



Microbial Testing Methods for Drinking Water

Shawn Dubois – Senior Scientist IDEXX

Agenda

- Introduction to indicator organisms
- Presence/Absence and Quantification
- Colilert, Colilert-18, and Colisure: Test for Total Coliform and *E. coli*
- EasyDisc: New tests for HPC
- Quality Control
- Questions

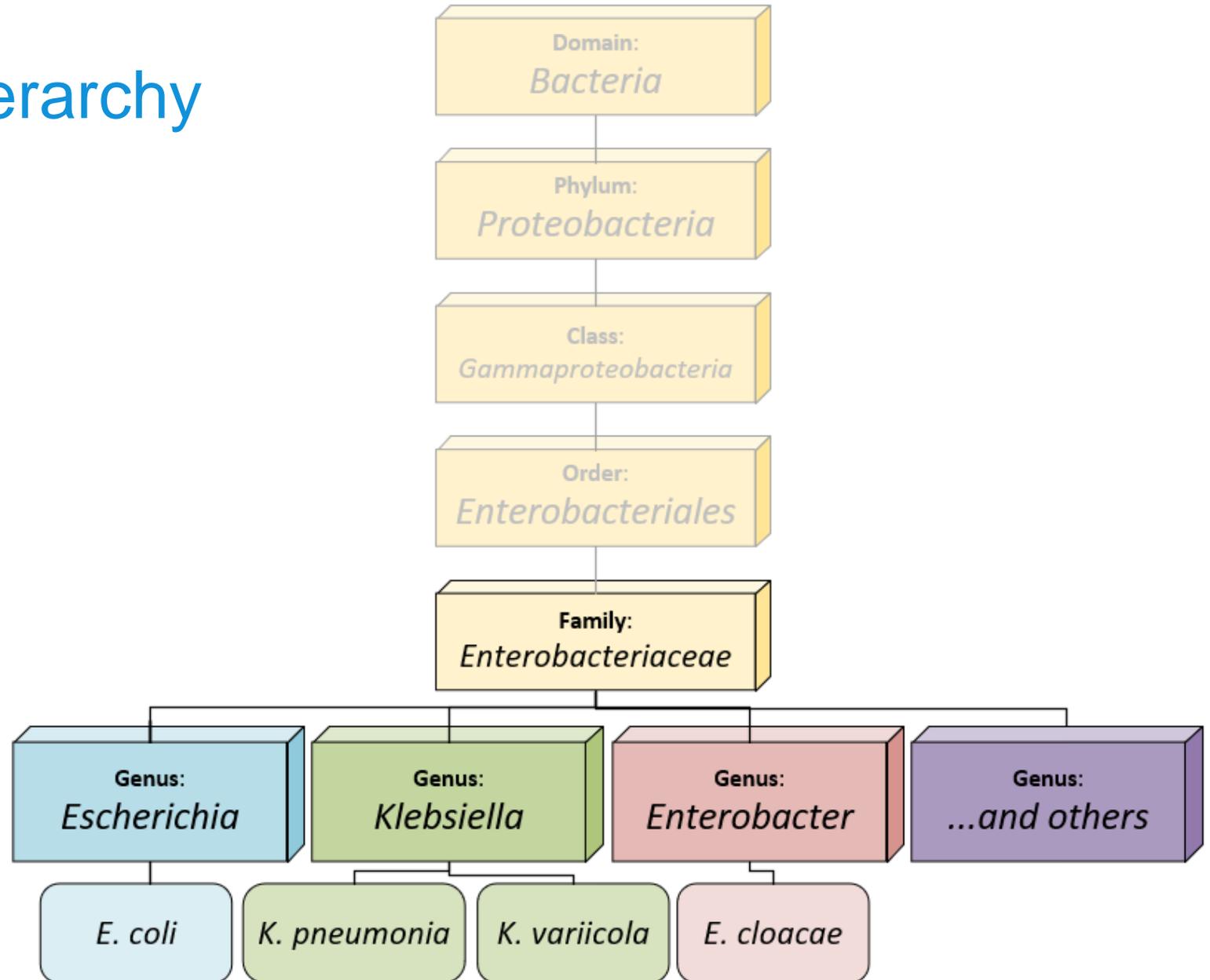


Requirements for an Indicator Organism



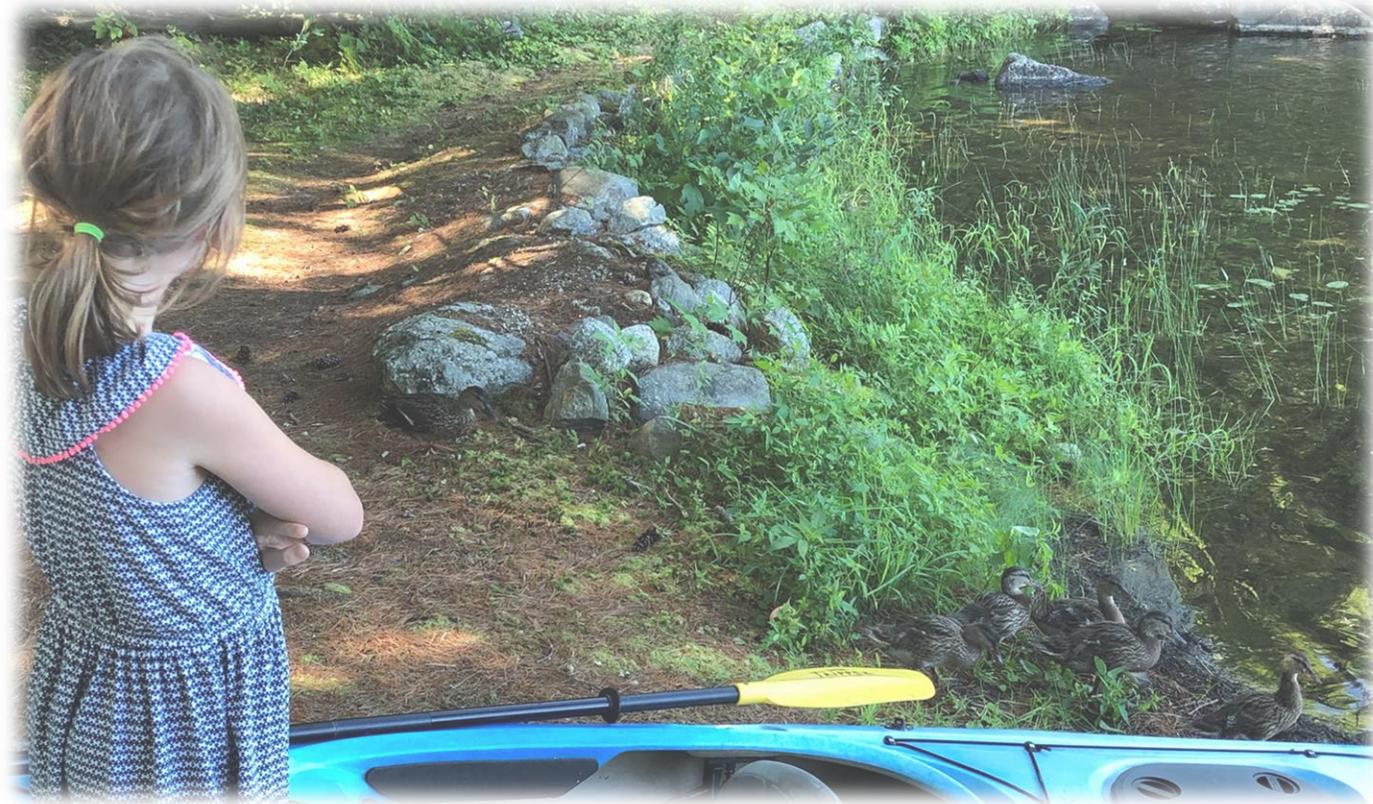
- Present when pathogens are present
- Absent in uncontaminated waters
- Present in higher numbers than pathogens in contaminated water
- Better survival in water than pathogens
- Easy and Safe to analyze
- Rapid results
- Inexpensive
- Accurate

