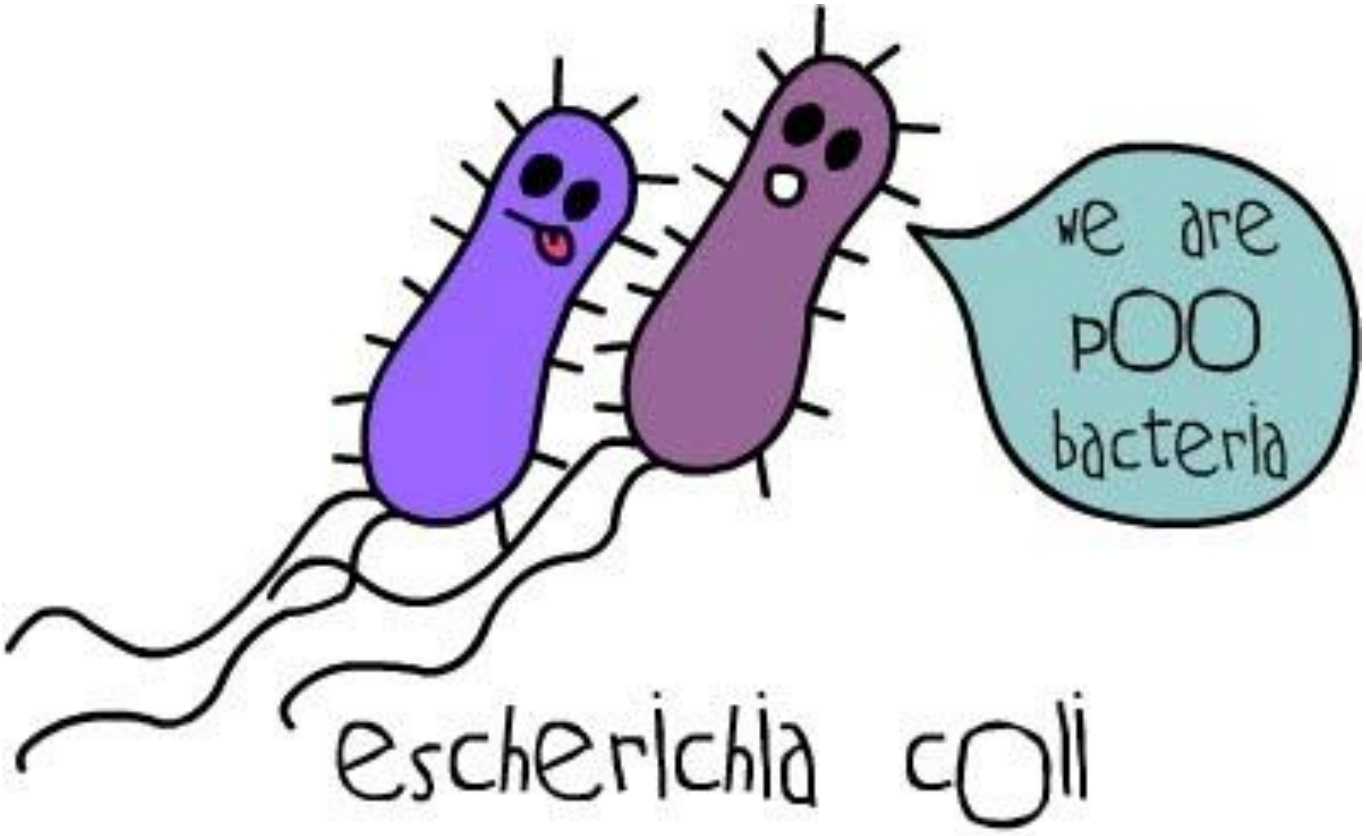


Fecal coliform and *E. coli* Analysis in wastewater by Quanti-Tray, Method 9223 B

Amy Staley

Alloway

E. coli happens



What is Total Coliform bacteria?

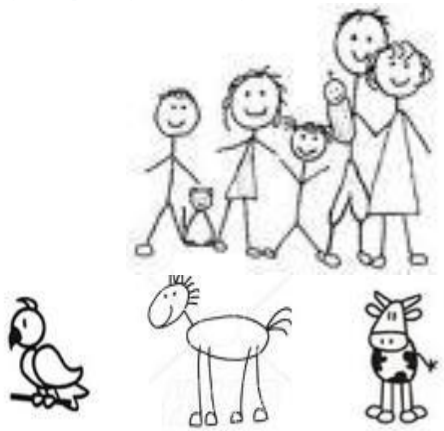
Total coliform bacteria characteristics:

- Aerobic or facultative anaerobes
- Gram negative; bacilli (rod shaped)
- Non-spore forming
- When incubated at 35 +/- 0.5 ◦C, can ferment lactose and produce gas within 48 hours.
- Can live in soil (predominantly environmental bacteria therefore not true indicators of fecal contamination)

E. coli Happens

Fecal Coliform Group

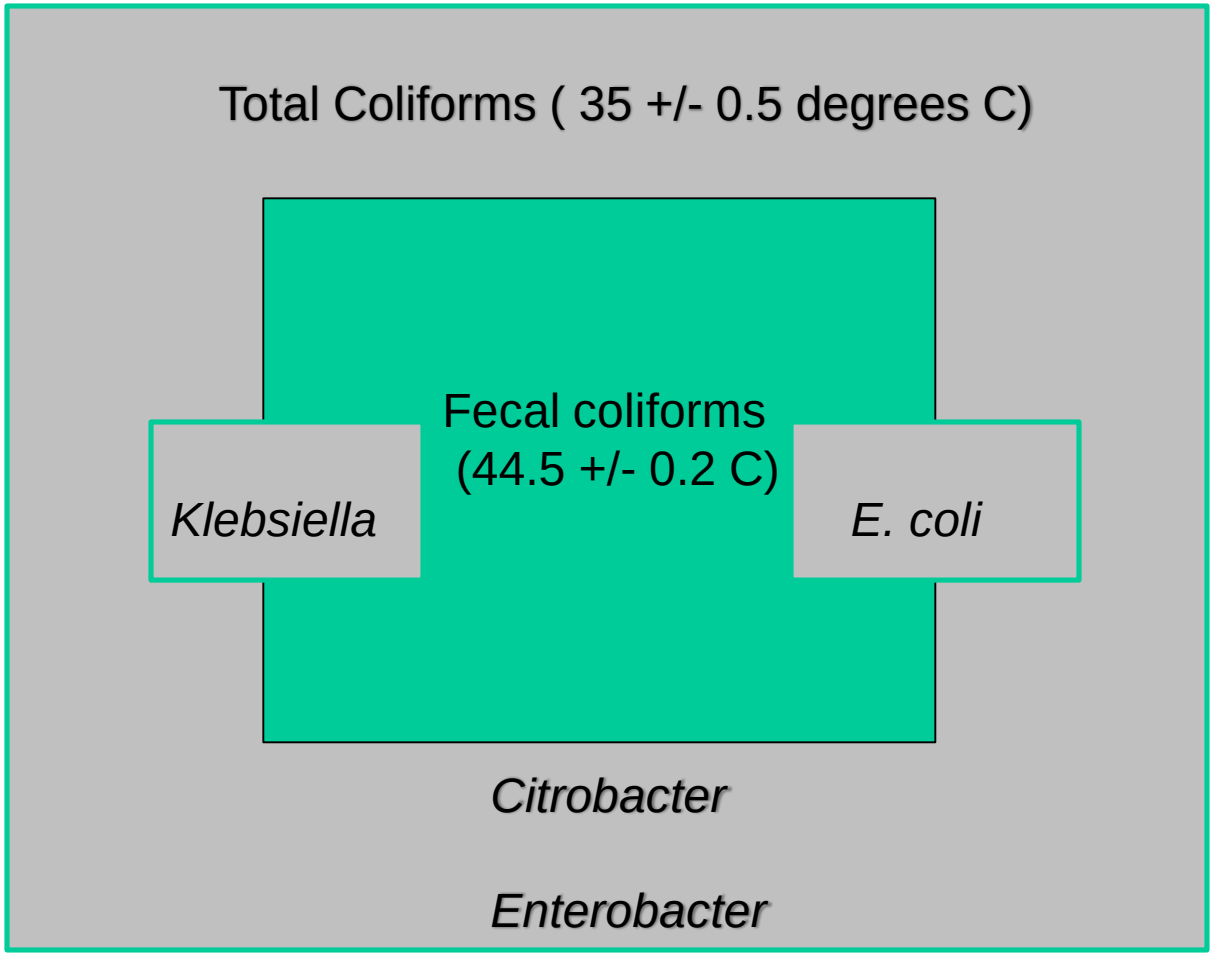
- group of **total coliform bacteria** found in intestinal tracts of warm-blooded animals.
- **Thermotolerant**: ideal temp 44.5 +/- 0.2° C



