



# The Monmouth County Department of Health

Lester W. Jargowsky, M.P.H. County Health Officer  
Bill Simmons, Environmental Programs Coordinator

## Recreational Bathing Water Monitoring in Monmouth County

Presented by Becky Cosgrove, MCHD Environmental Laboratory Director

# History of Recreational Bathing in Monmouth Co

- 1985 first ocean beach closures in Monmouth Co due to fecal coliform exceedances
- 1986 CCMP (20 year anniversary!)
- 1986 Pigeons on LB Pier
- 1988 3 weeks of closure (Asbury Park STP)
- 2002 Provisional closings in MC
  - Automatic closure after rain.
- 2004 enterococcus replaces fecal coliform as marine recreational bathing standard in NJ

# Genus Enterococcus

## Why this group as indicator?

- enterococci in water is an indication of fecal pollution and the possible presence of enteric pathogens
  - flora of the gi tracts of humans and other animals.
- Enterococcus typically more human specific than the larger fecal streptococcus group
- Enterococcus differ from streptococcus by being resilient/tolerant organisms
  - distinguished by their ability to survive in salt water and harsh conditions, therefore more closely mimic pathogens
- Strong correlation with bather GI illness

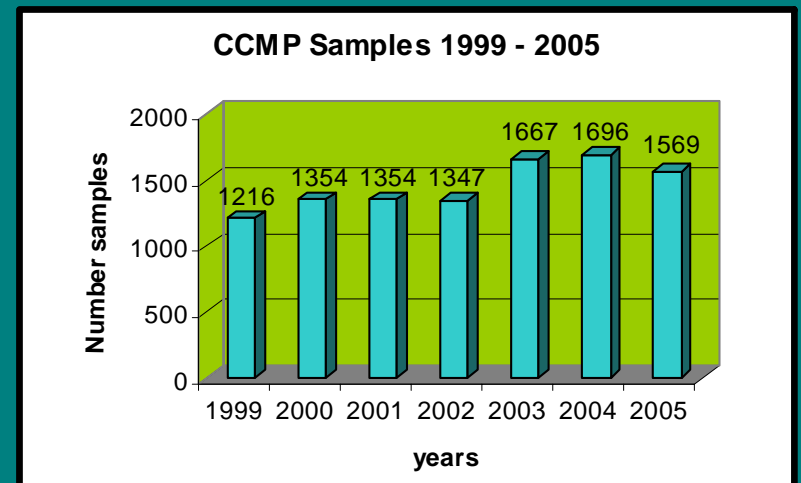
# Monmouth County Dept of Health Environmental Laboratory



- Elective CEHA activity
- Audited by NJDEP Office of Quality Assurance
- Certified for a limited list micro and chemical parameters
- BEACH Program: EPA Method 1600 for enterococcus in 2004


# Monmouth Co. Coastal Monitoring

- Keyport to Manasquan
  - Heavily impacted by Hudson/Raritan plume SB to LB
  - Shallow coastal lakes (9)
- Using Enterococcus as indicator, problem sites were generally the same ones
  - Wreck Pond outlet
  - Bayshore
  - Shark River sites