Coliform Bacteria Hierarchy



Coliform Genera

- *Escherichia*: Human and animal feces
- *Enterobacter*: Environment, feces
- *Klebsiella*: Environment, feces
- *Citrobacter*: Environment
- *Serratia*: Environment

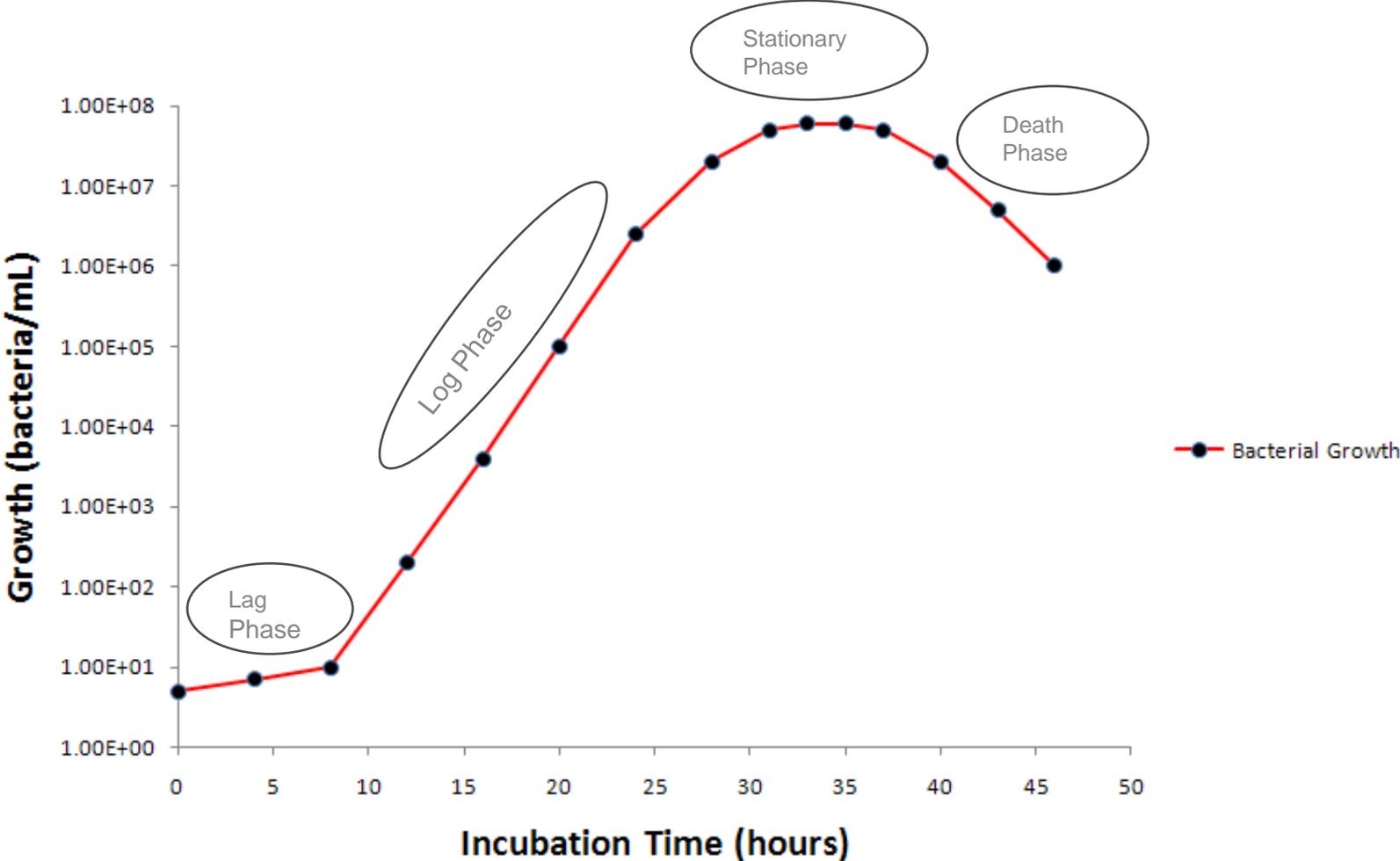


