

***E. Coli* as a Drinking Water Public Health Indicator**


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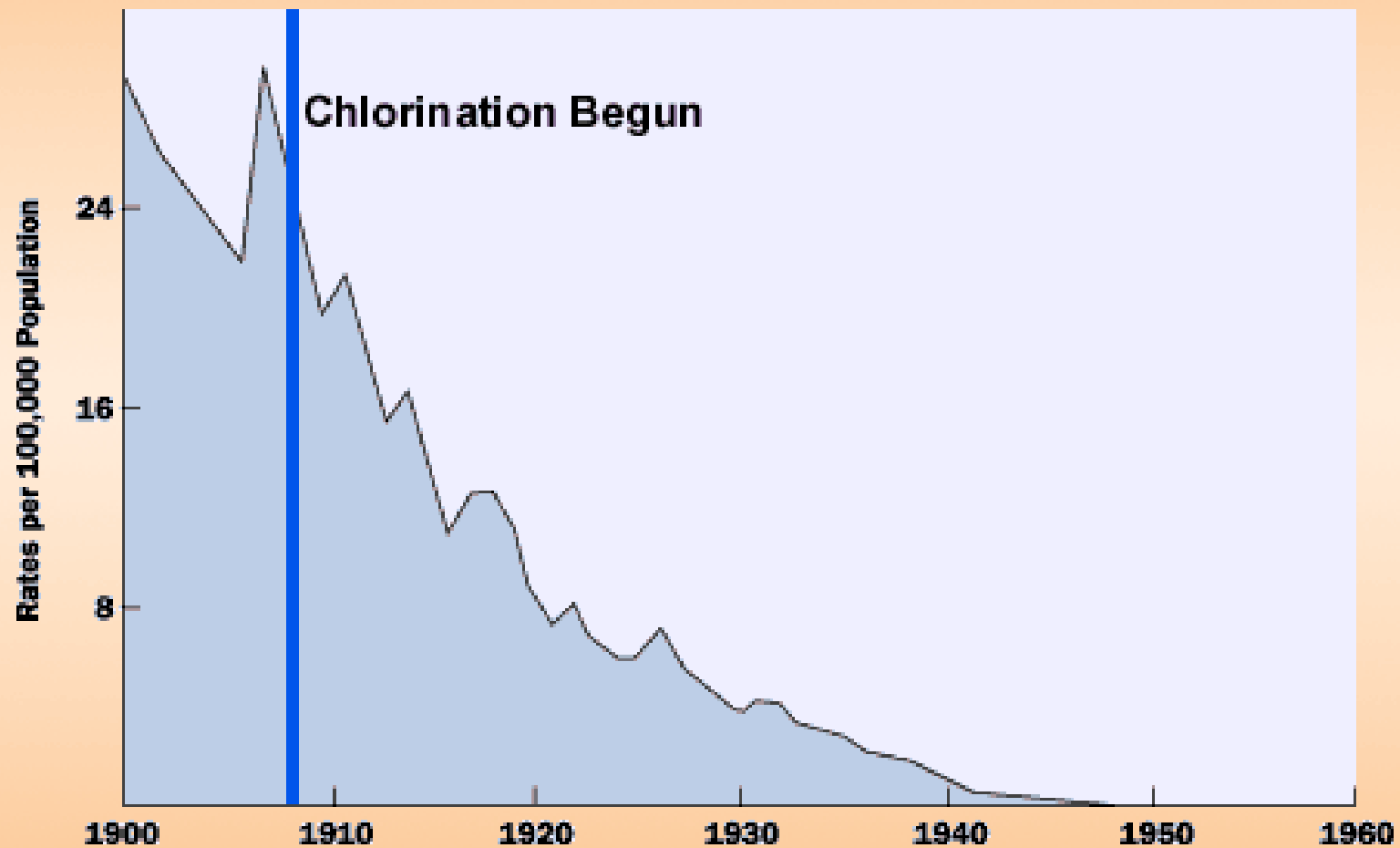
Brief Reminder of the History of Waterborne Disease

- Chlorination of public water systems and construction of wastewater collection and treatment wiped out Typhoid as a cause of death in the US
- Until 1920 dysentery from Typhoid fever and Cholera was the leading cause of death in the U.S.