E. coli

- a species of bacteria within the fecal coliform group
- dominant bacteria found in waste of humans and warm-blooded animals.
- produce a positive total coliform response
- possess an enzyme called (ß-glucoronidase) which releases fluorogen that is detected using a 365 nm UV lamp.
- Ideal temp 35 +/- 0.5 °C

Temperatures for growth



Purposes of Monitoring for Pathogens and Indicators

- Microbial pathogens are involved in human health issues. Therefore, monitoring is conducted for special purposes:
  - Drinking water safety
  - Disease outbreak investigation
  - Recreation management (ex. Beach closure)

E. coli as an indicator organism

- Why test for *E. coli* and not just fecal coliforms?
  - As NPDES permits have been renewed over the past few years, *E. coli* has been added. Fecal coliform requirements are being phased out and *E. coli* limits and monitoring requirements have been put in place.
  - *E. coli* has been shown to be a better predictor of the potential for impacts to human health from exposure to waste effluent and surface waters which contain wastewater effluent.

E. coli as an indicator organism

- Determines the sanitary quality of water
  * Polluted waters= high levels of total coliforms

- Impossible to test for ALL pathogenic microorganisms, so test for easily detectable indicator organisms.

E. coli as an indicator organism

- Hundreds of *E. coli* strains
  * Most are non pathogenic (some beneficial)
  * Some pathogenic strains

- Although generally not pathogenic, their presence indicates a *pathway* for human pathogens (ex. Viruses, bacteria, protozoa) to enter the water source.

E. coli as an indicator organism

- Ideal indicator organism for testing water for fecal contamination
  - Ability to survive for extended period of time outside of the body (especially in water)
  - Other fecal coliforms can arise from environmental factors (not always a result of waste contamination) ex. *Klebsiella pneumoniae* in pa

E. coli as an indicator organism

- Not all *E. coli* comes from humans
  - Different strains from different species (ex. Humans, birds, cows, etc…)
  - Most harmful pathogenic strain, shiga-toxin producing ***E. coli* O157:H7**, found in cow intestines
    - ex. Food poisoning
    - Can NOT be detected using standard fecal coliform methods.

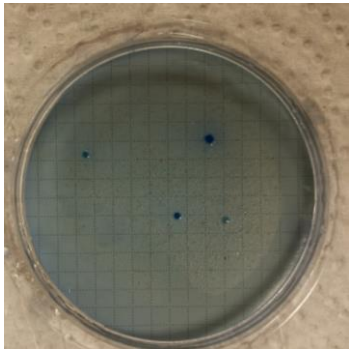
  - Differentiation may be necessary to pinpoint source of contamination
    - Performed by specialized labs.

Escherichia coli (*E. coli*)

- Recreational Water Quality : *E. coli* is a more accurate indicator of waste contamination than the fecal coliform group.
  - A positive relationship exists between *E. coli* density in recreational waters and numbers of observed gastrointestinal illnesses.
  - Lack of a positive relationship between fecal coliform group and gastrointestinal illness.
  - However, the absence of *E. coli* in water doesn't mean no pathogens present.

Fecal coliform testing methods

- EPA approved methods of testing for fecal coliform bacteria in wastewater include:



  * Membrane filter (MF) (CFU/100mL)
    - – Standard methods 9222

  - Multiple tube/ multi-well procedures (MPN/100mL)
    - Standard Methods 9221 C,E
    - Standard methods 9223 B (enzyme substrate)

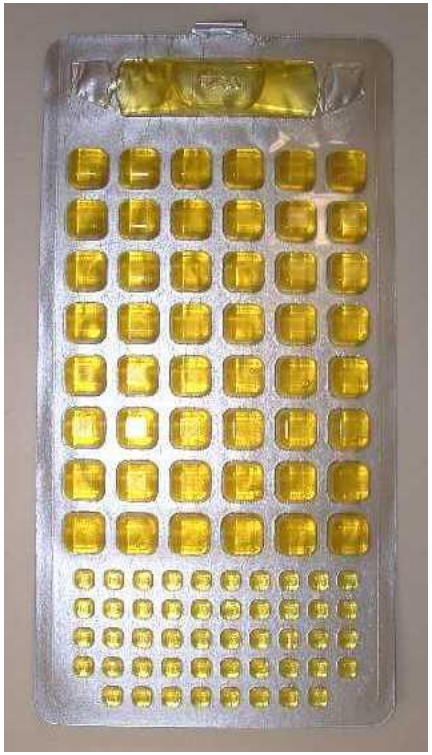
      Quanti-Tray/2000 using Colilert- 18 only

E. coli testing methods

- EPA approved methods for testing for *E. coli* in wastewater include:
  - Membrane filter (MF)
    - EPA Method 1603 (m-TEC media)
    - HACH Method 10029 (mColiblue 24 media)

E. coli Methods cont…

- Multiple tube/ multi-well procedures
  - Standard Methods 9223 B (Enzyme Substrate)
    - Quanti-Tray and Quanti-Tray/2000



Enzyme Substrate Methods

- Enzyme based methodologies detect both total coliforms, fecal coliforms, and *E. coli* simultaneously.
- Easy, rapid, accurate
- Widely accepted as standard for microbiological analysis of water and wastewater
- Enzymes for Quanti-Tray method: Colilert, Colisure, Colilert-18

Enzyme Substrate Methods

Colilert/ Colisure

- Enhancements for enzyme expression
  - * Traditional media provides a nutrient rich environment
  - * supports the growth of **both** target and non target organisms. (when non targets grow and mimic target organisms **false positives** occur)
  - * Growth of non targets can also **suppress** target organism and give false negative in traditional media
  - * To suppress non target organisms, traditional media often include high levels of salts, detergents and other selective agents which may inadvertently suppress target organisms and give **false negatives**.

Enzyme Substrate Methods

Colilert/ Colisure

- Ability to detect either presence/absence or to enumerate organisms.
  - Detects a single, viable organism per sample

- Suppression of non-coliforms
  - Suppresses up to 2 million heterotrophs per 100 ml during the specified incubation time **only**.

Enzyme Substrate Methods

Benefits of Quanti-Tray

- Detects down to one organism per 100 mL
- No dilutions (for counts to 200/100mL or 2,419/100 mL)
- Results in 18-28 hours
- No confirmation necessary
- If no dilutions are used: No glassware to purchase and clean

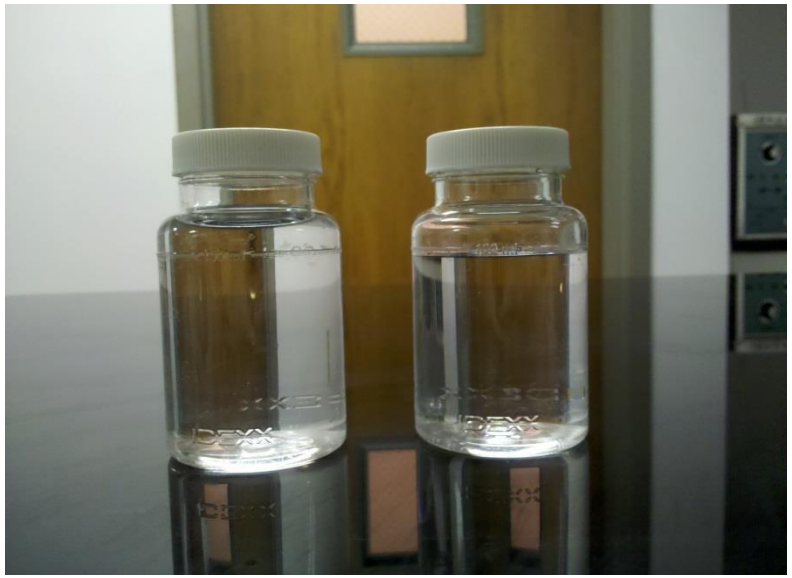
Procedure

- Turn on Quanti-Tray Sealer
  -Warm up time approx. 10 mins.

