

**Recreational Water Testing
Past, Present and Future**

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Objectives

- Introduction to Indicator Bacteria- *E.coli* & enterococci
- Recreation Water Regulation
- Past & Present Methods
- Proposed Legislation for Rapid Methods
- Future Methods
- Q & A

Some Facts & Figures on Beaches from NDRC*

- 2008 facts:
 - Closing & advisory days at oceans, bays & great lake resorts topped 20,000 for the 4th consecutive year
 - Two thirds of the closings & advisories were due to storm sewer run off
 - The percent of water samples exceeding the standard remained steady at 7% for '08,'07 & 8% for '06

*National Defense Resource Council

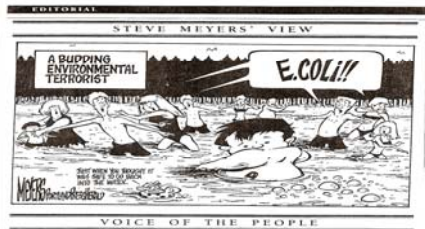
Some Facts & Figures on Beaches from NDRC, con't


•Examples of 2008 ranking of states for water quality

- VA 3rd
- NC 5th
- MD 8th
- FL 9th
- NJ 10th
- MA 15th
- ME 16th
- MI 17th
- SC 18th
- LA 30th
- [NRDC](http://www.nrdc.org/water/oceans/ttw/titinx.asp) (<http://www.nrdc.org/water/oceans/ttw/titinx.asp>)

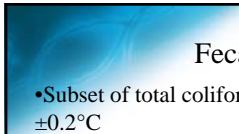
Some Facts and Figures for FL

- > 1000 miles of coastal beaches (Atlantic and Gulf)
- 634 public coastal beaches
- Most coastal swimmers in the Nation
- Peak season- April thru September
- Monitor beaches year round
- 3% of the samples tested > national standard
- Test for enterococci, single event not to exceed 104/100ml, geometric mean of 35/100ml (marine waters)
- Fecal coliforms single sample not to exceed 400/100ml (fresh waters)






Fecal Coliforms



Fecal Coliforms

- Subset of total coliforms- the ability to grow at $44.5^{\circ}\text{C} \pm 0.2^{\circ}\text{C}$
- The term fecal coliform is now referred to as thermotolerant bacteria- USEPA, Standard Methods, ISO methods (Europe) and in Australia & New Zealand as examples
 - not a definition of true fecal coliforms since other thermotolerant bacteria will also grow at this temperature
 - E.coli*, *Klebsiella*, some *enterobacter* and *citrobacter* will also grow and are environmental bacteria.



E.coli

Escherichia coli

- Named after the German Scientist who found this bacteria- Dr. Escherich
- A genus of Gram negative bacteria of the family Enterobacteriaceae
- A type of thermotolerant coliform bacteria commonly found in the intestines of warm blooded animals including humans
- Does not occur naturally in soil and vegetation
- May occur in soil and water as a result of fecal contamination

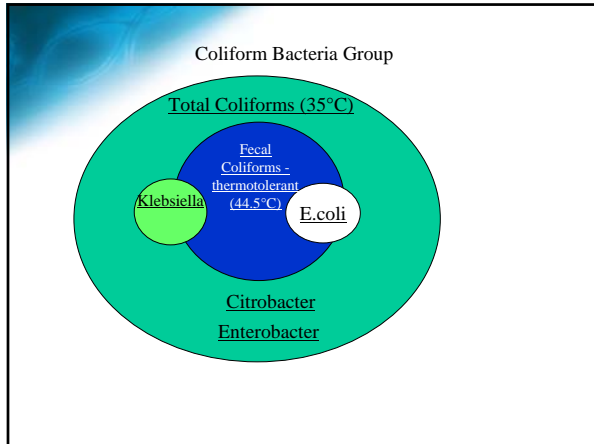
E. coli



Why Test for *E.coli*

Animal	# Tested	<i>E.coli</i>	Klebsiella spp	Enterobacter/ Citrobacter
Human	26	96.8%	1.5%	1.7%
Cow	15	99.9	-	0.1
Horse	3	100	-	-
Sheep	20	97	-	3
Pig	15	83.5	6.8	9.7
Average		94.5%		

Source: *E.coli*: Fecal Coliform A.P. Dufour, Special Technical Publication 65, ASKCM, Pp48-58, 1977



Definitions of Coliforms & E.coli

- Standard Methods for the Examination of Water and Wastewater, 21st Edition & On-Line
 - **9221 MTF**: Gram negative rod shaped bacteria that ferment lactose resulting in gas and/or acid formation (turbidity) within 48 h at 35C.
 - **9222 MF**: Gram negative rod shaped bacteria that develop red colonies with a metallic sheen within 24 h at 35C on m-Endo medium. Some members of the coliform group produce red colonies without a metallic sheen.