Typhoid Fever

- Headache
 - Malaise
 - High fever
 - May last for weeks
 - Life threatening
 - Treatable with antibiotics
- 

Death Rate for Typhoid Fever United States, 1900-1960



Source: U.S. Centers for Disease Control and Prevention, Summary of Notifiable Diseases, 1997.

Waterborne Disease Is Still A Major Health Problem

- How many people die from waterborne disease per day globally
 - 80,000 per day (WHO statistic)
 - 29,000,000 per year

Bacterial Agents Of Waterborne Disease

- *Salmonella*
 - *typhi* (Typhoid fever)
 - Salmonellosis
- *Vibrio cholerae*
- *Helicobacter pylori*
- *Campylobacter*
- *E. coli* O157

Parasitic Agents Of Waterborne Disease

- *Cryptosporidia*
- *Giardia*
- *Microsporidia*
- *Entamoeba*

Viral Agents Of Waterborne Disease

VIRUS	SOURCE	SYMPTOMS
Caliciviruses (Norwalk)	Feces	gastroenteritis, nausea, vomiting
Enteroviruses	Feces	febrile illness, diarrhea
Hepatitis A/E	Feces	fever, malaise, jaundice, nausea
Rotaviruses	Feces	vomiting and diarrhea in children
Adenovirus		

***E. coli* O157 Is A Good Example**

- Cramps
- No fever
- Bloody diarrhea
- Hemolytic uremic syndrome
 - Kidney failure
 - Death

New York Outbreak

- 1999 New York State *E. coli* O157 outbreak
 - Local fair
 - Once a year water system
 - cross connected to rain run-off
 - Young animals
 - Infected sisters
 - One death

E. coli O157




Indicator vs Specific Pathogen Testing


- In a perfect world we would test for all the pathogens in real time
 - Technology not available
 - Too expensive
 - Unknown emerging pathogens
- We are forced to use indicators

Indicators Instead of Pathogens

➤ Ideal indicator

- present in proportion to fecal contamination
 - works in all water types
 - safe to work with in the laboratory
 - no false positives or false negatives
 - survives in the environment at least as long as pathogens
- 
- The bottom right corner of the slide features a decorative graphic of several concentric, light blue circles that resemble ripples on water, set against the dark blue background.

Indicators In the US

- Coliforms
 - Fecal coliforms
 - *E. coli*
 - Enterococci
 - Coliphage
- 
- The bottom right corner of the slide features a decorative graphic of several concentric, light blue circles that resemble ripples on water, set against the dark blue background.

Coliforms

- Ferment lactose utilizing galactosidase
- Grow at 35 degrees C
- Grow in the presence of Brilliant green dye and bile salts
- Facultative gram positive rods
- *Enterobacter, E. coli, Klebsiella, Citrobacter, et al.*

Coliforms

➤ Advantages

- present in proportion to level of pollution
- safe to work with
- always present in fecal material
- cheap and easy to test for

➤ disadvantage

- often found when no evidence of fecal contamination can be identified

Fecal Coliforms

- Ferment lactose
- Thermotolerant (44.5 degrees C)
- *E. coli*, *Klebsiella*

Fecal coliforms

➤ Advantages

- present in proportion to level of pollution
- safe to work with
- always present in fecal material
- cheap and easy to test for

➤ disadvantage

- Can be found when no evidence of fecal contamination can be identified

E. coli

➤ Advantages

- always present in feces
- found in proportion to pollution
- safe to work with
- quick easy test available

➤ disadvantage

- may die-off faster than pathogens

Percentage of Total Coliforms That are *E. coli* In Feces

<u>Animal</u>	<u>E. coli</u>	<u>Animal</u>	<u>E. coli</u>
Cow	90%	Mouse	71%
Cat	92%	Pheasant	100%
Chicken	87%	Pig	63%
Dog	89%	Rat	100%
Human	82%	Turkey	88%