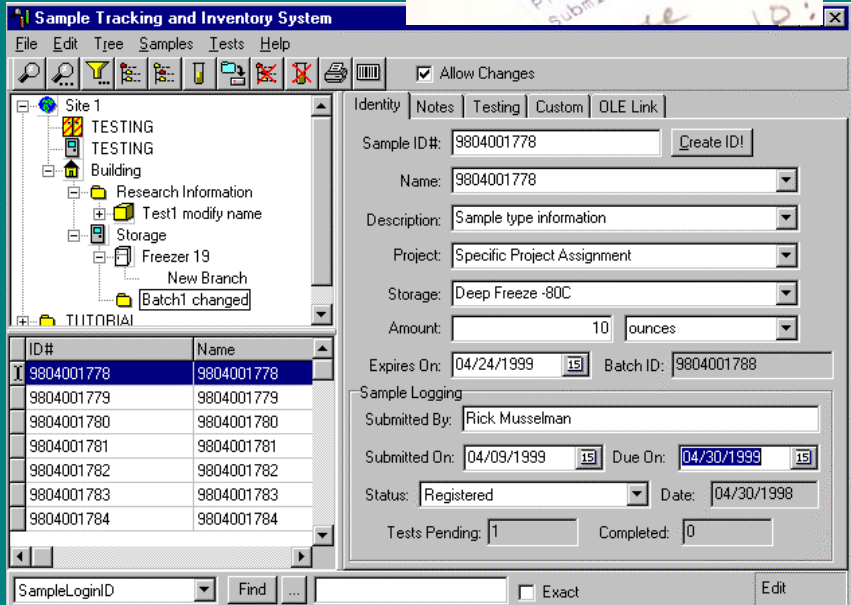
# Environmental Laboratory Sample Entry Sample Tracking and Inventory System(STIS) by ChemSW

- Windows database program bundled with a handheld scanner
  - password protection
  - data accessible to lab staff only
  - drop down lists for fields
    - consistent descriptions
- generates automated sample ID reflects current date
- Zebra barcode label printer
  - sample tags
  - bench sheet



The collage includes a handheld barcode scanner, a Zebra barcode label printer, and a close-up of a printed barcode label. The label contains the following information:

- ID#: A58G0063
- 70
- Project: CCMP
- Submitted: 5/16/2005



The screenshot shows the STIS software interface. The window title is "Sample Tracking and Inventory System". The menu bar includes File, Edit, Tree, Samples, Tests, and Help. The toolbar contains various icons for navigation and actions. The main window is divided into several sections:

- Tree View:** Shows a hierarchical structure starting with "Site 1", followed by "TESTING", "Building", "Research Information", "Test1 modify name", "Storage", "Freezer 19", "New Branch", and "Batch1 changed".
- Table:** A table with columns "ID#" and "Name". The first row is selected, showing ID# 9804001778 and Name 9804001778. Other rows show sequential IDs from 9804001779 to 9804001784.
- Form Fields:** Includes "Sample ID#" (9804001778), "Name" (9804001778), "Description" (Sample type information), "Project" (Specific Project Assignment), "Storage" (Deep Freeze -80C), "Amount" (10 ounces), "Expires On" (04/24/1999), and "Batch ID" (9804001788).
- Sample Logging:** Includes "Submitted By" (Rick Musselman), "Submitted On" (04/09/1999), "Due On" (04/30/1999), "Status" (Registered), and "Date" (04/30/1998).
- Test Counts:** "Tests Pending" (1) and "Completed" (0).

# Reading Plates

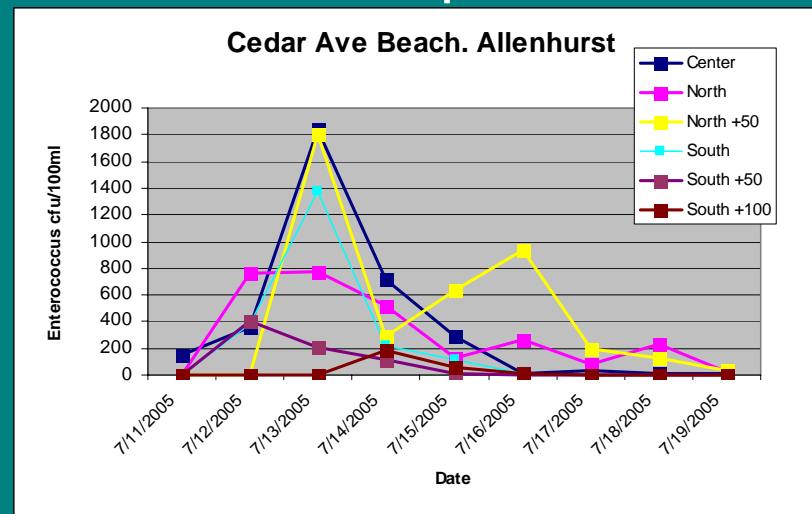


- Scan barcode on plate label
- Count colonies
- Record on benchsheet and read off result
- 2<sup>nd</sup> person for data entry into STIS