E. coli (non-pathogenic)

- 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
- Thermotolerant



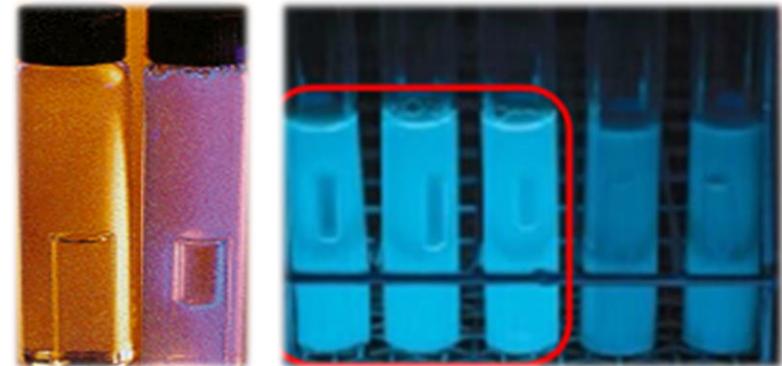
Coliform Bacteria Group



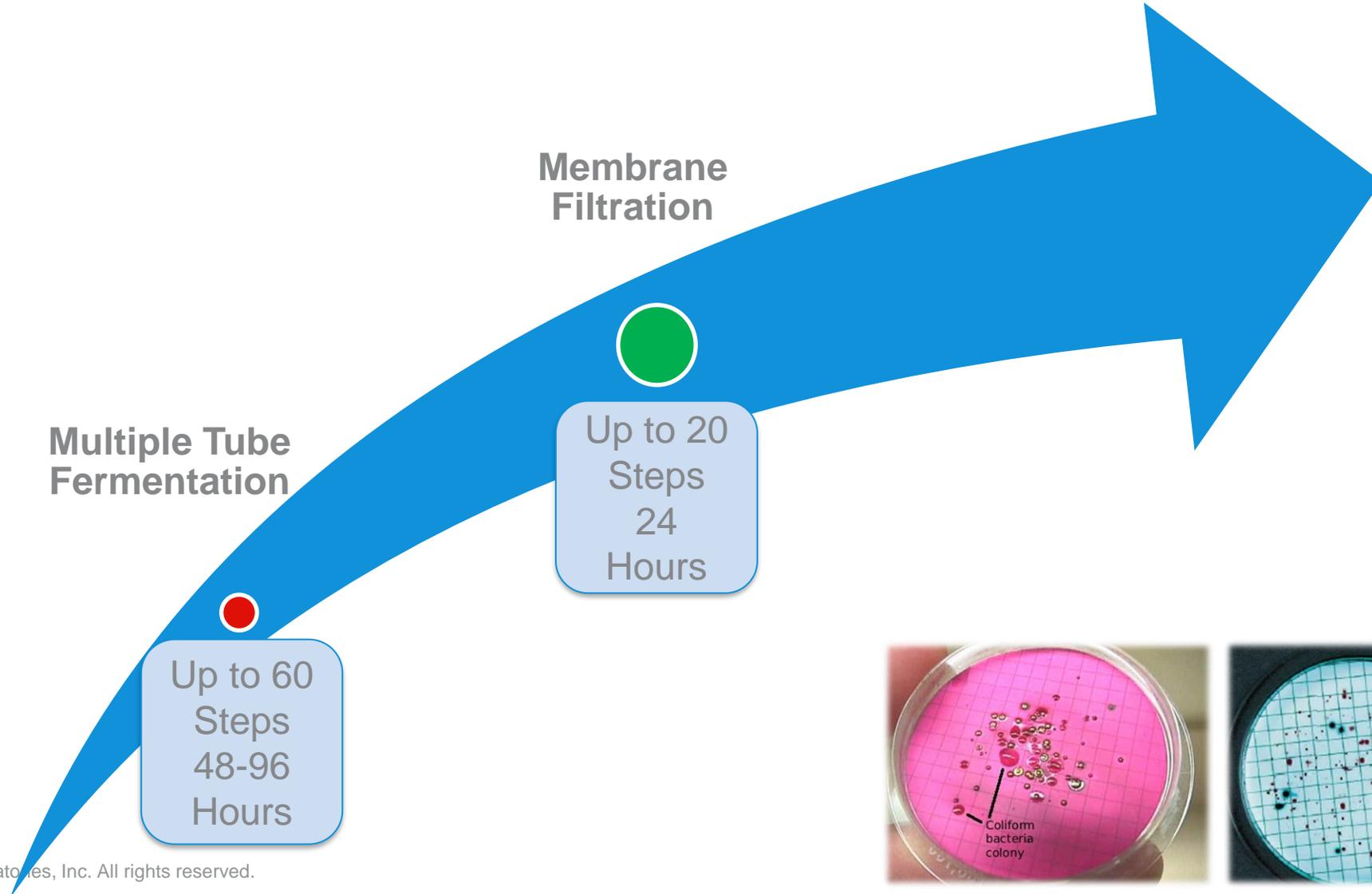
Drinking Water Methods – Evolution Over Time

