



Microbiological Test Products for Water

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Agenda

- Introduction to indicator organisms
- Evolution of water testing
- Enzymatic water test methods: P/A and Quantification
- Quality Control
- Questions



Requirements for an Indicator Organism

- Present when pathogens are present
- Absent in uncontaminated waters
- Present in higher numbers than pathogens in contaminated water
- $_{\odot}\,$ Better survival in water than pathogens
- Easy and Safe to analyze
- Rapid results
- o Inexpensive
- Accurate

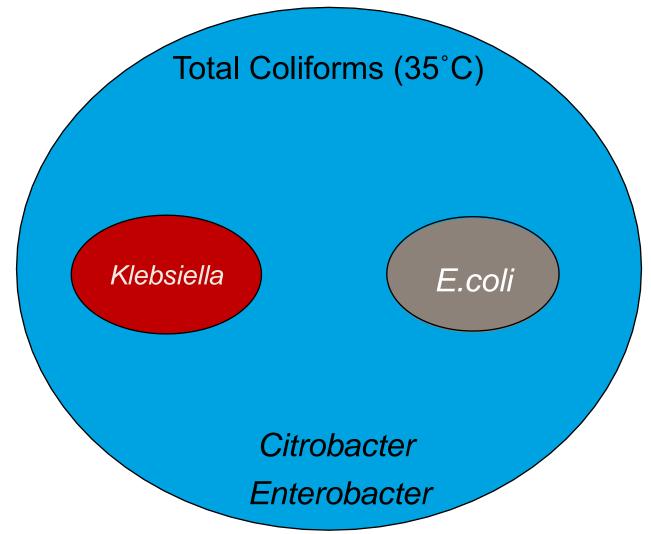


E. coli (non-pathogenic)

- Major inhabitant of gastrointestinal tract in warm-blooded animals, birds and humans
- Shed in feces at high levels
- Does not occur naturally in the environment
- Thus, true indicator of fecal contamination
- Gram negative
- o Thermotolerant