E. Coli – Pathogenic *E. coli* Dilemma

- Normal flora *E. coli*
- Enteropathogenic *E. coli*, enterotoxigenic *E. coli*, enteroinvasive *E. coli*, enteroaggregative *E. coli*, diffuse adherent *E. coli*, and enterohemorrhagic *E. coli*
- Education dilemma.
- Fecal indicator *E. coli* does not cause disease
- Routine tests do not detect pathogenic strains

History of the Science of Indicator Testing in Water

- Golden age of microbiology
 - 1890 *E. coli* characterized
 - Difficult series of identification tests
 - *E coli* recognized as an excellent fecal indicator
 - 1904 easy to perform surrogate test developed and named Total Coliform Test
 - 1900 brought to Wisconsin by Birge
 - 1918 accepted by U.S. government

History of the Science of Indicator Testing in Water

- 1960's realization that TC test had high level of false positivity in identifying fecal contamination
- Realization led to development of the fecal coliform test

History of the Science of Indicator Testing in Water

- 1980's realization that fecal coliform still had an unacceptable level of false positivity
- Advent of enzyme based tests allowed quick and easy identification of *E. coli*
- Finally able to detect *E. coli* 90 years after it was identified as an ideal indicator

The fermentation Tube:

- The Media: Lauryl Tryptose Broth (LTB)


- The fermentation tube



Read BG Tube Results

Negative control
tube is clear
showing no
growth.

Positive control
tube showing
turbidity (growth)
with gas.



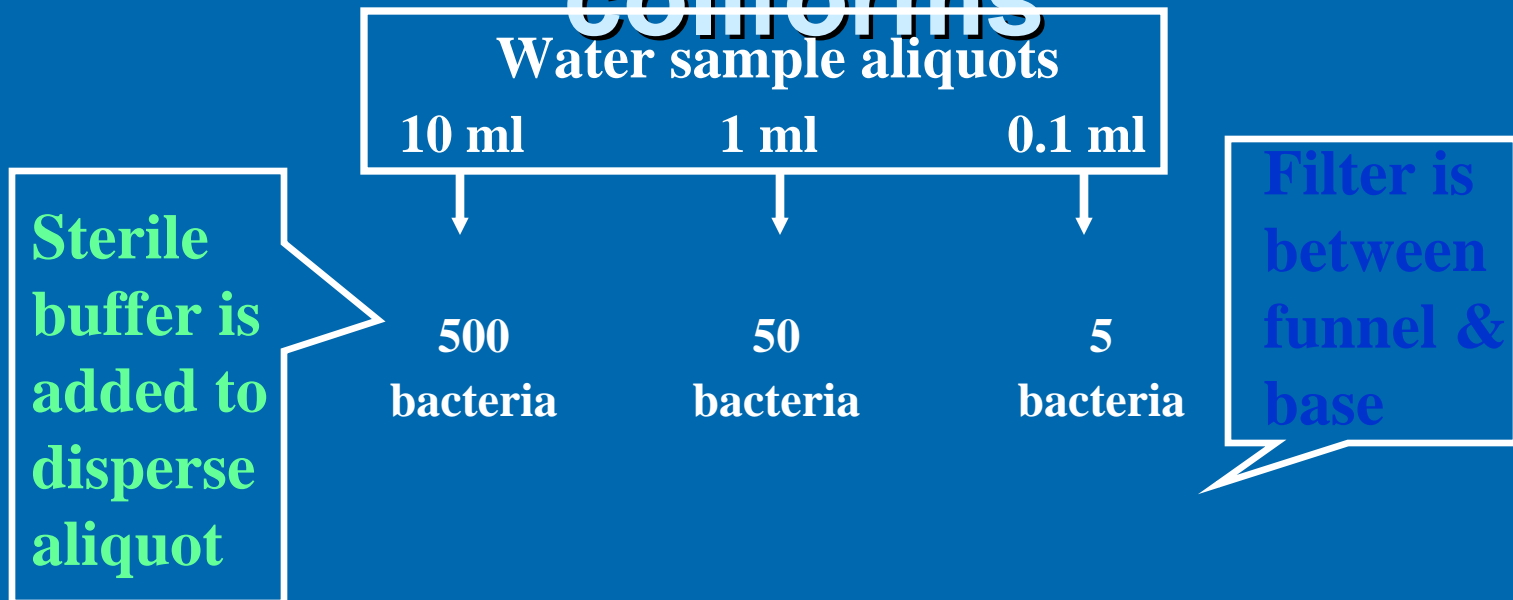
Ec Broth tubes with MUG and fermentation tubes:

E.coli

Fecal



Three aliquots of a 100 ml sample containing 5,000 coliforms



Vacuum manifold

Transfer filter with bacteria



mEndo

Media

NUT/MUG

- Lactose fermentation
- Aldehyde production
- Metallic green precipitate

• MUG

(4-methylumbelliferyl-b-D-glucuronide)

• *E. coli* hydrolyze MUG

(4-methyl-umbelliferone)

• Colonies fluoresce
under UV light

total coliforms

E. coli

E. coli colonies fluoresce

**NUT/MUG agar
under UV light**

**Count fluorescing
colonies-only`**

Colilert and Colilert-18 P/A Demonstration

E. coli make sample
fluoresce

Negatives remain
colorless

Coliforms turn
sample yellow

Colisure P/A Demonstration

E. coli make sample
fluoresce

Negatives remain
yellow

Coliforms turn
sample magenta

Colilert[®], Colilert[®]-18, Colisure[™] P/A Demonstration

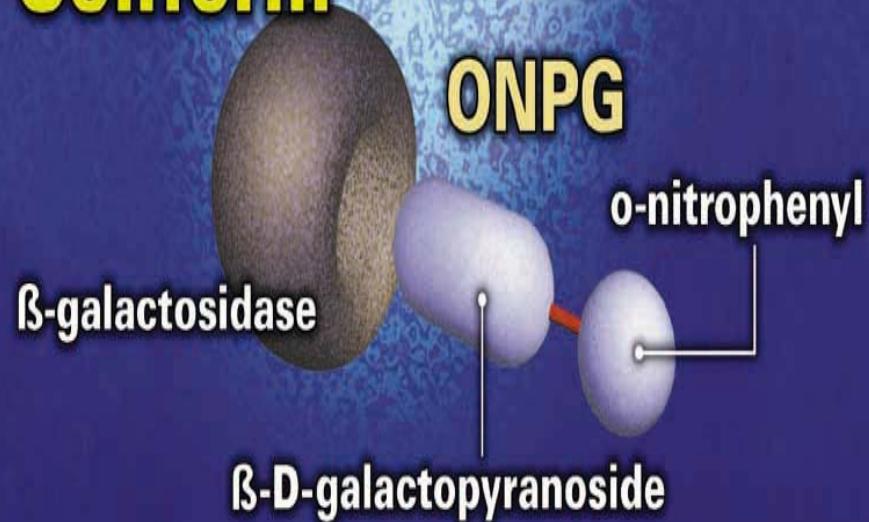
Add reagent to sample and incubate



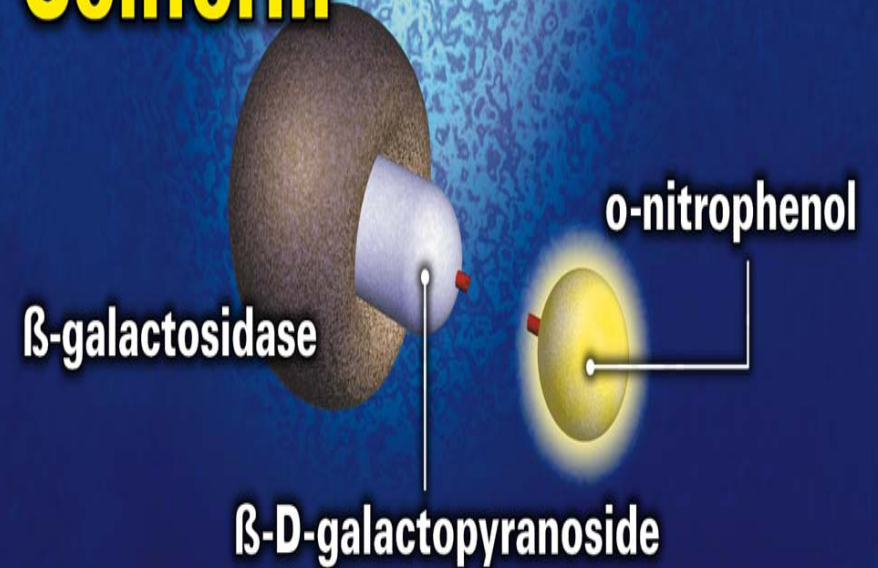
ONPG Positive Reaction

Colilert

Coliform



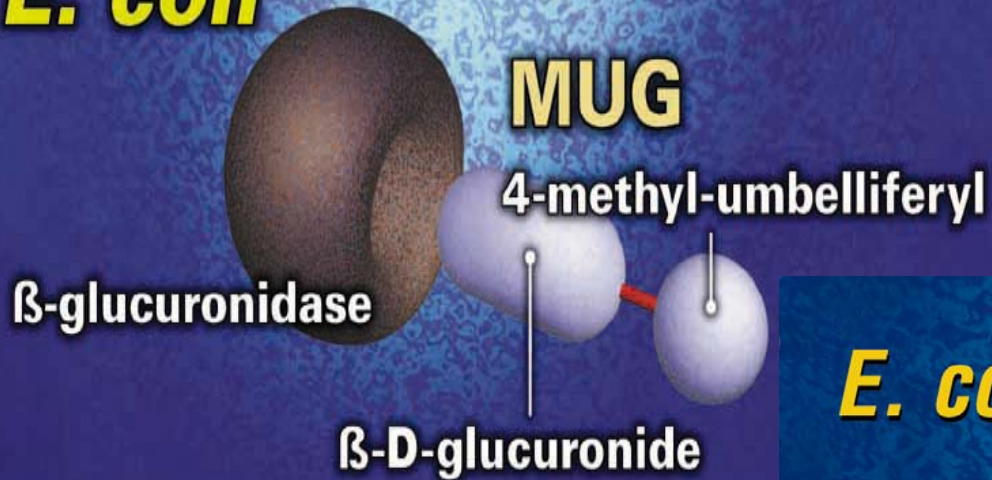
Coliform



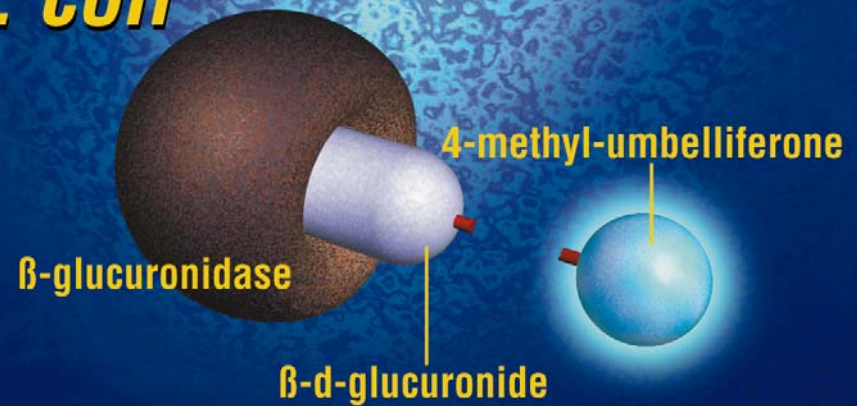
MUG Positive Reaction

Colilert

E. coli



E. coli



Quanti-Tray Demonstration



Add Colilert to sample
and shake to dissolve



Pour mixture into a
Quanti-Tray

Quanti-Tray Demonstration

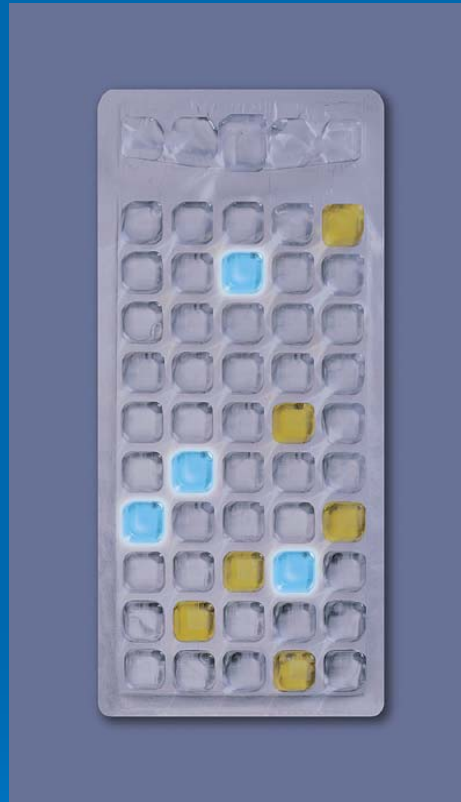


Seal and then incubate at
35°C for 24 hours



Count positive wells and
refer to MPN table

E.coli Positive Tray



Federal Regulations

Safe Drinking Water Act (SDWA)

1974, 1984, 1996

➤ 1989 Total Coliform Rule