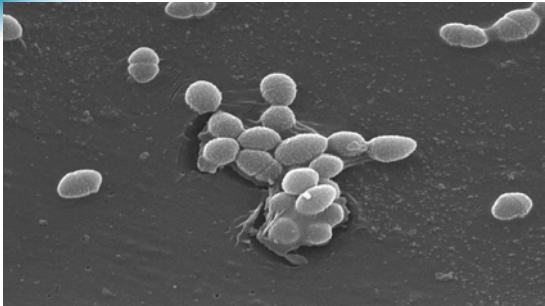
Definition of coliforms and *E.coli*

- **9223 Enzymatic:**
 - The total coliform group is defined as all bacteria possessing the enzyme β -D galactosidase which cleaves the chromogenic substrate resulting in the release of the chromogen.
 - *E.Coli* is defined as giving a total coliform response and possessing the enzyme β -D glucuronidase, which cleaves a fluorogenic substrate resulting in the release of the fluorogen.

Enterococcus vs Fecal Streptococcus

- Enterococci are the key subset of fecal streptococcus
- Fecal Streptococcus
 - 1) Enterococcus spp.:
E. faecalis, *E. faecium*, *E. gallinarum*, *E. avium*, *E. durans*, *E. casseliflavus*
 - 2) Non-enterococci streptococcus
S. bovis, *S. equinus*
- Defined as gram +, catalase -, grows in 6.5% saline, 40% bile salts, and at 10°C and 45°C.

E. faecalis as viewed through a Scanning Electron Microscope



Indicator vs Specific Pathogen Testing

- In a perfect world we would test for all the pathogens in real time
- Technology not available
- Too expensive
- Too time consuming to safeguard against unknown or emerging pathogen

Used with the permission of the WI State Laboratory of Hygiene, University of WI Board of Regents. No endorsement is implied.

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

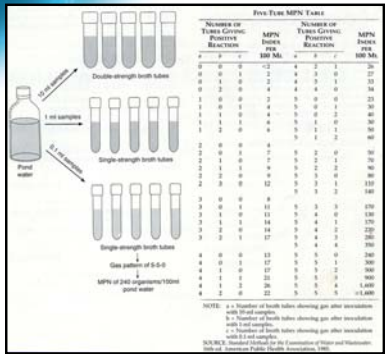
Analytical Methods for Biological Pollutants in the Ambient Water Rule

- Based on 1986 guidelines for E.coli & enterococci
 - EPI study found a direct correlation with gastrointestinal sickness with enterococci for MW and E.coli for fresh waters
- Methods include Colilert and Colilert-18, modified m-TEC for E.coli, Enterolert and m-EI (EPA 1600) for enterococci
- Final rule - 8/20/03

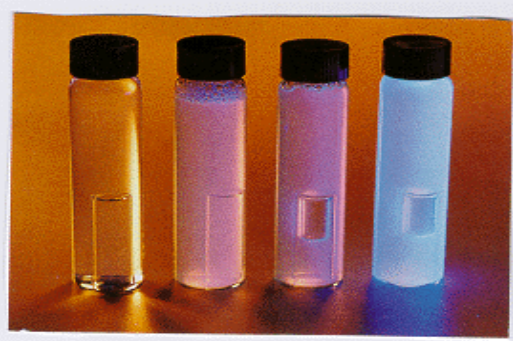
Past and Present Methods

Multiple Tube Fermentation-MPN

Most Probable Number Assay



Fecal Coliform/E. coli Multiple Tube Method -



Membrane Filtration

Membrane Filter Apparatus



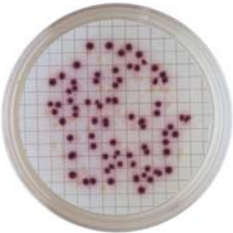


Membrane Filtration-
applicable to all methods

- May required media prep
- Extensive QC
- Minimum of 20 steps
- 20-80 colonies (20-60 for m-FC)
- Risk of confluent growth
- Risk of clogged filters
- Risk of overlapping colonies
- Risk of air bubbles under membrane
- Difficult to read