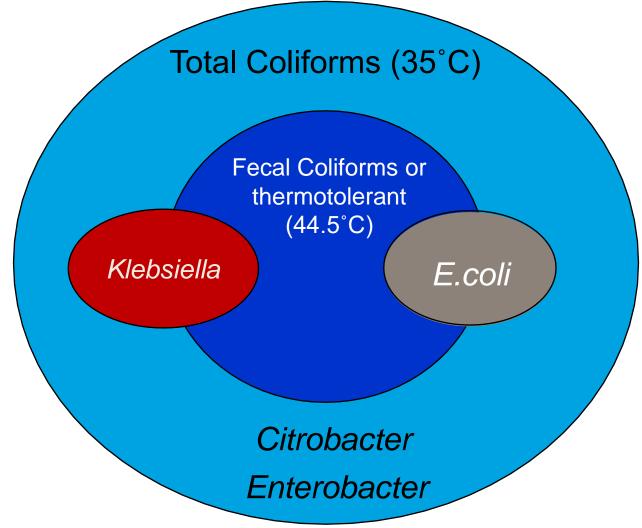


Coliform Bacteria Group



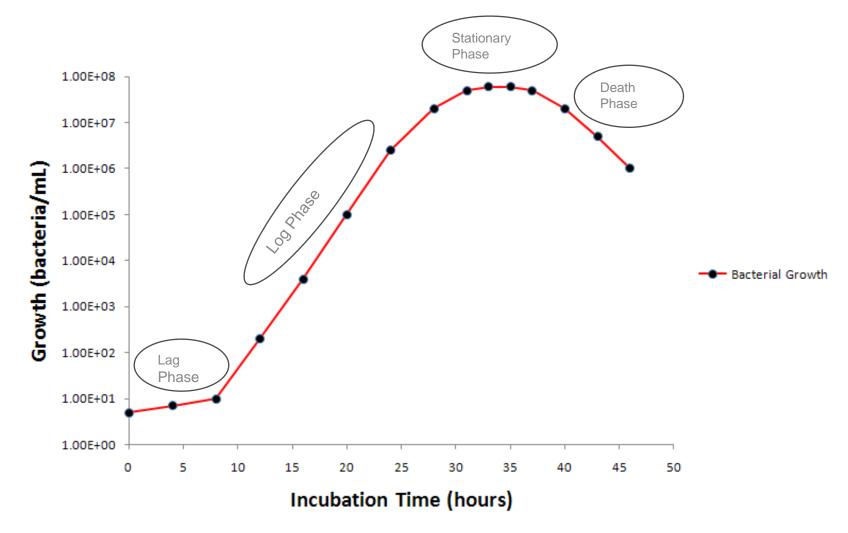


Coliform Bacteria Group

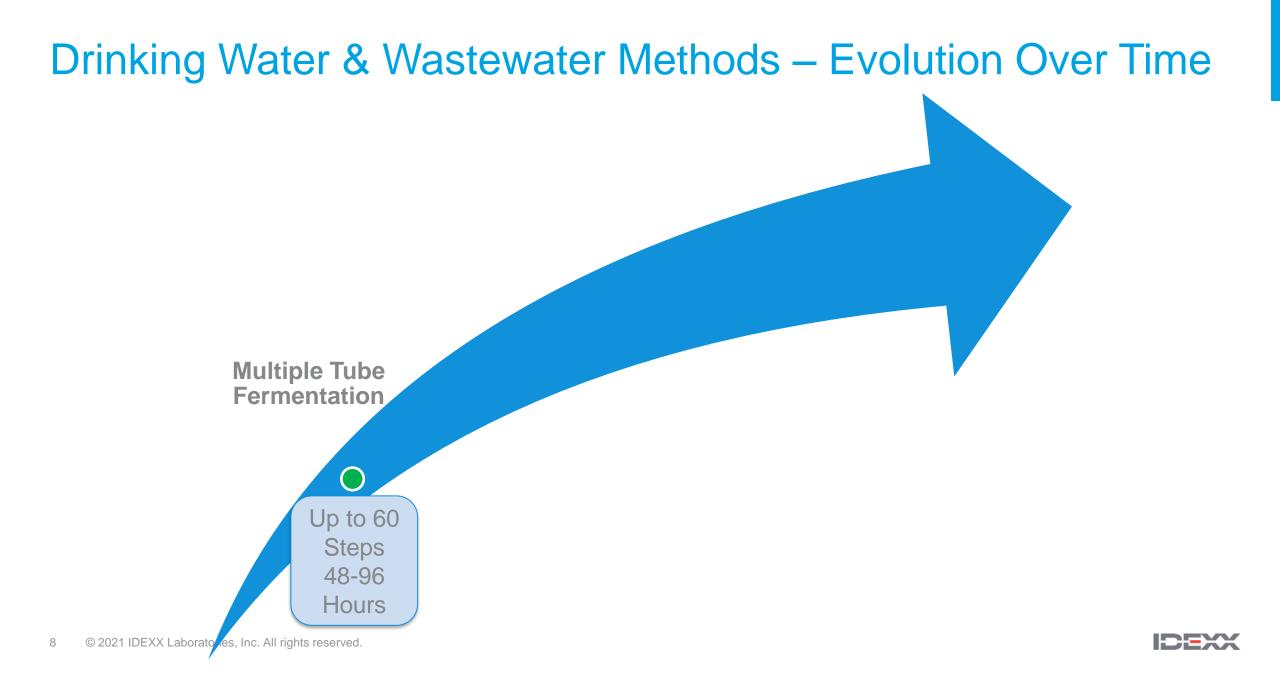




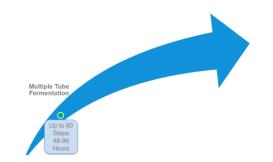
Coliform Bacteria Group

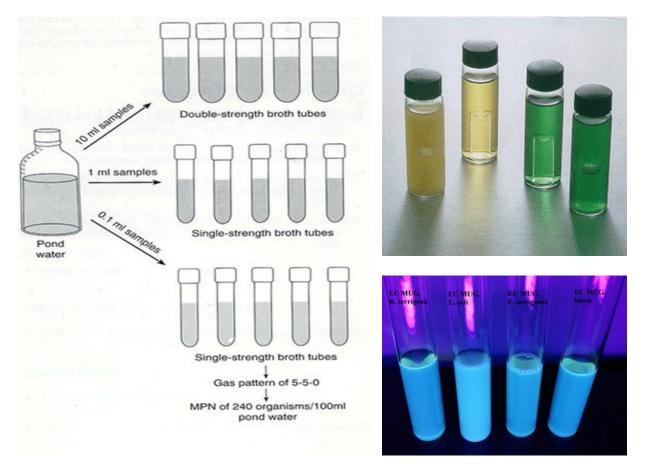






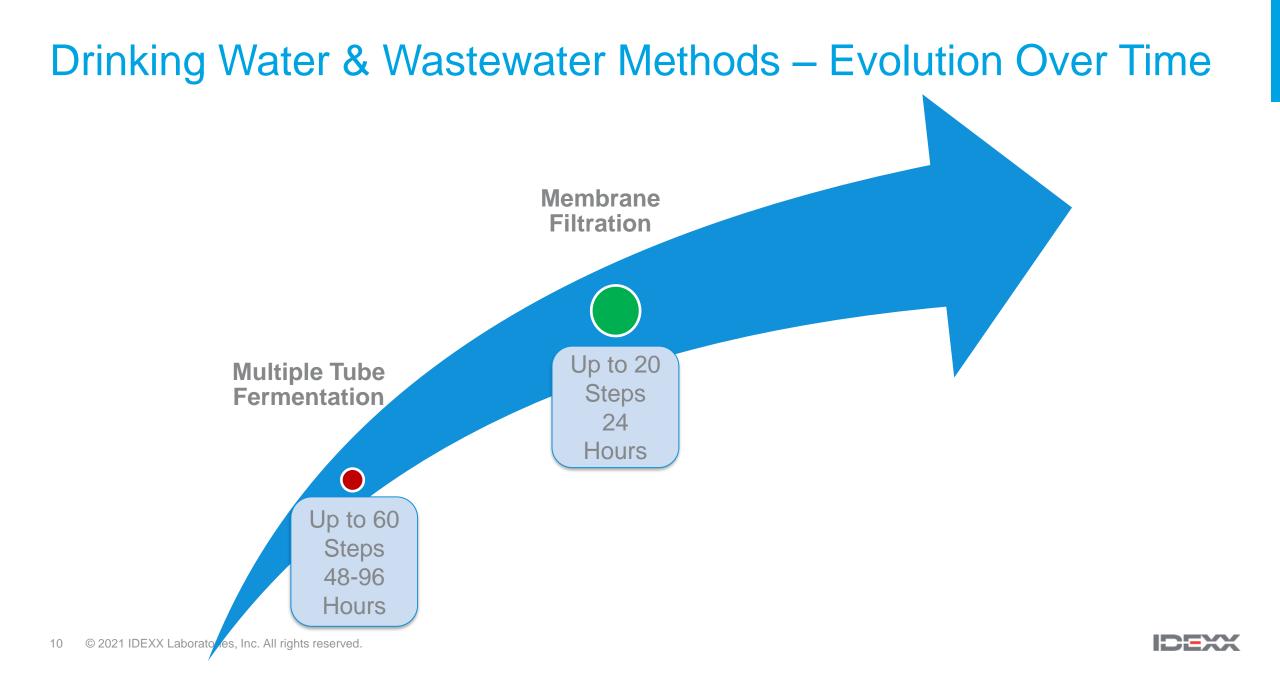
Multiple Tube Fermentation





- MTF is a Presumptive test
- Confirmation test is required for final results
- Media are either prepared or purchased and tested prior to testing samples
 - pH must be within tolerance
 - Tested prior to use with positive & negative controls
 - Media must be stored refrigerated
 - Discard prepared media after 2 weeks; purchased media cannot be used beyond the assigned exp. date
 - QC conducted with every test run



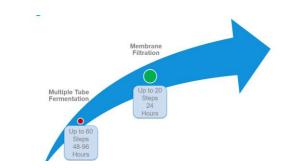


Membrane Filtration

Equipment needed:

- Manifold
- Sterile Filter funnel
- Pump & Tubing
- Collection flask
- Sterile forceps
- Filter/membrane
- Sterile media
- Incubator / Water bath