- Vigorously shake water sample bottle.
  -Interval between shaking and measuring the test portion should not exceed 3 minutes.

Procedure

Aseptically remove lid and adjust sample volume to the calibrated 100 ml line on sample container: (this is for use of 100 mls of sample)



Procedure

Need Dilutions?

Dilutions may also be used in which case you do NOT need to pour off excess water.

Test requires the use of 100 ml of sample:

- Ex. 1:10 dilution; use 10 ml sample: 90 ml blank water
- Final results must be multiplied by the applicable dilution factor.

Procedure

- Aseptically add 1 packet of Colilert reagent to the 100 ml test bottle

  **If sample “flashes” blue: excessive chlorine and invalid for analysis

- Re-cap the bottle and shake until reagent is mostly dissolved.
- Label back of tray with sample ID and dilution used

Procedure

- Use one hand to hold open the Quanti-Tray or Quanti-Tray/2000
  - Well side is facing the palm of the hand.
- Squeeze upper part of tray so it bends toward the palm.
- Gently pull foil tab to open the tray.
  - Avoid touching inside of tray or foil tab.
- Pour 100 ml sample into the tray.

Procedure

- Tap small wells 2-3 times to release air bubbles.
- Place tray with sample into rubber insert so that wells sit within the cutouts

- Place rubber insert on the input shelf of sealer.
- Slide rubber insert with tray into the sealer

Procedure

- For fecal coliform testing: Once sealed, incubate the tray/trays for 18 hrs – 22 hours (Colilert 18 only) in a water bath at 44.5 +/- 0.2°C

Procedure

- Using appropriate weighted rings, make sure the trays are weighted down so they are fully submerged under the water. (vinyl-coated lead ring Cat No. 1216K72 through Thomas Scientific shown in picture)

Procedure

- For E. coli testing: Once sealed, incubate the tray/trays for 24-28 hours (Colilert, Colisure) in a dry incubator at 35 +/- 0.5°C

- After the allotted time, if fluorescence is questionable for *E. coli*, incubate for an additional 4 hrs. Intensity of fluorescence indicates a positive result.

Counting and Calculations

- Quanti-Tray (51 wells) and Quanti-Tray/2000 (97 wells)

Counting Ranges:

Quanti-Tray: max. of 200 MPN/ 100 mls sample

Quanti-Tray 2000: max. of 2,419 MPN/ 100 mls sample

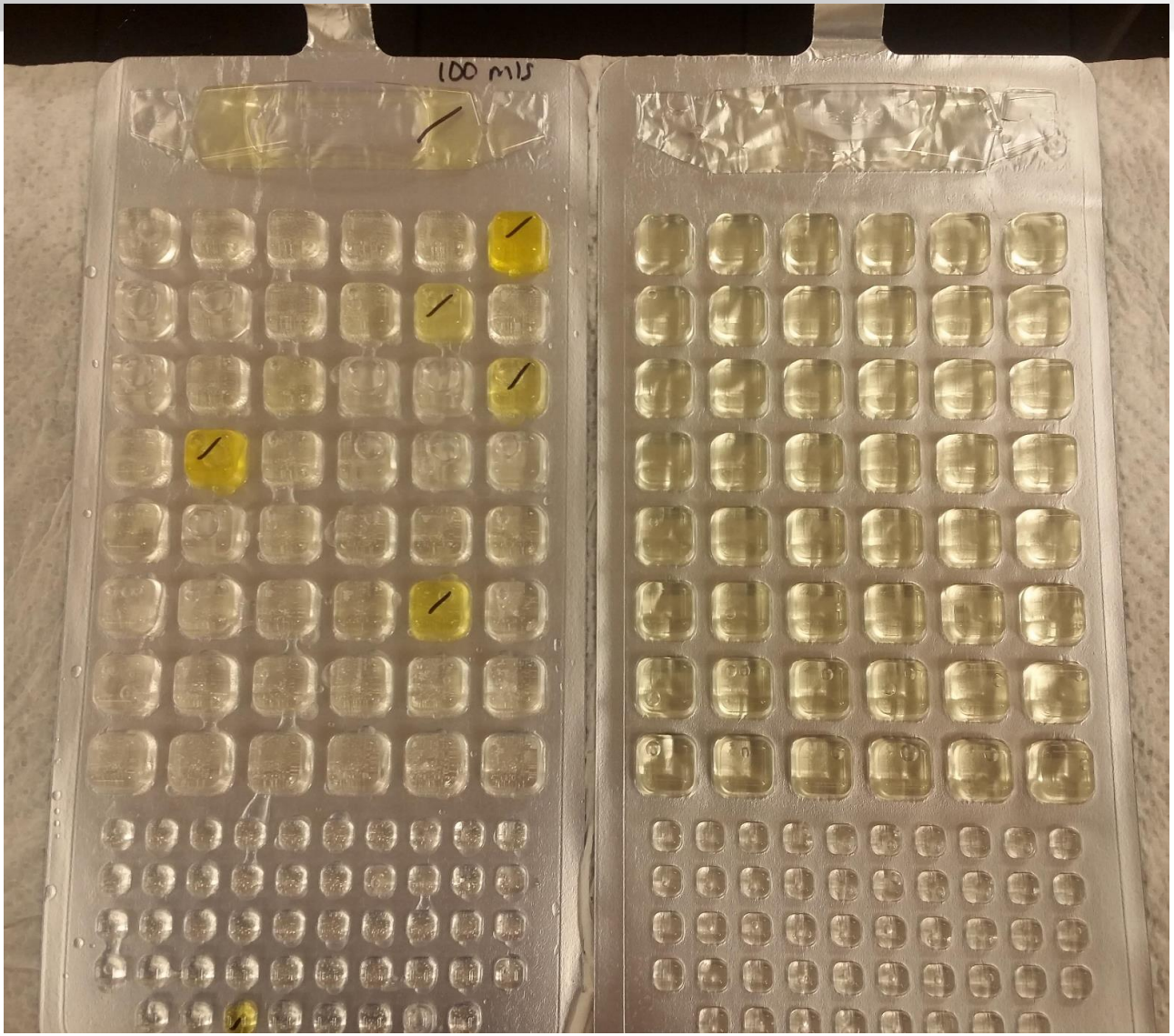
- Count both small and large yellow wells.