Multiple Tube
Fermentation

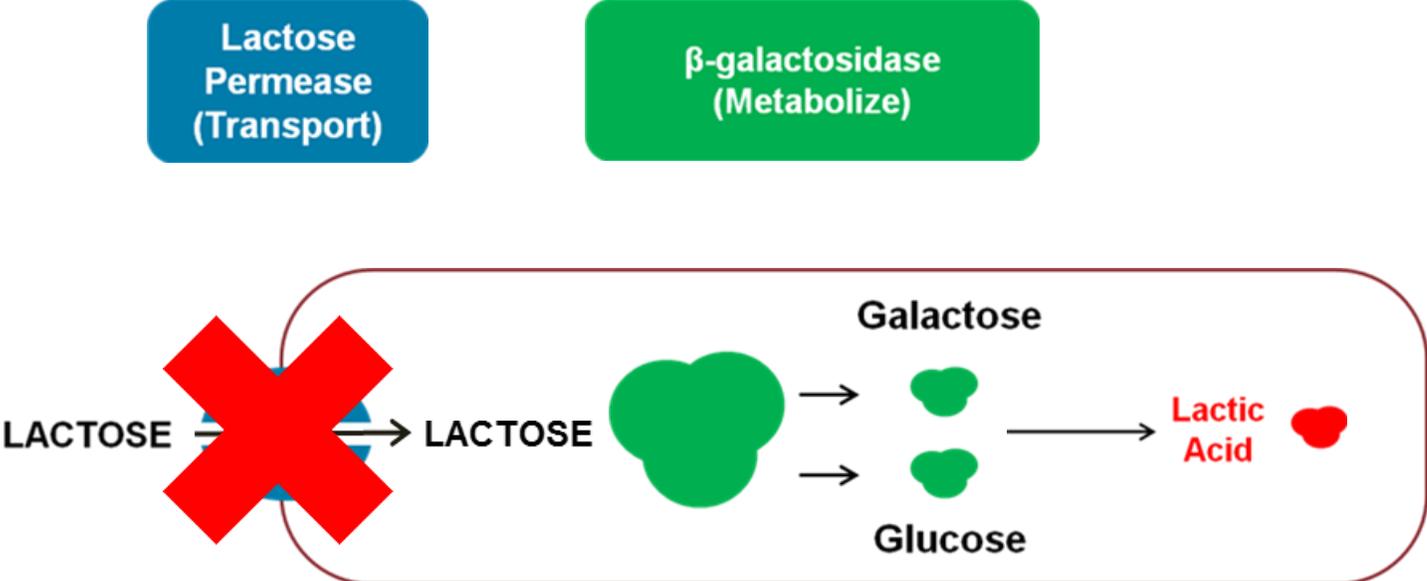
Up to 60
Steps
48-96
Hours



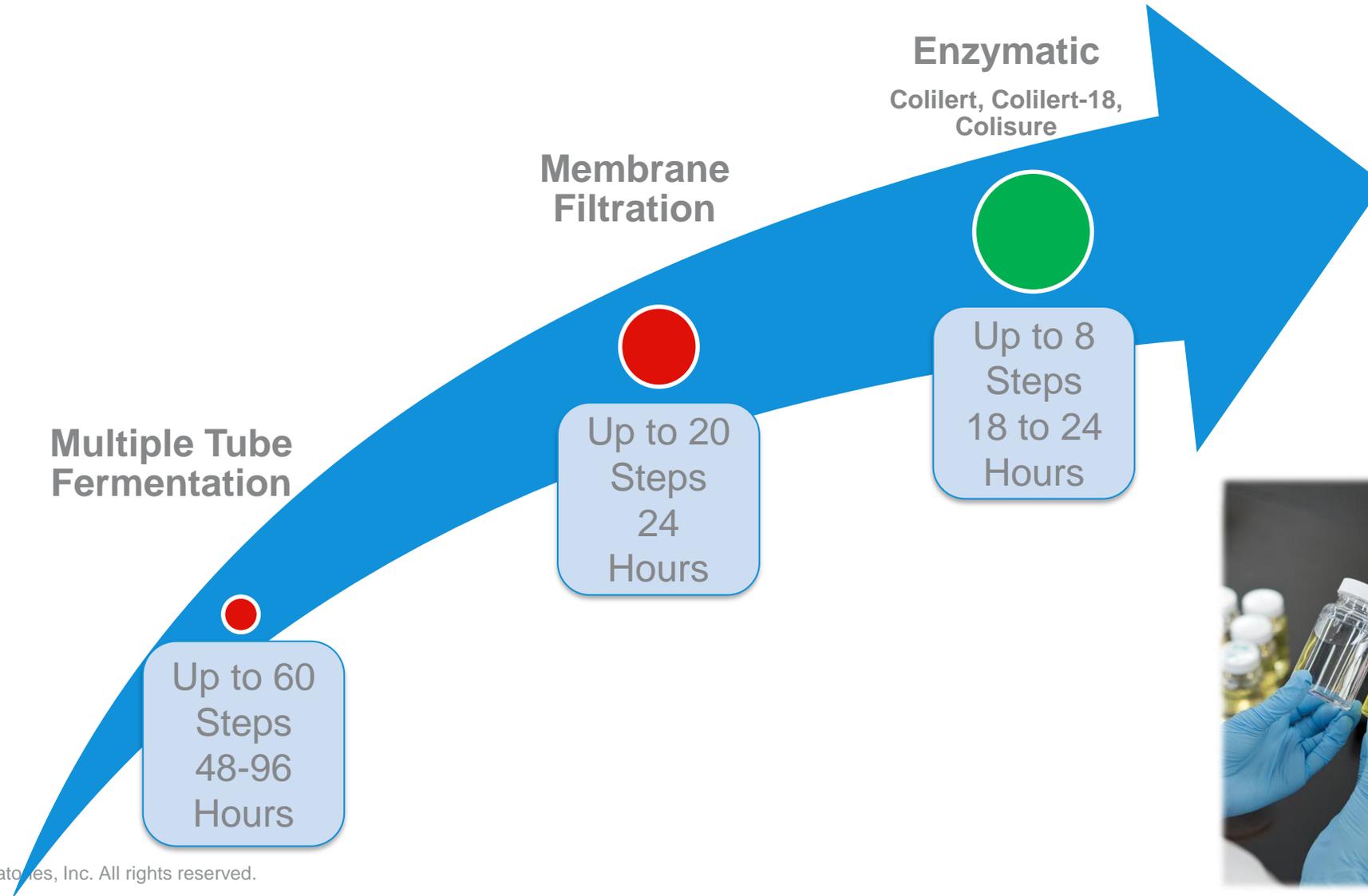
Drinking Water Methods – Evolution Over Time



Metabolic Process: Lactose



Drinking Water Methods – Evolution Over Time





IDEXX Enzymatic Testing Methods:

Colilert, Colilert-18, and Colisure

Very Simple Presence/Absence Procedure

Step 1



Add reagent to
water sample
and mix well

Step 2



Incubate vessel

Step 3

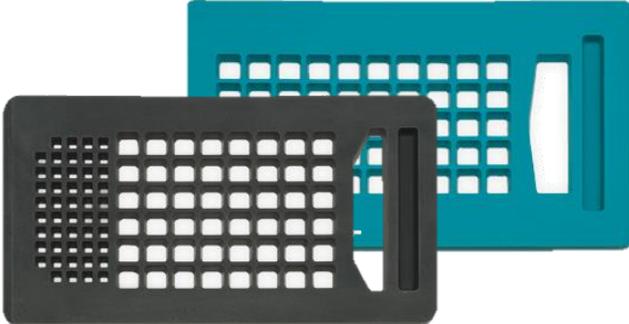


Read and record
results

Quantification Tools



Quanti-Trays



Quanti-Tray Inserts



Quanti-Tray Sealer PLUS

Procedure for Quantification



1

Add reagent to the sample



2

Pour into Quanti-Tray



3

Seal using Quanti-Tray Sealer PLUS



4

Incubate



5

Read and refer to MPN table

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*)