# July 2005 Mussels and Seagulls

- Contamination from huge amount of gulls feeding on mussels
- L shaped jetty to hold migrating sand
  - held in mussels and contamination
- Re-growth of bacteria
- Persisted 7 days
- Would have been interesting to know more re: species of entero





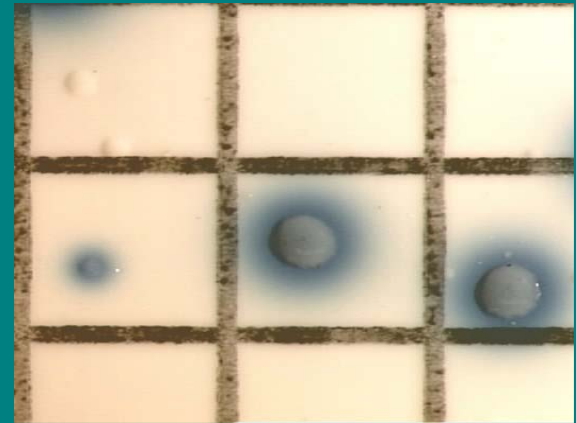
# EPA Method 1600 for Enterococci

- mEI agar
- Membrane filtration w  
24 hour incubation
  - 10 ml of sample to avoid crowded plates
  - 10 colonies is below  $10^4$  cfu/100ml
  - 11 colonies is above  $10^4$  cfu/100ml