*** <u>Use color comparator</u>** to confirm positive result.

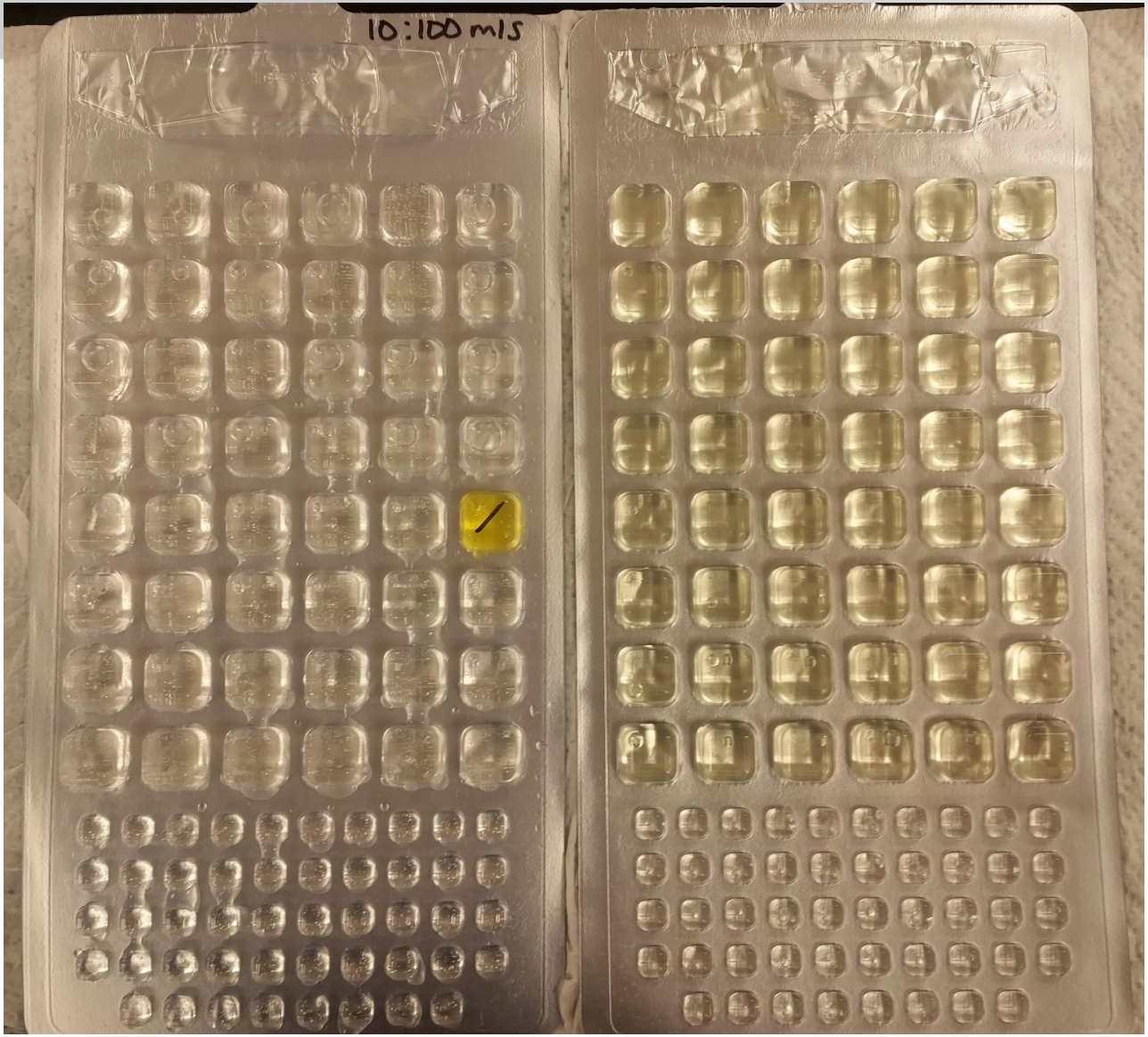
* Document these as total coliform positive or fecal coliform positive depending on you incubation temp and reagent used.

Blank vs. comparator

100 ml sample vs. comparator



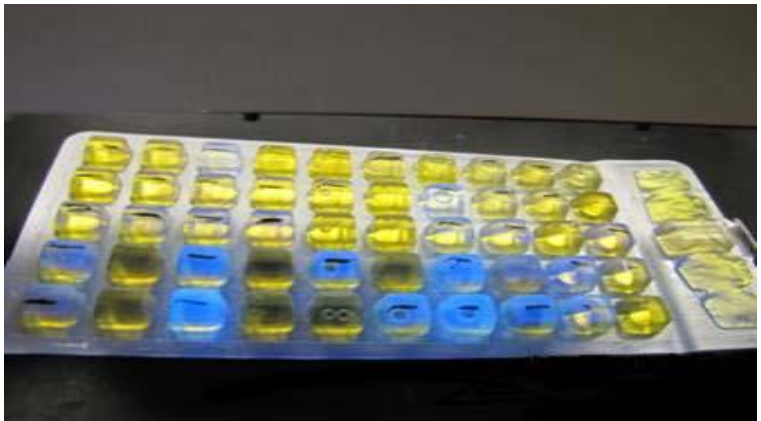
10:100 dilution vs. comparator



Counting and Calculations

For *E. coli* analysis:

- Use the UV lamp to check for fluorescence.
  - If no wells fluoresce, negative for E. coli
  - If wells do fluoresce, positive for E. coli
    - Count small and large fluorescing wells
    - Refer to table for MPN
- ** Wells must be both yellow and fluoresce for E. coli +