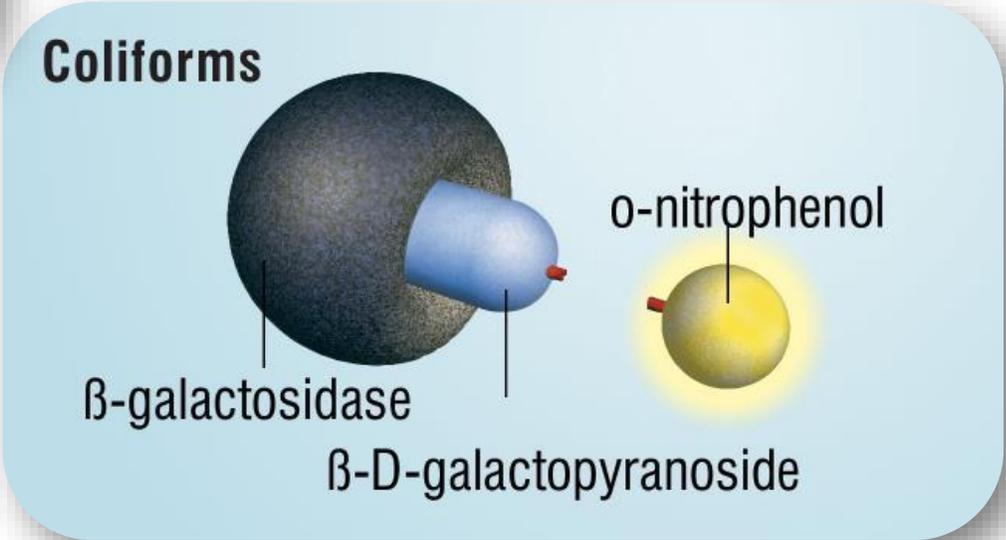
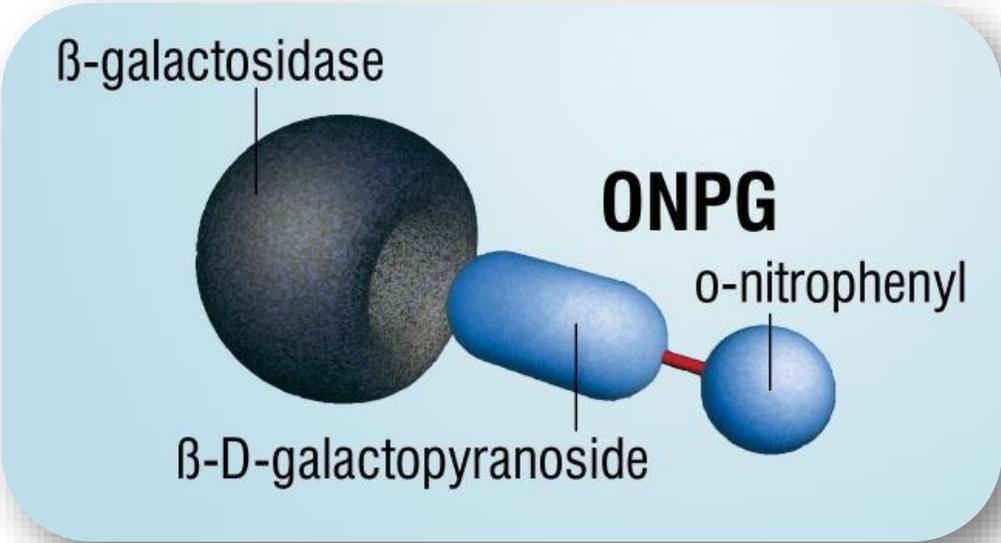
Colilert-18



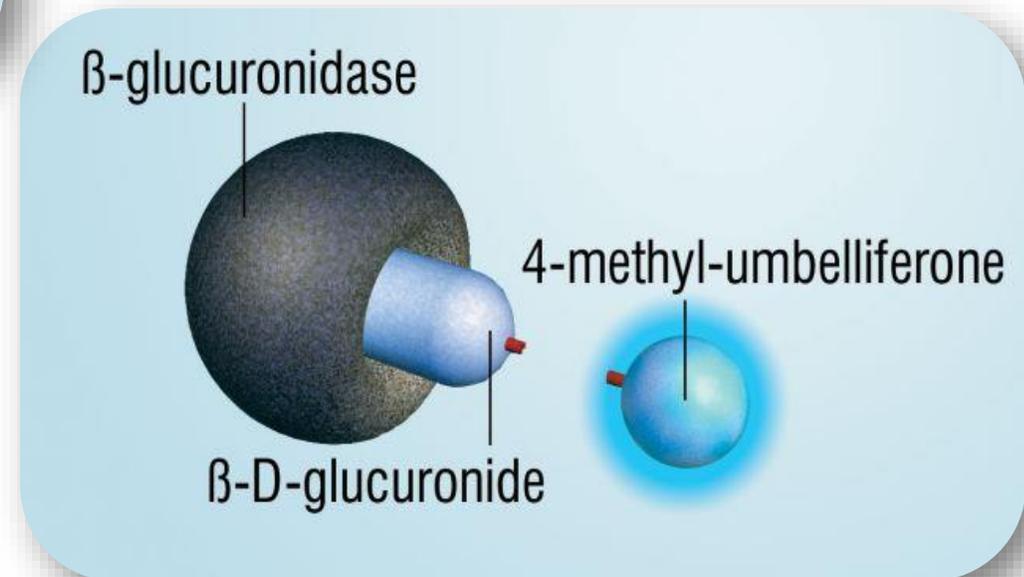
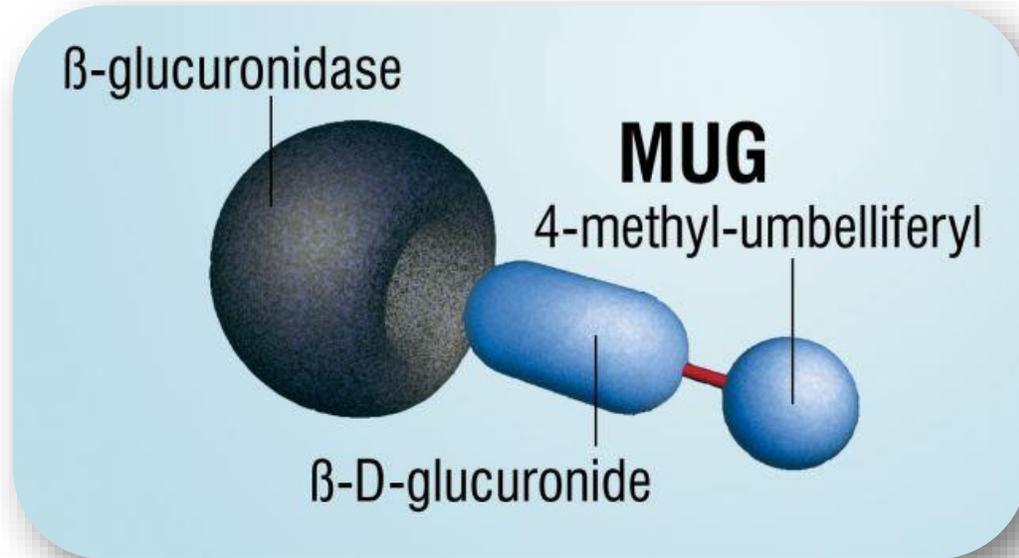
- 18 hour test
- Store at 2-25°C
- Total Coliform and *E. coli* (35°C)
- Fecal Coliform (44.5°C)