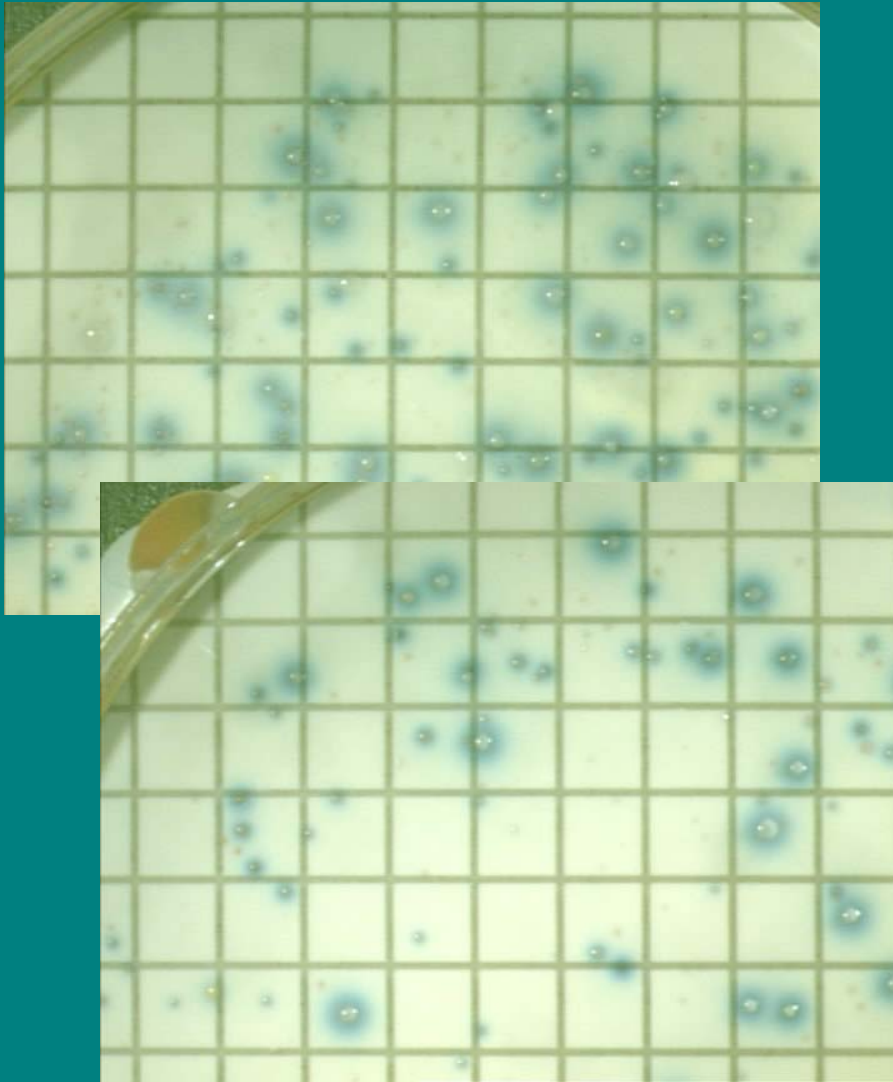


# Enterococci and false positive Aerococci

- Change in definition of typical colony
- September 2002 all colonies with blue halo counted as enterococcus
- July 2004 *Aerococcus sp.* (non-enterococcus) observed as false positive on coastal plates
- EPA clarified typical colony to be one with blue halo and diameter  $> 0.5$  mm
  - Stated intent to revise method with size criteria by the end of 2004
- NJDEP instructs to count blue halo colonies that are  $> 0.5$  mm diameter



# Range of Colony Sizes



- Range of sizes
  - Smaller than 0.5 mm
  - Larger than 0.5 mm
  - Often mix of colony sizes
- If not entero, then what?
- Is it Aerococcus?
- Is it fecal strep?
- why variable appearance?
  - where it comes from?
- Still in the learning phase for entero, especially near storm drains
- Balance protecting public health against unnecessary beach closures
- better decisions with more understanding

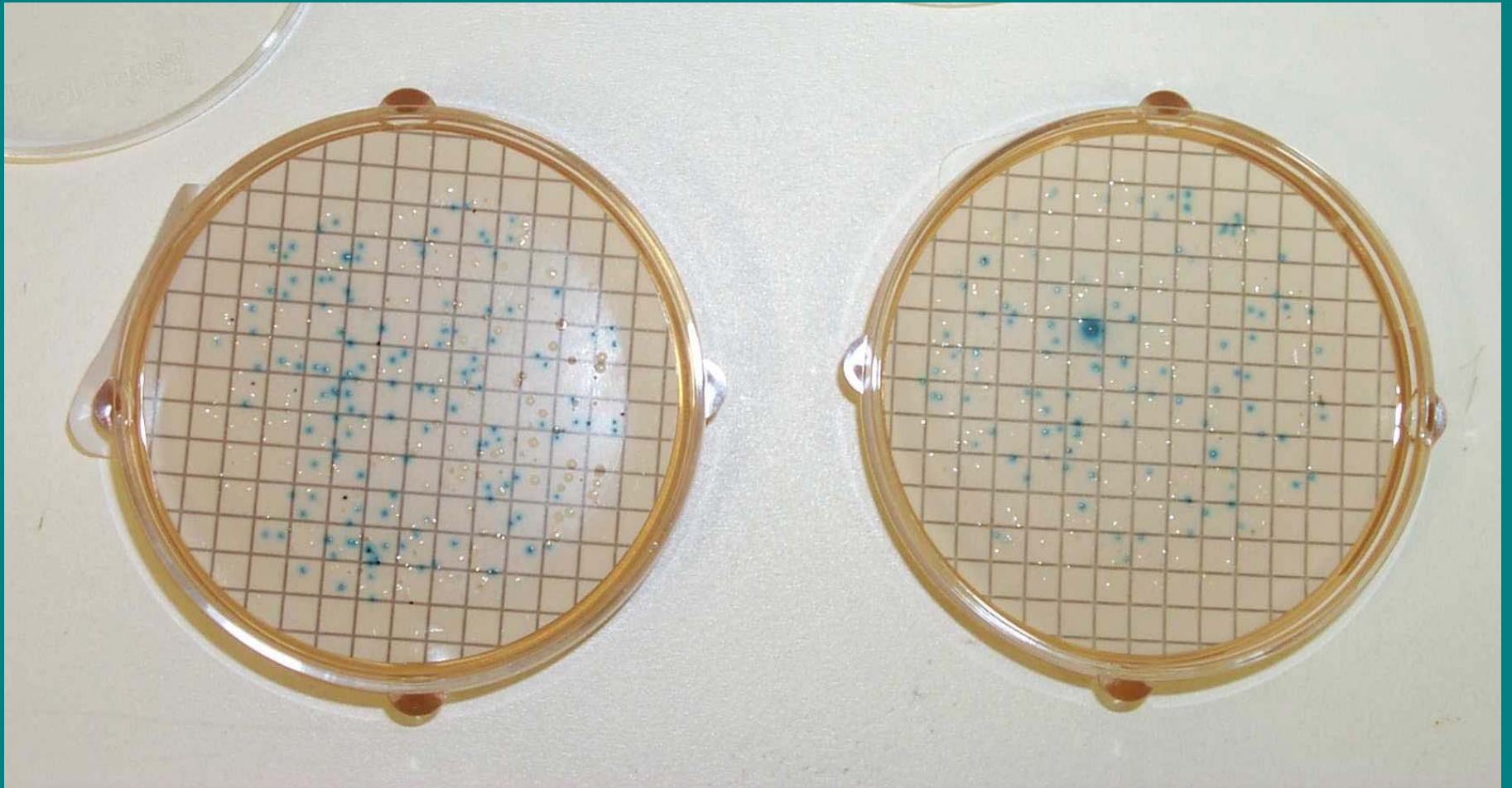
# Understanding the 0.5 mm size rule as it applies to various environmental sample types