Counting and Calculations

IDEXX Quanti-Tray*/2000 MPN Table

# Large Wells Positive	# Small Wells Positive																							
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
0	<1	1.0	2.0	3.0	4.0	5.0	6.0	7.0	8.0	9.0	10.0	11.0	12.0	13.0	14.1	15.1	16.1	17.1	18.1	19.1	20.2	21.2	22.2	23.3	24.3
1	1.0	2.0	3.0	4.0	5.0	6.0	7.1	8.1	9.1	10.1	11.1	12.1	13.2	14.2	15.2	16.2	17.3	18.3	19.3	20.4	21.4	22.4	23.5	24.5	25.6
2	2.0	3.0	4.1	5.1	6.1	7.1	8.1	9.2	10.2	11.2	12.2	13.3	14.3	15.4	16.4	17.4	18.5	19.5	20.6	21.6	22.7	23.7	24.8	25.8	26.9
3	3.1	4.1	5.1	6.1	7.2	8.2	9.2	10.3	11.3	12.4	13.4	14.5	15.5	16.5	17.6	18.6	19.7	20.8	21.8	22.9	23.9	25.0	26.1	27.1	28.2
4	4.1	5.2	6.2	7.2	8.3	9.3	10.4	11.4	12.5	13.5	14.6	15.6	16.7	17.8	18.8	19.9	21.0	22.0	23.1	24.2	25.3	26.3	27.4	28.5	29.6
5	5.2	6.3	7.3	8.4	9.4	10.5	11.5	12.6	13.7	14.7	15.8	16.9	17.9	19.0	20.1	21.2	22.2	23.3	24.4	25.5	26.6	27.7	28.8	29.9	31.0
6	6.3	7.4	8.4	9.5	10.6	11.6	12.7	13.8	14.9	16.0	17.0	18.1	19.2	20.3	21.4	22.5	23.6	24.7	25.8	26.9	28.0	29.1	30.2	31.3	32.4
7	7.5	8.5	9.6	10.7	11.8	12.8	13.9	15.0	16.1	17.2	18.3	19.4	20.5	21.6	22.7	23.8	24.9	26.0	27.1	28.3	29.4	30.5	31.6	32.8	33.9
8	8.6	9.7	10.8	11.9	13.0	14.1	15.2	16.3	17.4	18.5	19.6	20.7	21.8	22.9	24.1	25.2	26.3	27.4	28.6	29.7	30.8	32.0	33.1	34.3	35.4
9	9.8	10.9	12.0	13.1	14.2	15.3	16.4	17.6	18.7	19.8	20.9	22.0	23.2	24.3	25.4	26.6	27.7	28.9	30.0	31.2	32.3	33.5	34.6	35.8	37.0
10	11.0	12.1	13.2	14.4	15.5	16.6	17.7	18.9	20.0	21.1	22.3	23.4	24.6	25.7	26.9	28.0	29.2	30.3	31.5	32.7	33.8	35.0	36.2	37.4	38.6
11	12.2	13.4	14.5	15.6	16.8	17.9	19.1	20.2	21.4	22.5	23.7	24.8	26.0	27.2	28.3	29.5	30.7	31.9	33.0	34.2	35.4	36.6	37.8	39.0	40.2
12	13.5	14.6	15.8	16.9	18.1	19.3	20.4	21.6	22.8	23.9	25.1	26.3	27.5	28.6	29.8	31.0	32.2	33.4	34.6	35.8	37.0	38.2	39.5	40.7	41.9
13	14.8	16.0	17.1	18.3	19.5	20.6	21.8	23.0	24.2	25.4	26.6	27.8	29.0	30.2	31.4	32.6	33.8	35.0	36.2	37.5	38.7	39.9	41.2	42.4	43.6
14	16.1	17.3	18.5	19.7	20.9	22.1	23.3	24.5	25.7	26.9	28.1	29.3	30.5	31.7	33.0	34.2	35.4	36.7	37.9	39.1	40.4	41.6	42.9	44.2	45.4
15	17.5	18.7	19.9	21.1	22.3	23.5	24.7	25.9	27.2	28.4	29.6	30.9	32.1	33.3	34.6	35.8	37.1	38.4	39.6	40.9	42.2	43.4	44.7	46.0	47.3
16	18.9	20.1	21.3	22.6	23.8	25.0	26.2	27.5	28.7	30.0	31.2	32.5	33.7	35.0	36.3	37.5	38.8	40.1	41.4	42.7	44.0	45.3	46.6	47.9	49.2
17	20.3	21.6	22.8	24.1	25.3	26.6	27.8	29.1	30.3	31.6	32.9	34.1	35.4	36.7	38.0	39.3	40.6	41.9	43.2	44.5	45.9	47.2	48.5	49.8	51.2
18	21.8	23.1	24.3	25.6	26.9	28.1	29.4	30.7	32.0	33.3	34.6	35.9	37.2	38.5	39.8	41.1	42.4	43.8	45.1	46.5	47.8	49.2	50.5	51.9	53.2
19	23.3	24.6	25.9	27.2	28.5	29.8	31.1	32.4	33.7	35.0	36.3	37.6	39.0	40.3	41.6	43.0	44.3	45.7	47.1	48.4	49.8	51.2	52.6	54.0	55.4
20	24.9	26.2	27.5	28.8	30.1	31.5	32.8	34.1	35.4	36.8	38.1	39.5	40.8	42.2	43.6	44.9	46.3	47.7	49.1	50.5	51.9	53.3	54.7	56.1	57.6
21	26.5	27.9	29.2	30.5	31.8	33.2	34.5	35.9	37.3	38.6	40.0	41.4	42.8	44.1	45.5	46.9	48.4	49.8	51.2	52.6	54.1	55.5	56.9	58.4	59.9
22	28.2	29.5	30.9	32.3	33.6	35.0	36.4	37.7	39.1	40.5	41.9	43.3	44.8	46.2	47.6	49.0	50.5	51.9	53.4	54.8	56.3	57.8	59.3	60.8	62.3
23	29.9	31.3	32.7	34.1	35.5	36.8	38.3	39.7	41.1	42.5	43.9	45.4	46.8	48.3	49.7	51.2	52.7	54.2	55.6	57.1	58.6	60.2	61.7	63.2	64.7
24	31.7	33.1	34.5	35.9	37.3	38.8	40.2	41.7	43.1	44.6	46.0	47.5	49.0	50.5	52.0	53.5	55.0	56.5	58.0	59.5	61.1	62.6	64.2	65.8	67.3
25	33.6	35.0	36.4	37.9	39.3	40.8	42.2	43.7	45.2	46.7	48.2	49.7	51.2	52.7	54.3	55.8	57.3	58.9	60.5	62.0	63.6	65.2	66.8	68.4	70.0
26	35.5	36.9	38.4	39.9	41.4	42.8	44.3	45.9	47.4	48.9	50.4	52.0	53.5	55.1	56.7	58.2	59.8	61.4	63.0	64.7	66.3	67.9	69.6	71.2	72.9
27	37.4	38.9	40.4	42.0	43.5	45.0	46.5	48.1	49.6	51.2	52.8	54.4	56.0	57.6	59.2	60.8	62.4	64.1	65.7	67.4	69.1	70.8	72.5	74.2	75.9
28	39.5	41.0	42.6	44.1	45.7	47.3	48.8	50.4	52.0	53.6	55.2	56.9	58.5	60.2	61.8	63.5	65.2	66.9	68.6	70.3	72.0	73.7	75.5	77.3	79.0
29	41.7	43.2	44.8	46.4	48.0	49.6	51.2	52.8	54.5	56.1	57.8	59.5	61.2	62.9	64.6	66.3	68.0	69.8	71.5	73.3	75.1	76.9	78.7	80.5	82.4
30	43.9	45.5	47.1	48.7	50.4	52.0	53.7	55.4	57.1	58.8	60.5	62.2	64.0	65.7	67.5	69.3	71.0	72.9	74.7	76.5	78.3	80.2	82.1	84.0	85.9
31	46.2	47.9	49.5	51.2	52.9	54.6	56.3	58.1	59.8	61.6	63.3	65.1	66.9	68.7	70.5	72.4	74.2	76.1	78.0	79.9	81.8	83.7	85.7	87.6	89.6
32	48.7	50.4	52.1	53.8	55.6	57.3	59.1	60.9	62.7	64.5	66.3	68.2	70.0	71.9	73.8	75.7	77.6	79.5	81.5	83.5	85.4	87.5	89.5	91.5	93.6
33	51.2	53.0	54.8	56.5	58.3	60.2	62.0	63.8	65.7	67.6	69.5	71.4	73.3	75.2	77.2	79.2	81.2	83.2	85.2	87.3	89.3	91.4	93.6	95.7	97.8
34	53.9	55.7	57.6	59.4	61.3	63.1	65.0	67.0	68.9	70.8	72.8	74.8	76.8	78.8	80.8	82.9	85.0	87.1	89.2	91.4	93.5	95.7	97.9	100.2	102.4
35	56.8	58.6	60.5	62.4	64.4	66.3	68.3	70.3	72.3	74.3	76.3	78.4	80.5	82.6	84.7	86.9	89.1	91.3	93.5	95.7	98.0	100.3	102.6	105.0	107.3
36	59.8	61.7	63.7	65.7	67.7	69.7	71.7	73.8	75.9	78.0	80.1	82.3	84.5	86.7	88.9	91.2	93.5	95.8	98.1	100.5	102.9	105.3	107.7	110.2	112.7
37	62.9	65.0	67.0	69.1	71.2	73.3	75.4	77.6	79.8	82.0	84.2	86.5	88.8	91.1	93.4	95.8	98.2	100.6	103.1	105.6	108.1	110.7	113.3	115.9	118.6
38	66.3	68.4	70.6	72.7	74.9	77.1	79.4	81.6	83.9	86.2	88.6	91.0	93.4	95.8	98.3	100.8	103.4	105.9	108.6	111.2	113.9	116.6	119.4	122.2	125.0
39	70.0	72.2	74.4	76.7	78.9	81.3	83.6	86.0	88.4	90.9	93.4	95.9	98.4	101.0	103.6	106.3	109.0	111.8	114.6	117.4	120.3	123.2	126.1	129.2	132.2
40	73.8	76.2	78.5	80.9	83.3	85.7	88.2	90.8	93.3	95.9	98.5	101.2	103.9	106.7	109.5	112.4	115.3	118.2	121.2	124.3	127.4	130.5	133.7	137.0	140.3
41	78.0	80.5	83.0	85.5	88.0	90.6	93.3	95.9	98.7	101.4	104.3	107.1	110.0	113.0	116.0	119.1	122.2	125.4	128.7	132.0	135.4	138.8	142.3	145.9	149.5
42	82.6	85.2	87.8	90.5	93.2	96.0	98.8	101.7	104.6	107.6	110.6	113.7	116.9	120.1	123.4	126.7	130.1	133.6	137.2	140.8	144.5	148.3	152.2	156.1	160.2
43	87.6	90.4	93.2	96.0	99.0	101.9	105.0	108.1	111.2	114.5	117.8	121.1	124.6	128.1	131.7	135.4	139.1	143.0	147.0	151.0	155.2	159.4	163.8	168.2	172.8
44	93.1	96.1	99.1	102.2	105.4	108.6	111.9	115.3	118.7	122.3	125.9	129.6	133.4	137.4	141.4	145.5	149.7	154.1	158.5	163.1	167.9	172.7	177.7	182.9	188.2
45	99.3	102.5	105.8	109.2	112.6	116.2	119.8	123.6	127.4	131.4	135.4	139.6	143.9	148.3	152.9	157.6	162.4	167.4	172.6	178.0	183.5	189.2	195.1	201.2	207.5
46	106.3	109.8	113.4	117.2	121.0	125.0	129.1	133.3	137.6	142.1	146.7	151.5	156.5	161.6	167.0	172.5	178.2	184.2	190.4	196.8	203.5	210.5	217.8	225.4	233.3
47	114.3	118.3	122.4	126.6	130.9	135.4	140.1	145.0	150.0	155.3	160.7	166.4	172.3	178.5	185.0	191.8	198.9	206.4	214.2	222.4	231.0	240.0	249.5	259.5	270.0
48	123.9	128.4	133.1	137.9	143.0	148.3	153.9	159.7	165.8	172.2	178.9	186.0	193.5	201.4	209.8	218.7	228.2	238.2	248.9	260.3	272.3	285.1	298.7	313.0	328.2
49	135.5	140.8	146.4	152.3	158.5	165.0	172.0	179.3	187.2	195.6	204.6	214.3	224.7	235.9	248.1	261.3	275.5	290.9	307.6	325.5	344.8	365.4	387.3	410.6	435.2