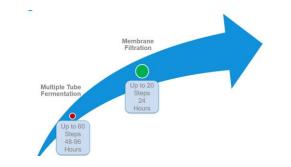


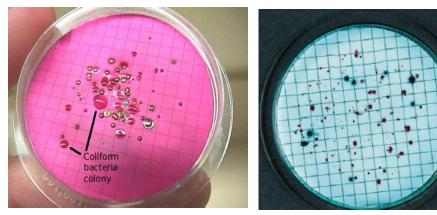






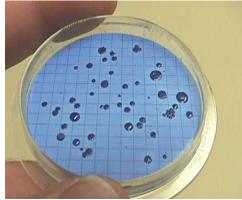
Membrane Filtration





m-ColiBlue

M-Endo



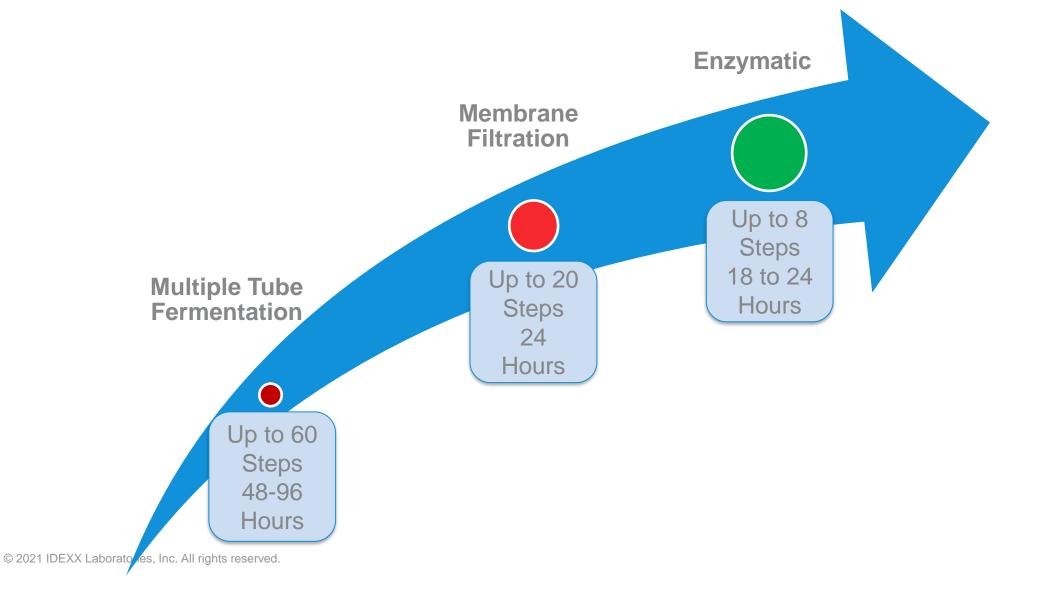
m-FC2021 IDEXX Laboratories, Inc. All rights reserved.

• Colonies of specific color / morphology are counted

- o Confirmations may be required
- \circ May required media prep, extensive QC
- Minimum of 20 steps
- o Blanks required
- Positive control required for each run
- 20-80 colonies for TC & EC; (20-60 for fecals)
- Risk of confluent growth, risk of clogged filters, risk of air bubbles under membrane
- Difficult to read, magnification required



Drinking Water & Wastewater Methods – Evolution Over Time



13



Enzymatic Testing Method Formats: Presence/Absence and Quantification

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Presence/Absence

Step 1



Step 2



Step 3



Add reagent to water sample and mix well Incubate vessel

Read and record results



Multiple Tube Quantification

Multiple Tube can be used for quantification using enzymatic tests



 $\circ~$ Refer to SM 9223 for 5 and 10 MPN tube procedure

• Refer to SM 9221 for MPN tables



Multi-well Quantification: Quanti-Tray System



Quanti-Tray Sealer PLUS



Multi-well Quantification: Quanti-Tray System





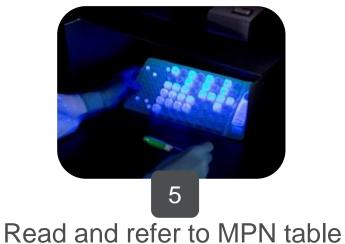


Add reagent to the sample

Pour into Quanti-Tray

Seal using Quanti-Tray Sealer PLUS







Colilert, Colilert-18, and Colisure: Total Coliform, *E. coli*, and Fecal Coliform Tests

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Colilert



- 24 hour test
- Store at 2-30°C
- Total Coliform and *E. coli*

- EPA approved, *Standard Methods* 9223B
 - Drinking Water
 - Ground Water
 - Recreational Water: Fresh/Marine Water
 - Wastewater (*E. coli*)



Colilert-18

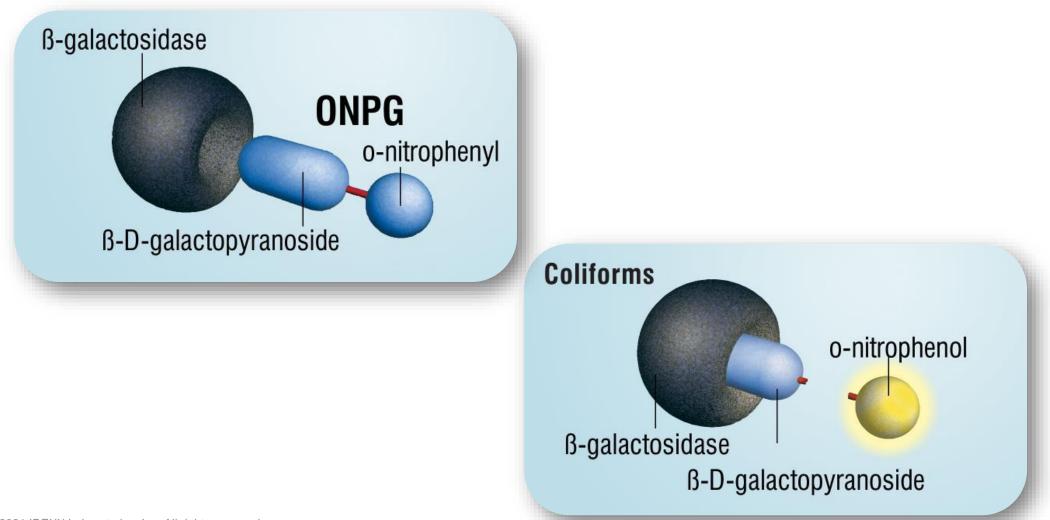


- 18 hour test
- Store at 2-25°C
- Total Coliform and *E. coli* (35°C)
- Fecal Coliform (44.5°C)