- Aerococcus(non-enterococcus) grows slowly(?) so colonies are smaller at 24 hours
- Method 1600 will grow fecal streptococci BUT
  - Rapid die off of *Streptococcus bovis* and *S. equinus* (fecal streptococci) outside of host(cow and horse, respectively)
- *E. faecalis* and *E. faecium* grow as larger colonies
  - Most contamination is *E. faecalis* or *E. faecium*(?) because of survival in saltwater
  - Are resistant organisms
  - Other Enterococcus spp. are bigger or smaller but are not usually significant portion(?)
- Variable in samples depending on the impacts

# Objective 1

- Recognize the false positive, *Aerococcus spp* on mEI agar by:
  - accurate measuring
  - recognition of colony morphology
  - biochemical confirmations

*Enterococcus* sp. or *Aerococcus* sp. or other



Main St. Ocean Grove. Bathing

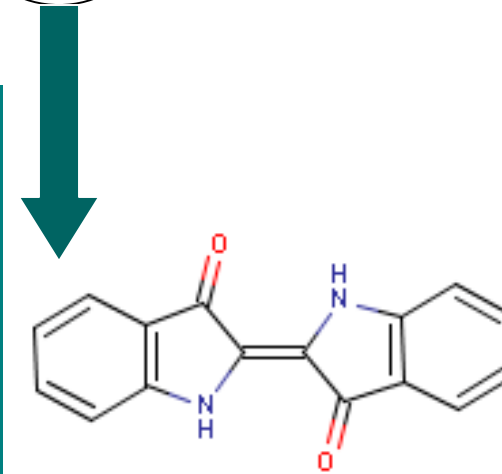
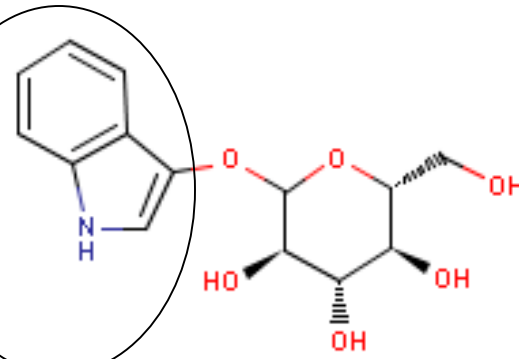
Myron / Wilson. Neptune. Non Bathing