Counting and Calculations

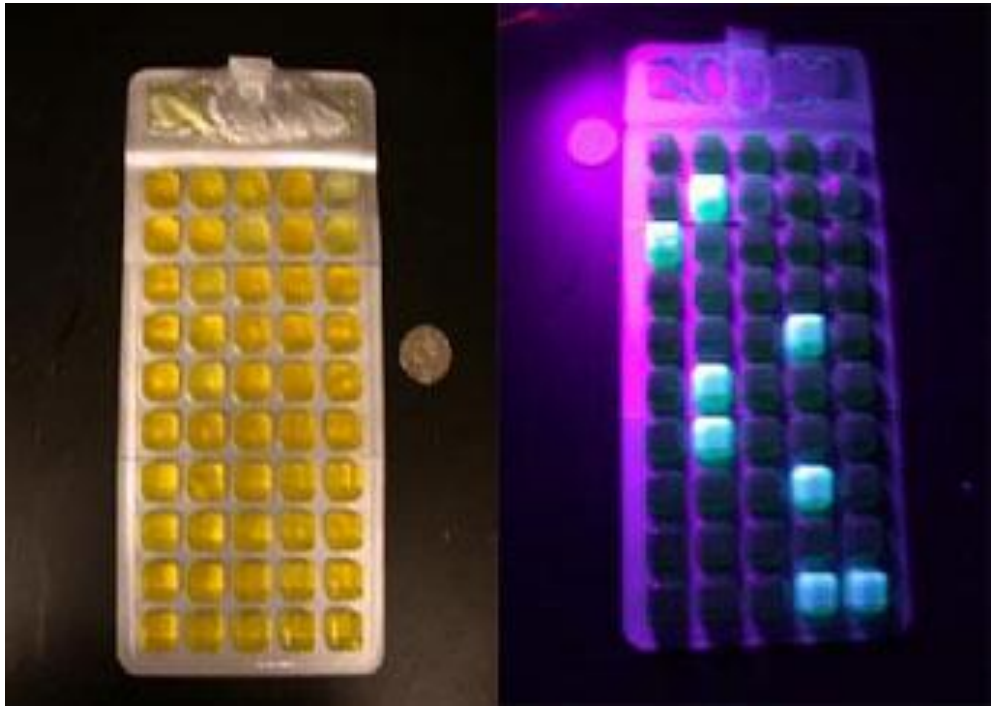
IDEXX
51-Well Quanti-Tray®
MPN Table

No. of wells giving positive reaction	MPN per 100 ml sample	95% Confidence Limits	
		Lower	Upper
0	<1.0	0.0	3.7
1	1.0	0.3	5.6
2	2.0	0.6	7.3
3	3.1	1.1	9.0
4	4.2	1.7	10.7
5	5.3	2.3	12.3
6	6.4	3.0	13.9
7	7.5	3.7	15.5
8	8.7	4.5	17.1
9	9.9	5.3	18.8
10	11.1	6.1	20.5
11	12.4	7.0	22.1
12	13.7	7.9	23.9
13	15.0	8.8	25.7
14	16.4	9.8	27.5
15	17.8	10.8	29.4
16	19.2	11.9	31.3
17	20.7	13.0	33.3
18	22.2	14.1	35.2
19	23.8	15.3	37.3
20	25.4	16.5	39.4
21	27.1	17.7	41.6
22	28.8	19.0	43.9
23	30.6	20.4	46.3
24	32.4	21.8	48.7
25	34.4	23.3	51.2
26	36.4	24.7	53.9
27	38.4	26.4	56.6
28	40.6	28.0	59.5
29	42.9	29.7	62.5
30	45.3	31.5	65.6
31	47.8	33.4	69.0
32	50.4	35.4	72.5
33	53.1	37.5	76.2
34	56.0	39.7	80.1
35	59.1	42.0	84.4
36	62.4	44.6	88.8
37	65.9	47.2	93.7
38	69.7	50.0	99.0
39	73.8	53.1	104.8
40	78.2	56.4	111.2
41	83.1	59.9	118.3
42	88.5	63.9	126.2
43	94.5	68.2	135.4
44	101.3	73.1	146.0
45	109.1	78.6	158.7
46	118.4	85.0	174.5
47	129.8	92.7	195.0
48	144.5	102.3	224.1
49	165.2	115.2	272.2
50	200.5	135.8	387.6
51	> 200.5	146.1	infinite

IDEXX Sales and Technical Support
1-800-321-0207 or 1-207-856-0496
www.idexx.com/water

Reagents and Standards

- Colilert or Colilert 18
  - Snap packs for sample size 100 ML
  - Sample turns yellow when total coliform bacteria and fecal coliform bacteria are present and fluoresces blue to indicate the presence of *E. coli*



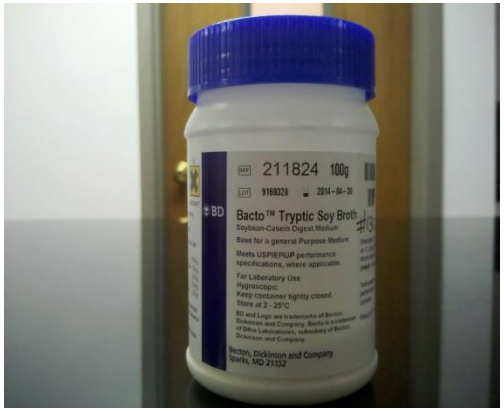
Reagents and Standards

- Distilled Water/RODI Water
  - Do **NOT** use phosphate buffered rinse water with this method
- pH Buffers
  - For calibration of pH meter used for checking newly prepared TSB media
- DPD reagent packets
  - For determination of residual chlorine (QC for new sterile water)
- Conductivity Standard
  - For Calibration of conductivity meter (QC for new sterile water)

Reagents and Standards

- Bacterial Cultures
  - QC for new reagent packs
  - Ex: Microbiologic Kwik Stiks

- TSB media (tryptic soy broth)
  - QC for bottles
    - Can be purchased premade or as a dry media



TSB dry media

Reagents and Standards

- Autoclave Biological Indicator Checks
  - QC for Autoclave
- Clorox Bleach
  - Disinfection of counter and spills
- Colilert comparator
  - Pre-dispensed in either types of Quanti-Tray
  - Used for determination of positive result

BT Sure biological indicator

Equipment Needed for Method 9223 B

Autoclave

- Sterilize TSB media for 15 minutes at 119º - 121

- Sterilize blank water

  < 500 ml = 30 mins

  > 500 ml = 45 mins

Equipment

Autoclave Supplies

- Autoclave tape
- Autoclave bags : run waste cycle 45 mins at proper temp.
- Autoclave biological indicator (monthly QC)
- Log book: record time in/out, temp., cycle time

Autoclave tape

Autoclave bags

BT Sure Biological Indicator

Equipment

Refrigerator

- Storage of reagents at 0º-5º C
  - TSB media
  - Bacterial cultures
    - ex. Kwik Stiks

Refrigerator 0-5ºC

Equipment

Oven

- Sterilize measuring glassware for 2 hrs. at 180º C
  - pipettes
  - graduated cylinders

- Supplies:
  - aluminum foil

Equipment

Incubator

- Incubate Quanti-Trays for *E. coli* analysis at 35ºC +/- 0.5º C for indicated amount of time

Incubator 35º C +/- 0.5º

Equipment

Testing Supplies

- Quanti-Tray sealer and rubber inserts

- Quanti-Tray (51 wells)
  range: 1-200 MPN/100 mls
  OR
- Quanti-Tray 2000 (97 wells)
  range: 1-2419 MPN/100 mls

Quanti-Tray Sealer and rubber inserts

Quanti-Tray / Quanti-Tray 2000

Equipment

Testing Supplies (cont.)