- EPA approved, *Standard Methods* 9223B
 - Drinking Water (Standard Methods 9223B, ISO 9308-2)
 - Ground Water (Standard Methods 9223B, ISO 9308-2)
 - Recreational Water (Standard Methods, ISO 9308-2)
 - Wastewater (E. coli) (Standard Methods 9223B)
 - Wastewater (Fecal) (EPA approved)

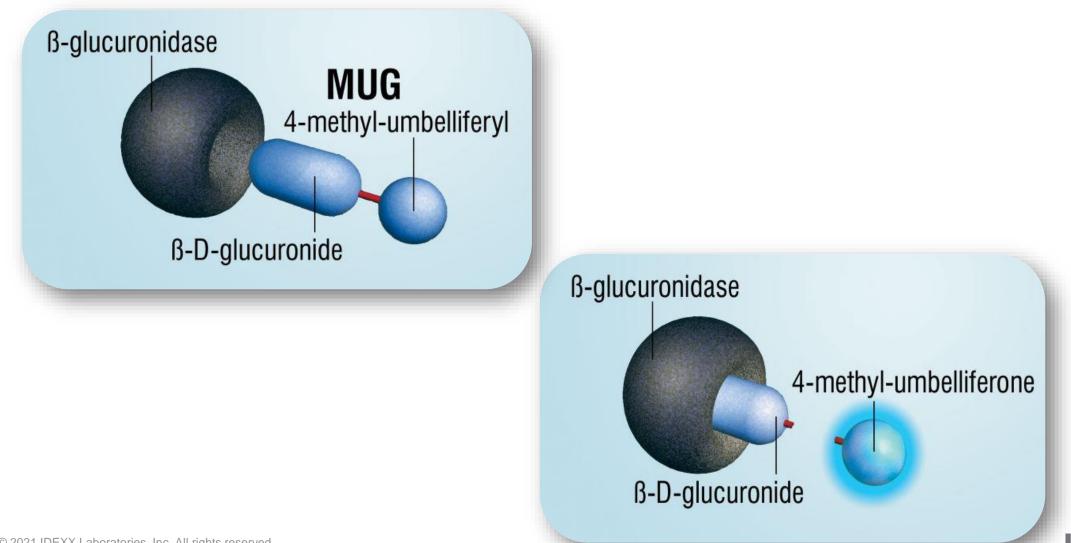


Colilert and Colilert-18: Total coliform and fecal coliforms





Colilert and Colilert-18: *E. coli*





Colilert and Colilert-18: Incubation - Total coliform and *E. coli*



Temperature

- Total coliforms / *E.coli*:
 - 35 ± 0.5°C

Time

- Colilert: 24-28 hours
- Colilert-18: 18-22 hours



Colilert and Colilert-18: Reading Results



Yellow color indicates positive for Total Coliforms



Fluorescence and yellow color indicates positive for *E. coli*.

Read *E. coli* under 365nm long wavelength 6 watt UV light



Colilert-18: Incubation - Fecal coliform



Temperature

- Fecal coliform:
 - 44.5 ± 0.2°C

Time

• Colilert-18: 18-22 hours



Colilert-18: Reading Results - Fecal Coliforms



Yellow color indicates positive for Fecal Coliforms



Colilert and Colilert-18: Comparator

- Comparator is used when reading results
 - Available in both Quanti-Tray and P/A
- Comparator provides the minimum yellow (and fluorescence) for positive results