- 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



Colilert and Colilert-18: *E. coli*



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



Temperature

- Colilert:
 - $35 \pm 0.5^{\circ}\text{C}$
- Colilert-18:
 - Pre-warm in a water bath at 44.5°C for 7-10 min or 35°C for 20 min – **P/A format only**
 - $35 \pm 0.5^{\circ}\text{C}$

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

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

51-Well Quanti-Tray MPN Table

51-Well Quanti-Tray MPN Table

Number of wells giving positive reaction per 100 mL sample	Most Probable Number (MPN)	95% Confidence Limits	
		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
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

Number of wells giving positive reaction per 100 mL sample	Most Probable Number (MPN)	95% Confidence Limits	
		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

Colilert and Colilert-18: Comparator

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



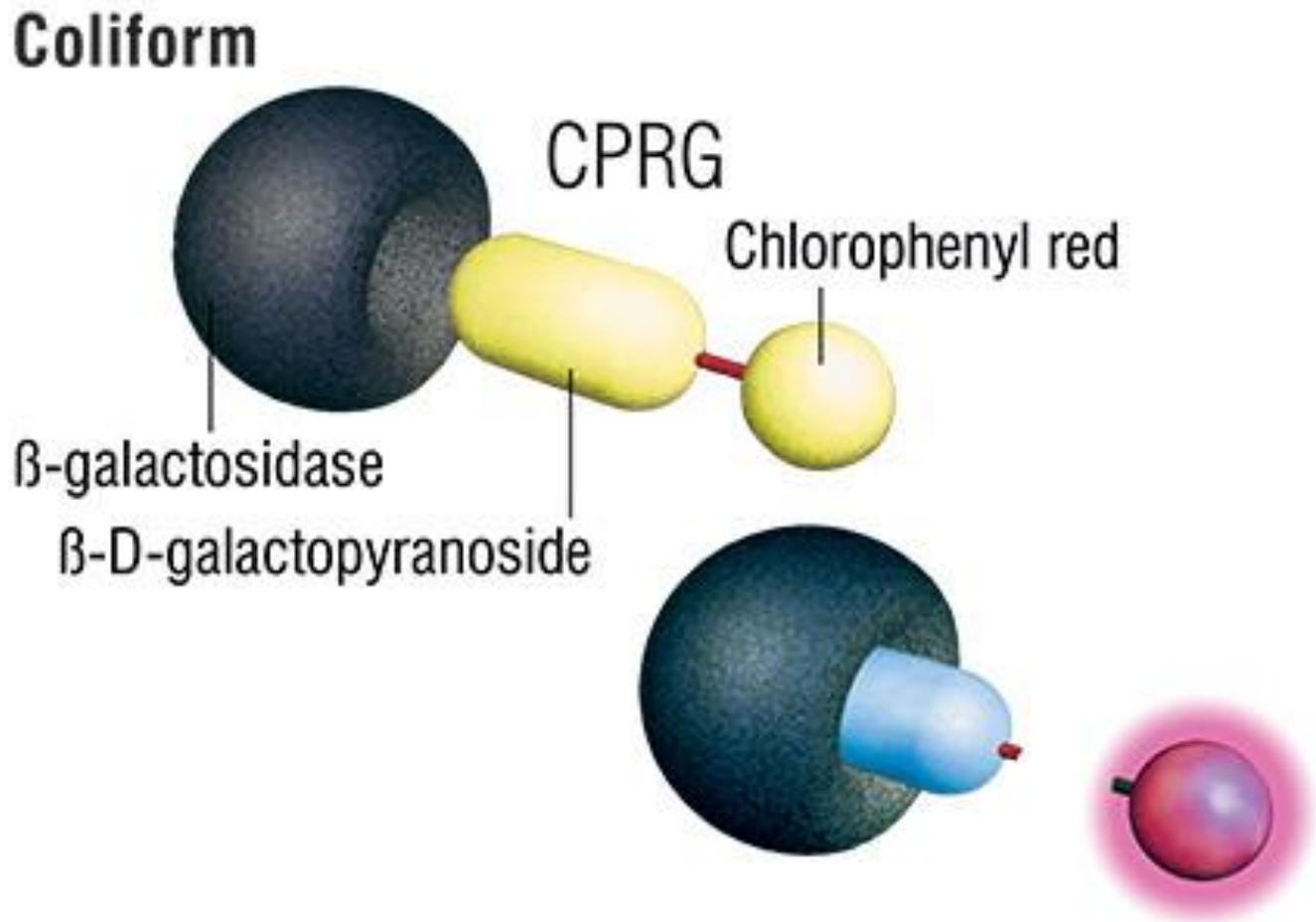
Colisure



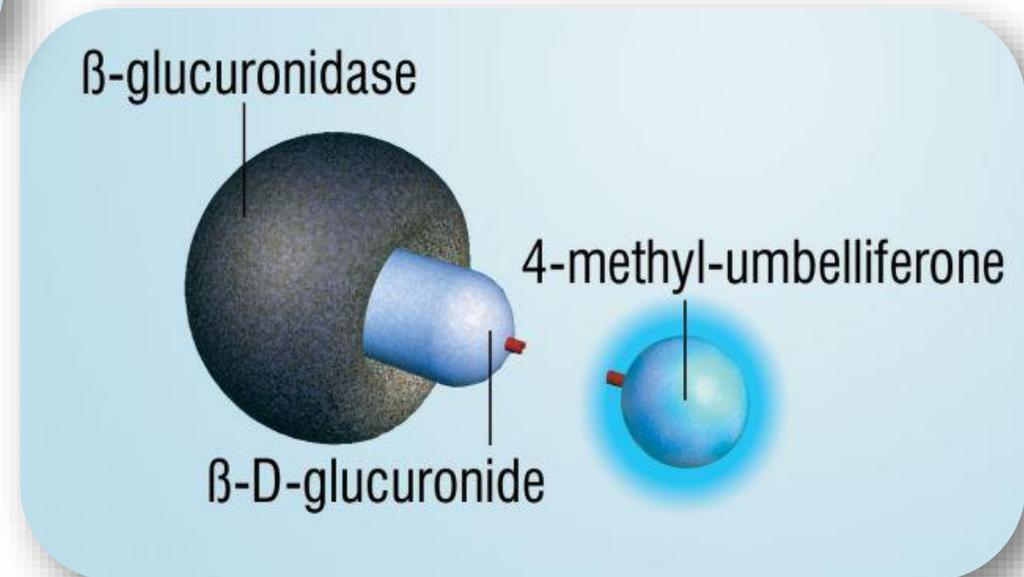
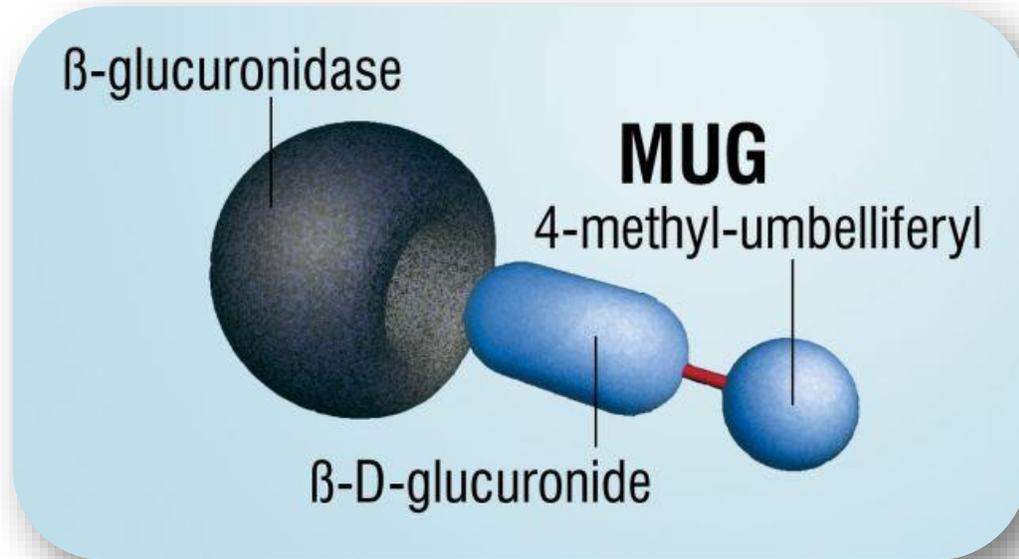
- 24 to 48 hour test
- Store at 2-25°C
- Total Coliform and *E. coli*

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

Colisure: Total coliform



Colisure: *E. coli*



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



Temperature

- Colisure:

- $35 \pm 0.5^{\circ}\text{C}$

Time

- 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



EasyDisc:

Heterotrophic Plate Count (HPC) Tests

Heterotrophic Plate Count (HPC)

- Unlike other indicator organism testing...
- HPC is used to indicate general water quality:
 - Adequate disinfection control
 - Biofilm and clogged filters
 - Water aesthetic (taste, odor, turbidity)
 - Biofouling and biocorrosion
- HPC not used to detect specific bacteria, or their sources

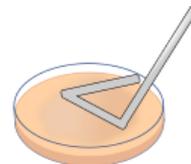
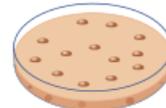


Pour Plate and Spread Plate Methods

Pour Plate

- 1 Sample added to empty petri dish 
- 2 Pour warm agar over the sample 
- 3 Swirl plate to mix and incubate 
- 4 Bacterial colonies grow throughout the medium 

Spread Plate

- 1 Sample poured onto solid medium 
- 2 Spread sample evenly over surface 
- 3 Incubate 
- 4 Bacterial colonies grow on medium surface 

Media preparation

Quality control

Excess waste

Sample preparation

Results interpretation

**Protocols may vary by region*

Plate Media



Plate Count Agar
(PCA)

- Used for compliance testing
- Potable drinking water and source water testing



Reasoner's 2 Agar
(R2A)

- Non-compliance testing
- Potable, medical, dental and pharmaceutical waters

EasyDisc

- 47mm plate reduces waste by 50%
- Flexible white top ensures a tight seal, eliminating condensation and providing a clear background for reading results
- Plates were designed to be nested together for safe stacking
- Media is dehydrated right on the plate allowing for a longer shelf life (12 months)



EasyDisc

1

Remove lid and add 1 mL sample to the EasyDisc plate.



3

Continue incubating using the times and temperatures specific to each test.

	Incubation temperature(s)	Incubation time(s)
EasyDisc PCA	33-37°C	45-51 hours
EasyDisc R2A	20-28°C	5-7 days

2

Gently swirl the plate to distribute the liquid, replace the lid, and incubate at room temperature for 20 minutes.