- MCLG of zero
- EPA Proclaims Absence of TC = pathogen free water
- <5% positivity allowed
- EPA says a single coliform detection does not necessarily represent a health risk
- A separate MCL for fecal coliform or *E. coli* is not needed

1989 Total Coliform Rule (Continued)

- Required Fecal coliform or *E. coli* testing on all TC positives
- Required public notification
 - More rapid notification for acute (fecal contamination) events
- Policy conflict – no MCL on trigger for acute violation

Federal Regulations

Safe Drinking Water Act (SDWA)

1974, 1984, 1996

➤ 2000 Public Notification Rule

- Tier 1, 2 and 3 violations
- Total coliform detection is no longer considered an immediate public health threat

Federal Regulations

Safe Drinking Water Act (SDWA)

1974, 1984, 1996

- 1996 Information Collection Rule
 - Allowed exemption from *Cryptosporidium/Giardia* testing based on Fecal coliform or *E. coli* testing results

Federal Regulations

Safe Drinking Water Act (SDWA)

1974, 1984, 1996

- **Surface Water Treatment Rule, Interim Enhanced Surface Water Treatment Rule, Long Term 1 and Long Term 2 Enhanced Surface Water Treatment Rule**
 - Specifies only *E. coli* as the fecal contamination indicator

Federal Regulations

Safe Drinking Water Act (SDWA)

1974, 1984, 1996

- 2006 Ground Water Rule
 - Allows *E. coli*, enterococci or coliphage as fecal indicators
 - Conflicting statements on TC vs fecal indicators as protective of public health

Other Federal Agencies