Colisure

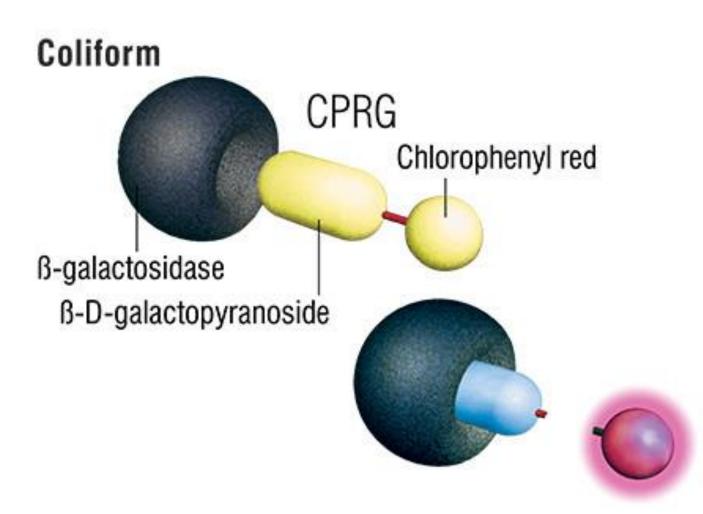


- 24 to 48 hour test
- Store at 2-25°C
- Total Coliform and E. coli

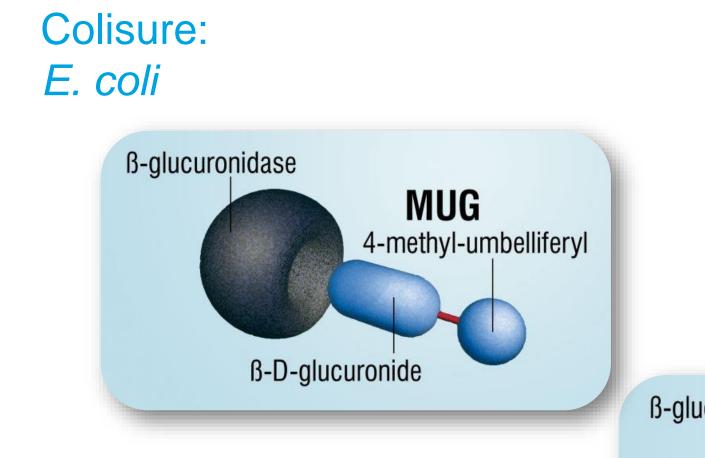
- EPA approved, Standard Methods 9223B
 - Drinking water
 - Ground water

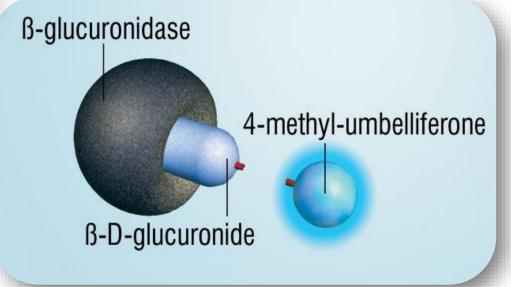












Colisure: Incubation - Total coliform and *E. coli*



Temperature

- Total coliforms/*E.coli*:
 - 35 ± 0.5°C

Time o 24-48 hours



Colisure: Reading Results



Magenta color indicates positive for Total Coliforms



Fluorescence and magenta color indicates positive for *E. coli*

Read *E. coli* under 365nm long wavelength 6 watt UV light



51-Well Quanti-Tray MPN Table

51-Well Quanti-Tray' MPN Table

34

Number of wells giving positive reaction per 100	Most Probable Number	95% Confidence Limita		
mL sample	(MPN)	Lower	Upper	
0	<1	0.0	3.	
1	1.0	0.3	5.	
2	2.0	0.6	7.	
3	3.1	1.1	9.	
4	4.2	1.7	10.	
5	5.3	2.3	12.	
6	6.4	3.0	13.	
7	7.5	3.7	15.	
8	8.7	4.5	17.	
9	9.9	5.3	18.	
10	11.1	6.1	20.	
11	12.4	7.0	22	
12	13.7	7.9	23.	
13	15.0	8.8	25	
14	16.4	9.8	27.	
15	17.8	10.8	29.	
16	17.8	11.9	29.	
17	20.7	13.0	33.	
	22.2	14.1	35.	
18				
19	23.8	15.3	37.	
20	25.4	16.5	39.	
21	27.1	17.7	41.	
22	28.8	19.0	43.	
23	30.6	20.4	46.	
24	32.4	21.8	48.	
25	34.4	23.3	51.	
26	36.4	24.7	53.	
27	38.4	26.4	56.	
28	40.6	28.0	59.	
29	42.9	29.7	62.	
30	45.3	31.5	65.	
31	47.8	33.4	69.	
32	50.4	35.4	72.	
33	53.1	37.5	76.	
34	56.0	39.7	80.	
35	59.1	42.0	84.	
36	62.4	44.6	88.	
37	65.9	47.2	93.	
38	69.7	50.0	99.	
39	73.8	53.1	104.	
40	78.2	56.4	111.	
41	83.1	59.9	118.	
42	88.5	63.9	126.	
43	94.5	68.2	135.	
44	101.3	73.1	146.	
45	109.1	78.6	158.	
46	118.4	85.0	174.	
47	129.8	92.7	195.	
48	144.5	102.3	224.	
49	165.2	115.2	272.	
50	200.5	135.8	387.	