- Pre-sterilized clear sample bottles with dechlorination chemicals
- Squeeze bottles for blank water
  - used for dilutions
- Enzymes
  - ex. Colilert
- Long wave UV lamp

Sterile sample bottle


Squeeze bottles


Long wave UV lamp

365-366 nm

Equipment

Measuring Items

- Sterile graduated cylinders
- Sterile pipettes
- Balance for weighing dry media
  - if preparing TSB

Sterile graduated cylinders

Sterile pipettes

Balance

Equipment

Misc. Items

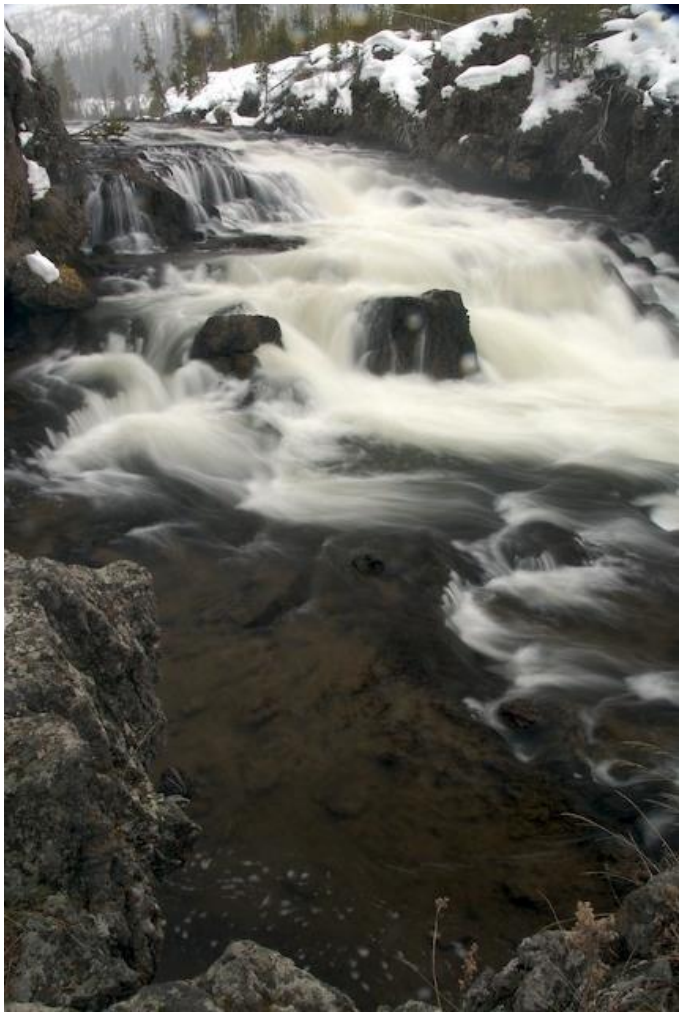
- Pipette washer
- Conductivity meter
  -QC of blank water
- pH meter
  -for checking pH of TSB

Pipette washer

Conductivity meter

pH meter

Sample Collection



- Samples to be representative of the water being tested
  - Use aseptic technique for collection
- Keep sample container closed until collection begins
  - Avoid contact with inside of bottle and/or cap
- Collect directly into sterile container containing de-chlorinate agent
  - Do not rinse the bottle
- Leave air space to allow for mixing

Sample Collection

- If not analyzed immediately:
  - Cool sample to <10°C
- Ideally analysis within 2 hours of collection is preferred
- Sample must be analyzed within 8 hours of collection for wastewater analysis.

Quality Control

Daily QC

Method Blank (ww batch)

- Once per batch (every 10 samples)

Duplicate (ww batch)

- One sample per batch

Incubator Temperature checks

- Twice daily - 4 hours apart

Refrigerator Temperature

- Once per day

Quality Control

Laboratory Micro Temperature Check Form 109-0

Month/Year Twice Daily

Equipment	Analyst	Date	Time	QC Incubator # 1	Fridge	Analyst	Date	Time	QC Incubator # 1	
Range °C				34.5 - 35.5	0-5ºC				34.5 - 35.5	
1	ANS	2/3/11	08:00	34.5	2.0					
2	ANS	2/3/11	13:00	35.0						
3										
4										
5										
6										
7										
8										
9										
10										
11										
12										

Quality Control

Monthly QC

Autoclave Biological Indicator Checks

- Verifies autoclave is sterilizing properly
- BT sure

Reagent Water Analysis (Blank Water)

- Residual Chlorine – Not Detected
- Conductivity - < 2 µmhos/cm

Quality Control

Autoclave Biological Indicator Check
WWTP

Analyst	Reagent Number B/T Indicator	Autoclave Equipment Number	Date Autoclaved	Date/Time In incubator	Date/Time Out incubator	Color Purple, Yellow or cloudy	Pass or Fail

Instructions: (Perform once per month)

1. Run one indicator through an autoclave sterilization cycle
2. Compress the plastic vial
3. Incubate at 57 C for 48 hours
4. Document Color of indicator after 48 hours
5. Document "Pass" if Purple color exists without any cloudiness
6. Document "Fail" if yellow color exists or cloudiness is present
7. Notify the Laboratory Manager immediately if test fails
8. Items autoclaved in cycle that fails this test should not be used.

Quality Control

Bacteriology Distilled Water pH & Chlorine Check

Frequency: Monthly

1. Check deionized water used in bacteriology lab for pH and residual chlorine.
2. DO NOT stir the sample while testing for pH, per EPA letter dated 3-23-92.
3. Results should be documented in the bacteriology log book then transferred to this form.

Determination of pH:

Meter/electrode serial #: _____

Date performed: _____

Analyst: _____

pH Obtained : _____(su)

pH Limits: 5.50 - 7.50 (su)

Buffer control #s: _____

Determination of Residual Chlorine:

Date performed: _____

Analyst: _____

Chlorine: _____(Detected / Not Detected)

Limits: Not Detected

DPD control #: _____

Form 5971-1

Quality Control

Bacteriology Distilled Water Conductivity Check

Frequency: Monthly

1. Rinse the conductivity electrode with RO/DI water.
2. Decant sufficient KCl solution in a beaker to submerge the electrode tip.
3. Read the conductivity of the solution while gently swirling.
4. Adjust the meter to read the known valueby using the up and down arrows.
5. Rinse the electrode with RO/DI water.
6. Decant sufficient fresh RO/DI water (500 mL) in a beaker to submerge the electrode tip.
7. Read the conductivity of the RO/DI water while gently swirling.

Date performed: _____

Analyst: _____

Conductivity: _____@ 25°C

Limits: <2.0 u mhos @ 25°C

Control # for KCl Solution _____

Meter/Electrode Serial # _____

Form 5972-2

Quality Control

Quarterly QC

- Autoclave Timer Calibration
- Autoclave External Thermometer Calibration

Quality Control

Form 127- 0

Autoclave Timer Check
WWTP

Frequency: Quarterly

Autoclave Equipment Number _____

Determine correction setting for autoclave timer as outlined below.