- No other federal agencies require testing for total coliforms
- USDA, USGS, FDA and CDC all have *E. coli* based regulations in place

Australia	Thermo-tolerant coliforms or <i>E. coli</i>	2003	TC not required
Canada	<i>E. coli</i>	2004	TC used to detect operational failures
European Union	<i>E. coli</i> or enterococci	2003	TC non mandatory
WHO	Thermo-tolerant coliforms or <i>E. coli</i>	2006	TC not accepted

Non Fecal Pathogen Issue

- Biofilm pathogens
 - *Legionella*, *Mycobacteria* and the protozoans *Acanthamoeba* and *Naegleria fowleri*
- Total coliforms emanate from Biofilms and therefore are indicative of biofilm pathogens
 - Not always true, could be risky practice
- Biofilm pathogens require specific testing.

Literature Based Caveats Regarding the Continued Use of Total Coliform as a Fecal Contamination indicator

- Growth in distribution systems
- Ubiquitous occurrence in nature where fecal contamination is not evident.
- Not reliable indicators in actual waterborne disease outbreaks

Literature Support For *E. coli* As the Ideal Indicator of Choice

- Advent of fast, reliable enzyme detection technologies
- *E. coli* is a major constituent of the fecal flora of warm blooded animals.
- Fecal contamination events rarely occur without detectable *E. coli*.
- *E. coli* survives but does not multiply in the aquatic environment
- Almost all *E. coli* detections in drinking water can be traced to a contamination event.

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