Number of wells giving	Most Probable Number	95% Confidence Limits				
mL sample	(MPN)	Lower	Upper			
0	<1	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			

Example:

- Positive Wells: 4
- MPN: 4.2

Quanti-Tray / 2000 MPN Table

IDEXX Quanti-Tray /2000 MPN Table

35

v	Large Wells												Small Wei		
Po	ositive	25	26	27	28	29	30	31	32	33	34	35	36	37	38
	•	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
	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
	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
	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	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
	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	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
	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
	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
	9	38.1	39.3	40.5	41.6	42.8	44.0	45.2	48.4	47.6	48.8	50.0	51.2	52.4	/ 53
	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
_	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
	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
	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	80.2	61
	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
	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
_	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
	17	52.5	53.9	55.2	56.6	58.0	59.3	60.7	62.1	63.5	64.9	66.3	67.17	69.1	70
	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
		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
	19 20								69.2				75.2		
_		59.0	60.4	61.9	63.3	64.8	66.3	67.7		70.7	72.2	73.7		76.7	78
	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
	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
	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
	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
_	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
	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
	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
	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
	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
_	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
	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
	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
	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
	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
	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
	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
	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
	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
	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
	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
T	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
	42	164.3	168.6	172.9	177.3	181.9	188.5	191.3	196.1	201.1 /	206.2	211.4	216.7	222.2	227
	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
	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
	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	32
-	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
	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
		4476.0	distant.	-		-manual tot	of the lot	1000			Table T		Turke her	THE R. L	110
	48	344.1	360.9	378.4	396.8	416.0	436.0	458.9	478.6	501.2	524.7	549.3	574.8	601.5	629

41	153.2	157.0	160.9	164.8	168.9	173.0	177.2
42	164.3	168.6	172.9	177.3	181.9	186.5	191.3
43	177.5	182.3	187.3	192.4	197.6	202.9	208.4
44	193.6	199.3	205.1	211.0	217.2	223.5	230.0
45	214.1	220.9	227.9	235.2	242.7	250.4	258.4
46	241.5	250.0	258.9	268.2	277.8	287.8	298.1
47	280.9	292.4	304.4	316.9	330.0	343.6	357.8
48	344.1	360.9	378.4	396.8	416.0	436.0	456.9
49	461.1	488.4	517.2	547.5	579.4	613.1	648.8

Example:

- Positive Wells: 48
 Large and 25 Small
- MPN: 344.1



Is there a difference? The simple answer is no!

- It is based on the method used
 - MPN (Most Probable Number) used for bacteria grown in a liquid medium (e.g., Colilert)
 - CFU (Colony Forming Units) used for bacteria grown on a solid medium (e.g., agar)
- It is the label or unit associated with the numerical result
- Reported as MPN/100 mL or CFU /100 mL based on the method used for testing between the 2 reported units
- Both methods have a lower and upper 95% confidence limit



Quanti-Tray- Dilutions

- The Quanti-Tray system reduces the needs for dilutions due to the increased counting range
- If the expected MPN value is greater than 200/100mL or 2419/100mL, the sample can be diluted prior to addition of reagent
- Sample is diluted with sterile DI water (diluent)
- Reagent is added to the diluted sample
- Dilution examples that can be done:
 - 1:1 dilution = 50 ml of sample + 50 mL of diluent
 - 1:10 dilution = 10 mL of sample + 90 mL of diluent



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Quality Control

Quality Controls

- Positive & negative control with each new reagent
 - National collection type strains (i.e. ATCC)
 - IDEXX offers QC product for total coliform / E. coli and fecal coliform

Test	Code	Contents	Bacteria
Total Coliforms- <i>E. coli</i>	WQC-TEC	3 each	E. coli K. variicola P. aeruginosa
Fecal Coliforms	WQC-FC	3 each	E. coli P. aeruginosa



IDEXX Certificates of Analysis

Important to have a copy for your records and audits

• Obtain a C of A for all our products from our website

- <u>https://www.idexx.com/en/water/resources/certificates-analysis-water/</u>
- Indicates all the testing performed for the specific product and lot
- All products are under quarantined until the batch records are reviewed and approved by QA
- Once approved by QA, the specific product and lot is released for distribution
- ISO Certification for: 9001,17025, and 14001