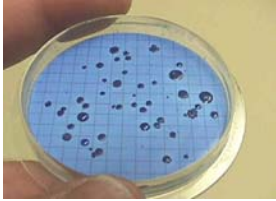
Modified m-TEC for E. coli

- Contains 5-bromo-6-chloro-3-indolyl- β -D-glucuronide
- Selective inhibitory chemicals that can effect the growth of sub-lethal injured bacteria.
- 2 hours of incubation at 35°C followed by 22 hours at 44.5°C
- Positive reaction is red to magenta color colonies
- Extensive QC requirements



m-FC

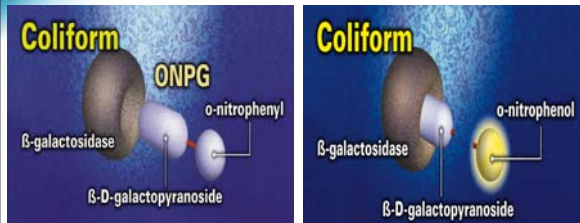
- Enriched lactose medium
- Contains aniline blue
- Larger colonies than m-Endo; range is 20-60 colonies
- Incubate in water bath at 44.5 ±0.2°C for 24 hours in plastic bag
- Blue colonies are positive
- Atypical- grey to green colonies
- Confirm as per section 9020 Standard Methods



Defined Substrate Technology®

ONPG Positive Reaction

Colilert & Colilert-18



MUG Positive Reaction

Colilert & Colilert-18

E. coli
MUG
4-methyl-umbelliferyl
 β -glucuronidase
 β -D-glucuronide

E. coli
4-methyl-umbelliferone
 β -glucuronidase
 β -d-glucuronide

Quanti-Tray Demonstration

Add Colilert to sample
and shake to dissolve

Pour mixture into a
Quanti-Tray

Quanti-Tray Demonstration

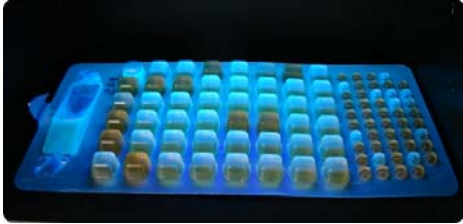
cont.

Seal and then incubate at
35°C for 24 hours

Count positive wells and
refer to MPN table

E.coli- Blue Fluorescence- Quanti-Tray
under a 365nm UV Light

(courtesy of Kim Phillips, Houston Health Department)



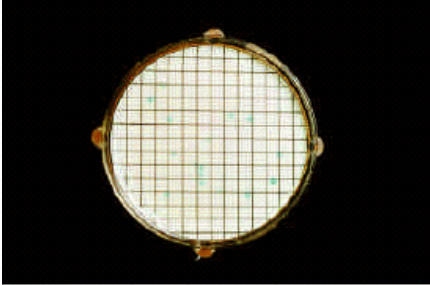
Enterococci
MF and Defined
Substrate Technology®

MF- Enterococci

-mEI Agar (EPA 1600) based on enzymatic method

- Indoxyl β -D Glucoside
- 24 hours at 41°C
- Count all colonies regardless of color with a blue halo as enterococci
- Very small colonies; difficult to interpret
- Colonies <0.5mm are not counted
- Time consuming to prepare and expensive

Method 1600- m-EI



Enterolert™ Demonstration



Add reagent



Seal in a Quanti-Tray
Incubate at 41.5C for 24 hrs

Enterolert™ Demonstration



Count fluorescent wells
and refer to MPN table

Enterococci con't

•Confirmation procedure:

- Transfer colonies to BHI plate (Brain Heart Infusion)
- Incubate for 24 hours at 35°C; transfer a smear to 2 slides
 - Catalase test and a gram stain
- Transfer a loop from the BHI plate and incubate for 24hours at 35°C
 - Transfer a loop onto a BEA plate; Incubate 48 hours at 35°C
 - Transfer a loop into BHI tubes for 48 hours at 10°C
 - Transfer a loop into BHI/6.5% NaCl for 48 hours at 35°C
- Catalase negative (no bubbles from H₂O₂), growth on BEA and turbidity in the BHI & BHI/NaCl confirm enterococci

Why new faster methods? Events and proposed legislation

NRDC and Other Groups

•*The Environmental Protection Agency has agreed to update its 20-year-old beachwater quality standards by 2012. The legal settlement requires EPA to:*

- Conduct new health studies and swimmer surveys
- Approve a water-testing method that will produce same-day results (easier said than done!)
- Protect beachgoers from a broader range of waterborne illnesses
- NRDC is urging Congress to pass the Clean Coastal Environment and Public Health Act, which would require states to begin using rapid-water tests within one year of EPA validation

Proposed National Legislation

- Clean Coastal Environment and Public Health Act of 2009
 - Senate version:
 - Not later than October 15, 2012, the Administrator of the Environmental Protection Agency shall complete an evaluation and validation of a rapid testing method for the water quality criteria and standards for pathogens and pathogen indicators
 - **RAPID TESTING METHOD:** The term ‘rapid testing method’ means a method of testing the water quality of coastal recreation waters for which results are available as soon as practicable and not more than 2 hours after the commencement of the rapid testing method.