1. Set autoclave timer to operate for 50 minutes.
2. Use a lab clock as a reference and record autoclave timer reading after 15, 30 and 45 minutes. Enter reading in column (C) below.
3. Complete calculations in table below and post instructions to obtain desired exposure on autoclave.
   - Column C = autoclave timer reading
   - Column D = 50 - column C
   - Column E = column D ÷ column A

4. Complete documentation in bacteriology log book.

A	B	C	D	E
Time Interval	Timer Setting	Timer Reading	Elapsed = (50 - C)	Ratio = (D ÷ A)
15	50			
30	50			
45	50			
			Average Ratio:	

Setting is obtained by multiplying the desired exposure time by the average ratio.

Quality Control

Autoclave Thermometer Calibration

Frequency: Quarterly

1. Place the calibrated maximum registering thermometer in the autoclave.
2. Run a 15 minute cycle using slow exhaust and monitor the exterior thermometer for the maximum reading during the cycle. Record maximum external reading below (°F).
3. After the cycle is completed record the maximum internal temperature on the maximum registering thermometer.
4. Add the correction factor for the maximum reading thermometer to obtained corrected maximum temperature. See thermometer calibration for correction factor.
5. Convert the internal thermometer reading from °C to °F.
6. Calculate the correction factor for the external thermometer.

Maximum registering thermometer reading _____(°C)

Correction for Max. registering thermometer _____(°C)

Corrected maximum temperature in autoclave _____(°C)

Corrected maximum temperature in autoclave

°F = (1.8)(°C) + 32 _____(°F)

External thermometer maximum reading _____(°F)

External thermometer correction factor _____(°F)

7. Label the external thermometer on the autoclave with the correction factor.

Form 5963-1

Quality Control

Annual QC

Thermometer Calibrations

- Includes the MRT

Reagent Water Contamination Analysis

- Cd, Cr, Cu, Pb, Ni, Zn

Balance Service Check

- Outside Contractor

Quality Control

Bacteriology RO/DI Water Contamination Check

Frequency: Annually

1. Otain a sample bottle for stock.
2. Fill bottle wit RO/DI water.
3. Submit water to a laboratory for the listed metals.
4. Immediately report any values that exceed limits to the Technical Director.

Contracted Lab_____

Date sent_____

Metal	Limit (mg/L)	Result	Pass / Fail
Cadmium	<0.05		
Copper	<0.05		
Chromium	<0.05		
Nickel	<0.05		
Lead	<0.05		
Zinc	<0.05		
Total	<0.10		

Form 5975-2

Quality Control

Form 132-0

Maximum Registering Thermometer (MRT) Calibration
WWTP

Frequency: Annually
Equipment ID: _____

1 Place the reference NIST calibrated MRT in a 25 ml graduated cylinder containing 10 mL reagent water
2 Place the daily working MRT thermometer in the same 25 ml graduated cylinder containing 10 mL reagent water
3 Run a 15 minute cycle using slow exhaust
4 After the cycle is complete and pressure is @ 0 psi, open the autoclave door. and remove the graduated cylinder containing the MRT's
5 After five minutes record the temperature of each MRT below
6 Calculate the correction factor for the daily working MRT thermometer
7 Label the daily working MRT with the correction factor, date calibrated, and analyst initials.
8 Apply correction factors to every temperature documented

NIST Reference MRT Serial Number:	

Thermometer	Ser. No.	NIST Reading	Test Reading	Correction °C

Completed By: _____ Date: _____

Quality Control

QC per each new lot ***prior to use***

Sample bottle sterility checks: each new lot

- Use TSB media (Tryptic Soy Broth)
  - Test 1% of each box received for growth

Quality Control

- QC per each new lot ***prior to use***
  - TSB media check
    - 1 positive control (using E. coli) ,
    - 1 negative control (no inoculation)

Quality Control

Colilert check

(Each new lot received)

Innoculation with 3 control bacteria:

One control bacteria **must** be *E.coli*

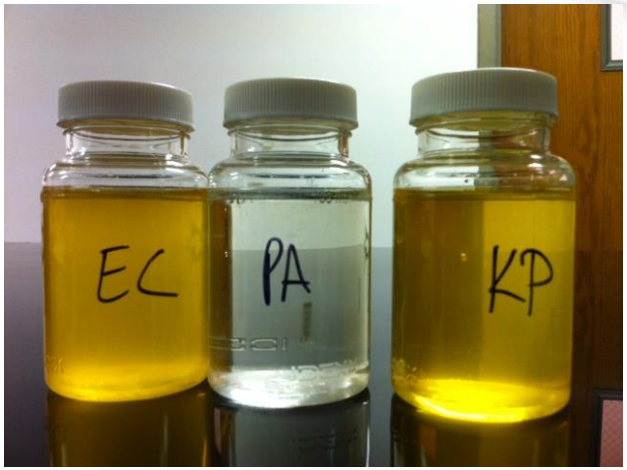
total coliform (+), E. coli (+)

One control bacteria can be *Pseudomonas aeruginosa* (or other non-coliform)

total coliform (-), E. coli (-)

One control bacteria can be *Klebsiella pneumoniae* (or other coliform)

total coliform (+), E.coli (-)



Quality Control

Bottle Sterility Check
WWTP

Analyst	Reagent Number TSB	Reagent Number Sample Bottle	Date In	Time In	Date Out	Time Out	Pass or Fail

Instructions: (Minimum 1 bottle per each new lot)

1. Aseptically transfer 25 ml of single strength TSB to a sample container using a sterile pipette.
2. Incubate the sample bottle at 35 ± 0.5 C for 24 hours and check for growth
3. Growth will be indicated by even the slightest turbidity in the TSB
4. If the sample container is opaque the TSB must be poured into a glass vessel after incubation in order to look for turbidity
5 Document "Pass" if no turbidity is detected.
6 Document "Fail" if turbidity is detected. Notify the laboratory director immediately.

Quality Control

Form 155-0

TSB Media Positive Check
WWTP

Frequency: Each New Lot

Positive Control Procedures

1 Before using each new lot of TSB media it must be checked for positive growth
2 For the positive control check use the E. coli microorganism from Fisher (23-0035004)
3 Take one E. coli pellet and transfer it to a bottle containing 99 ml of sterile phosphate buffer water (that has been slightly warmed). Ensure the pellet is dissolved.
4 Incubate bottle for 30 minutes at 35 C
5 Remove from incubator and shake vigorously
6 Using a sterile loop, transfer one loop of the above solution to a sterile sample bottle containing 25 mls of TSB.
7 Swirl the sterile loop in the TSB media.
8 Transfer the bottle with the TSB to the incubator and incubate for 24 hours at 35 ± 0.5 C
9 Growth will be indicated by even the slightest turbidity in the TSB
10 Document "Pass" if turbidity is detected
11 Document "Fail" if turbidity is not detected
12 The TSB must Pass (show signs of turbidity). If it does not, notify the laboratory supervisor immediately and contact the supplier of the TSB. The TSB must not be used if it fails this check

TSB Media

Lot Number of TSB Media	Reagent Number TSB Media	Date Received	Date Opened	Date/Time In Incubator	Date/Time Out of Incubator	Analyst	*Result* Pass or Fail

Requirements:

Organism	Result
E. coli	Must show turbidity

Quality Control

MMO-MUG Quality Control Record

To be recorded for each new lot

Laboratory	Alloway	Year	2011	Page #	25

Date	Analyst/Testing Lab	Lot # of MMO-MUG Reagent	Reagent: Colilert/Colisure	Test Results		
				E. coli	*Klebsiella*	*Pseudomonas*
3/10/11	ANS, AETS	DCA 500	Colilert	TC +, EC +	TC+, EC-	TC-, EC-

Quality Control Thoughts

- Without quality control is your data defensible?
- Alloway is a full service laboratory and we are committed to helping you.
  - At Alloway we can help you:
    - Set up your lab for E. coli
    - Train your analysts
    - Perform many of the required QC for E. coli testing