QC Certificates

QUALITY CO	QUALITY	QUALITY C 120mL Sterile	QUALITY CONTROL CERTIFICATE				
Product and Company Contact Information Product Catalog Number: WP0201-18 Part Number: 98-08876-00 Lot Number: TEST123 Technical Support Inquiries: 1-207-556-4496 1-800-321-0207 (US/CAN) 00-800-4339-9111 (Europe) Email: water@idexx.com	Product and Company Contact Information Product Catalog Number: WP020I-18 Part Number: 98-08876-00 Lot Number: TEST123 Technical Support Inquiries: 1-207-556-4496 1-800-321-0207 (US/CAN) 00-800-4339-9111 (Europe) Email: water@idexx.com	Product and Company Contact Information Product Catalog Number: WV120SBST-200 Part Number: 98-09221-00 Technical Support Inquiries: 1-207-556-4496 1-800-321-0207 (US/CAN) 00-800-4339-9111 (Europe) Email: water@idexx.com	Product and Company Contact Information Product Catalog Number: WQT-2K Lot Number: EL014 Part Number: 98-21675-00 Expiration date: 12 May 2018 Technical Support Inquiries: Manufacturer: 1-207-556-4496 IDEXX Laboratories, Inc. 1-800-321-0207 (US/CAN) One IDEXX Drive 00-800-4339-9111 (Europe) Westbrook, ME 04092 USA Email: water@idexx.com Fax: 1-207-556-4630				
White Properties Powder Appearance** White to off-white, free flowin pH Tested for pH range 7.0 - 7.6. Product Performance Product Performance	Fecal Coliforms** Representative Quanti-tray samples of this lot have bee with reads at 18 hours for target organisms and 22 hou Target Bacteria Result Excherichia coli Yellow color	Physical Properties Fill Line Accuracy Lot has been	Physical Properties				
Coliforms and E. coli Representative samples of this lot have been tested with the o 0.5°C with reads at 18 hours for target organisms and 22 hou approximately 20-50 cfu/100mL. Quantitative testing is perf to spread plate counts using Tryptic Soy Agar with or withou	Escherichia coli Yellow color ATCC 11775 / WDCM 00090 Non-Target Bacteria Result Pseudomona: aeruginosa No color chan ATCC 10145 / WDCM 00024	to within ± 2. Sterility Lot has been In accordance assurance lev	Product Sterility: This lot was sterilized with Ethylene Oxide using a process cycle that has been validated in accordance with the standards of ANSI/AAMI/ISO 11135 (2007), Method C (Overkill Method). Biological indicator strips with a minimum population of 1 X 10 ⁶ spores of <i>Bacillus atrophaeus</i> per strip were processed with the lot and tested negative for growth.				
count. The passing range is ≥ 0.5 to ≤ 1.4 (i.e. greater than o Note: Testing performed in accordance with ISO 11133:201 Target Bacteria P/A Result	Coliform-free Lot has been : irradiated prov	Appearance Absence of n					
Escherichia coli ATCC 25922 / WDCM 00013 ATCC 11775 / WDCM 00090		Sodium Thiosulfate Content Lot is able to Methods for i	 Average volume of overflow well: > 10.0 mL 				
Klebsiella pneumonine Yellow color chang ATCC 31488 / WDCM 00206 no fluorescence Enterobacter aerogenes: Yellow color chang ATCC 13048 / WDCM 00175 no fluorescence Non-Target Bacteria P/A Bacult	This product was performance tested and has met al This information is released by IDEXX Quality Assu Name: Tom Bannen, Sr. Quality Assurance Specialist	Fluorescence Test Result: Negat	 <u>Volume ratio large wells to small wells</u>: between 11:1 and 13:1 <u>Dve Test</u>: sealed with 100 mL dye and checked for absence of leaks <u>Seal Integrity</u>: trays sealed with 100 mL water, Colisure*, and anti-foam: wells withstood 18 PSI of pressure without leaking 				
Non-Target Bacteria P/A Result Pseudomonas aeruginosa No color change, no ATCC 10145 / WDCM 00024	Signature: Tom BANNEN Date: June 12, 2015	This product was performance tested and has r This information is released by IDEXX Quality					
*Colifert and Quanti-Tray are trademarks of IDEXX Laboratories, Inc. or it **Parameter does not fall under the IDEXX ISO/IEC 17025:2005 \$ IDEXX Wa ISO	*Colliert and Quanti-Tray are trademarks of IDEXX Laboratories, in **Parameter does not fall under the IDEXX ISO/IEC 17025:1	Name: Lisa Johnson, Sr. Quality Assurance Speci Signature: Lion Johnson	Signature: Jury Johuson "Quanti-Tray and Colinure are trademarks or registered trademarks of IDEXX Laboratories, Inc. or its affiliates in the United States and/or other countries.				
Teeling Laboratory -QA-088_C, CO #	Teeling Laboratory Accreditation #AT-1031 -0A-008_0	fm-QA-093_8, CO #067741, Effective Date: 5/29/2013	htts:GA-082_R_CO-6907741, Effective Date: 50/00013 Monday, June 01, 2015				

Questions







For more information on how IDEXX can streamline your testing contact:

Boyd Hawkins - Inside Sales Account Manager

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