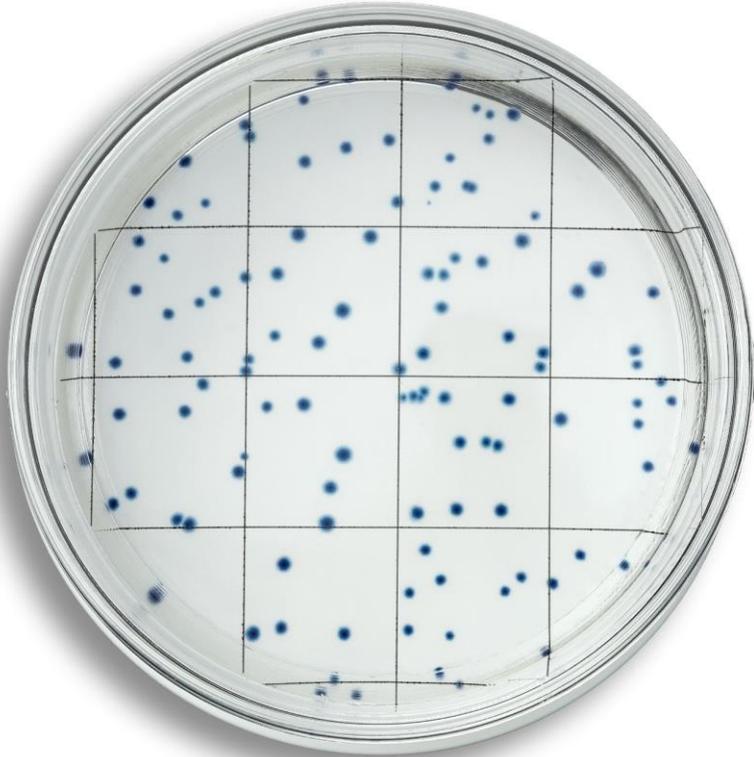
4

Count all colonies.



- No media preparation and less than 1 min hands-on time

EasyDisc



- Definitive end-point makes reading colonies easier and faster
- Laser-etched grid aides in colony counting
- Lot and catalog information are laser etched into the base



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 HPC

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

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
 - <https://www.idexx.com/en/water/resources/certificates-analysis-water/>
 - 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 CONTROL CERTIFICATE

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

Physical Properties
 Powder Appearance** White to off-white, free flowing
 pH Tested for pH range 7.0 - 7.6.

Product Performance
Coliforms and E. coli
 Representative samples of this lot have been tested with the following methods at 37.0°C with reads at 18 hours for target organisms and 22 hours for non-target organisms. Quantitative testing is performed by spread plate counts using Tryptic Soy Agar with or without methylene blue. The passing range is ≥ 0.5 to ≤ 1.4 (i.e. greater than or equal to 0.5 and less than or equal to 1.4).
 Note: Testing performed in accordance with ISO 11133:2013

Target Bacteria	P/A Result
<i>Escherichia coli</i> ATCC 25922 / WDCM 00013 ATCC 11775 / WDCM 00090	Yellow color change and positive fluorescence \geq control
<i>Klebsiella pneumoniae</i> ATCC 31488 / WDCM 00206	Yellow color change and no fluorescence
<i>Enterobacter aerogenes</i> ATCC 13048 / WDCM 00175	Yellow color change and no fluorescence
Non-Target Bacteria	P/A Result
<i>Pseudomonas aeruginosa</i> ATCC 10145 / WDCM 00024	No color change, no fluorescence

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 **Parameter does not fall under the IDEXX ISO/IEC 17025:2005 certification.



 IDEXX Water Quality
 ISO 17025
Testing Laboratory
 Accreditation #AT-1931

QUALITY CONTROL CERTIFICATE

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

Fecal Coliforms**
 Representative Quanti-tray samples of this lot have been tested with reads at 18 hours for target organisms and 22 hours for non-target organisms.

Target Bacteria	Result
<i>Escherichia coli</i> ATCC 11775 / WDCM 00090	Yellow color change
Non-Target Bacteria	Result
<i>Pseudomonas aeruginosa</i> ATCC 10145 / WDCM 00024	No color change

Coliform-free
 Lot has been tested and found to be coliform-free after irradiation.

**This product was performance tested and has met all quality control specifications required for release.
 This information is released by IDEXX Quality Assurance:**
 Name: Tom Bannen, Sr. Quality Assurance Specialist

Signature: 
 Date: June 12, 2015

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Testing Laboratory
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QUALITY CONTROL CERTIFICATE

120mL Sterile

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

Physical Properties
Fill Line Accuracy
 Lot has been tested and found to be within $\pm 2\%$.

Sterility
 Lot has been tested and found to be sterile in accordance with assurance level. No growth after 48 hours incubation at 35°C \pm 0.5°C with sterile Tryptic Soy Broth.

Appearance
 Absence of non-sterile particles.

Sodium Thiosulfate Content
 Lot is able to neutralize residual chlorine. Methods for testing are available.

Fluorescence Test
 Result: Negative

**This product was performance tested and has met all quality control specifications required for release.
 This information is released by IDEXX Quality Assurance:**
 Name: Lisa Johnson, Sr. Quality Assurance Specialist

Signature: 
 Date: June 01, 2015

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Testing Laboratory
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QUALITY CONTROL CERTIFICATE

Quanti-Tray*/2000

Product and Company Contact Information
 Product Catalog Number: WQT-2K
 Part Number: 98-21675-00

Technical Support Inquiries:
 1-207-556-4496
 1-800-321-0207 (US/CAN)
 00-800-4339-9111 (Europe)
 Email: water@idexx.com

Lot Number: EL014
Expiration date: 12 May 2018

Manufacturer:
 IDEXX Laboratories, Inc.
 One IDEXX Drive
 Westbrook, ME 04092 USA
 Fax: 1-207-556-4630
 idexx.com/water

Physical Properties
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 1×10^6 spores of *Bacillus atrophaeus* per strip were processed with the lot and tested negative for growth.

No growth after 48 hours incubation at 35°C \pm 0.5°C with sterile Tryptic Soy Broth.

Representative samples of this lot have been tested by IDEXX Laboratories for the following requirements and passed:

- Volume of 96 wells: 98 \pm 2.0 mL.
- Average volume of overflow well: > 10.0 mL.
- Volume ratio large wells to small wells: between 11:1 and 13:1
- Dye Test: sealed with 100 mL dye and checked for absence of leaks
- Seal Integrity: trays sealed with 100 mL water, Colisure*, and anti-foam: wells withstood 18 PSI of pressure without leaking

**This product was performance tested and has met all quality control specifications required for release.
 This information is released by IDEXX Quality Assurance:**
 Name: Lisa Johnson, Sr. Quality Assurance Specialist

Signature: 
 Date: Monday, June 01, 2015

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Questions



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