Clean Coastal Environment and Public Health Act of 2009

- House version:
 - Requires rapid monitoring at highest priority beaches
 - Post results within 2 hours of receiving a water quality sample
- Concerns from labs about this proposal:**
 - Cost of equipment
 - Personnel to train on newer methods
 - May need to renovate lab for the new equipment/method
 - Newer methods are developed that could possibly obsolete recently purchased equipment in 1-2 years.

Future Methods

- Predictive Modeling
- IMS-ATP
- Molecular Methods- qPCR

Predictive Modeling

- **Great Lakes Predictive Modeling** (2009 Beach Conference)
 - Used to estimate when bacteria levels are above or below the water quality standard so beaches may be closed or advisories issued in a timely manner.
 - Variables used to predict are:
 - wave length/height
 - sun light
 - 24 hour rainfall
 - temp, humidity
 - wind speed & direction
 - water temp.
 - Turbidity, conductivity and pH of water
 - Need to determine the variables for each location

Predictive Models con't

- Data is sent to a computer & a program is applied to determine outcome. A mean, lower and upper interval are established. If lower interval is above the unacceptable level, risk is high, if at the higher interval, risk is low.
- Examples of risk factors are CSO, gull population, sewage outflow, layout of beach

Predictive Models con't

- **Quantitative Microbial Risk Assessment** (2009 Beach Conference, Dr. Joan Rose, Nick Asbolt)
 - Developed over the last decade and used to address both probability of infection and community risks
 - Can be used to fill in “gaps” where there is no epidemiologic data on beach type and health outcome and to aid in selecting appropriate criteria
 - Use this approach to assist in beach notifications
 - Can be used to set priorities for improving the safety of water and setting public policy

Basics of PCR

- Developed by Dr. Kary B. Mullis
- Polymerase Chain Reaction
 - Technique that rapidly amplifies and copies predetermined regions of the DNA (target)
 - Can amplify from a single DNA molecule
 - DNA made up of 4 main pairs
 - Adenine
 - Thymine
 - Guanine
 - Cytosine

PCR con't

- PCR requires close attention to detail and also exclusion of contamination by DNA from the environment- A separate area required for prep of sample and another to test the sample
- Results in 2-4 hours
- Estimate start up cost depends on equipment
 - Estimate from \$30,000-\$50,000
 - Estimated cost/sample \$20-\$25
- At this time cannot differentiate between live and dead bacteria
- At the 2009 Beach Conference, USGS presented a paper on qPCR for E.coli & enterococci

PCR con't

- Probes different between manufacturers, may require a license fee
- Appears EPA leaning towards qPCR for enterococci; however it is not final!
 - Evaluating several beaches presently
 - Completing epidemiological studies
- Beta site testing in CA to develop QC/QA procedures for the method
- Debate at meeting relating to cost, where is the \$ for this?
- Concern if purchase equipment today and 1 year from now, new technology is introduced what will the outcome be?

IMS/ATP Method

- Paper by R.N.Bushon & al; Rapid Detection of E.coli and enterococci in Recreation Water using IMS/ATP
 - A viable alternate to PCR
 - Detects live cells via ATP
 - Easier and simpler than PCR and can be done as a routine test
 - Cost of equipment : start up ~\$6000; cost/sample \$15-\$20
 - Time for testing 2-3 hours
 - IMS -need antibodies and not all are available for all strains
- Method binds the bacteria to antibodies that are attached to magnetic beads to target the bacteria.
- Bacterial concentration is determine by measuring the amount of ATP present in the sample.

Liquid Crystal Biosensor

- Under development by Crystal Diagnostics
 - Developing a rapid microbial detection platform to detect the presence of microbes and pathogens
 - Test is based on using liquid crystals as a biosensor
 - Time for test < 30 mins
- Will this be the future system to replace existing micro methods?

IDEXX Support

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- #2 Technical Service
- #3 Select extension

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That's all folks!

Thank You

Questions