# Blue Halos

## Enzyme Action

- All major enterococci sp. produce enzyme, B glucosidase
- In addition, *Aerococcus viridans* (Family Streptococcaceae) produce enzyme B glucosidase
- Indoxyl B-D glucoside in mEI medium is cleaved
  - Blue halo is formed when Indigo blue complex precipitates and diffuses into surrounding media

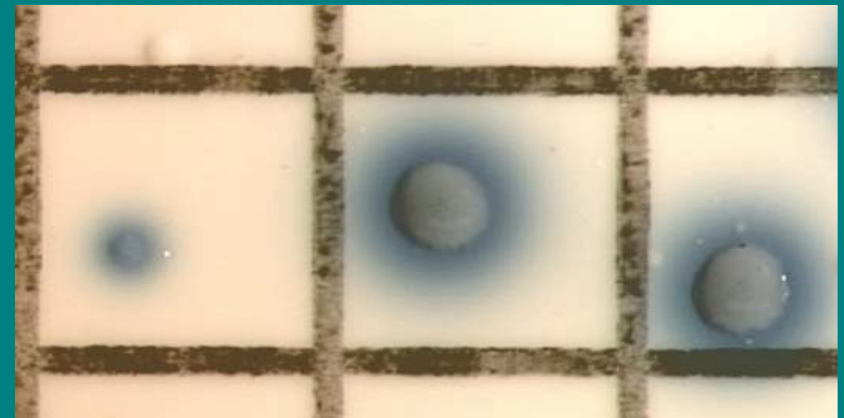
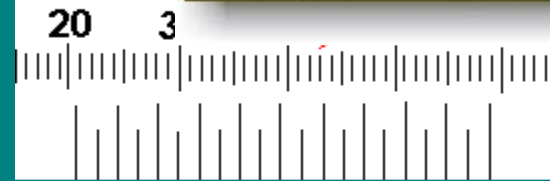
Indoxyl B-D glucoside



indigo blue

# Accurate measurement of colonies

- Method 1600
  - mag of 2-5X or stereoscopic microscope
- Reticles or "eyepiece micrometers" in eyepiece microscope
  - graduations are arbitrary
- reticle is calibrated against a stage micrometer for each objective
- Make table so "units" can be converted to mm

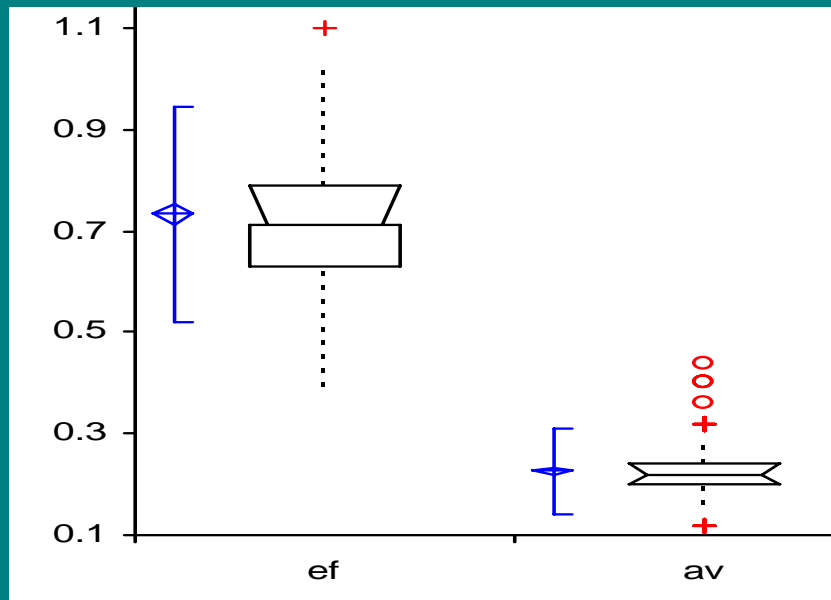




# American Type Culture Collection

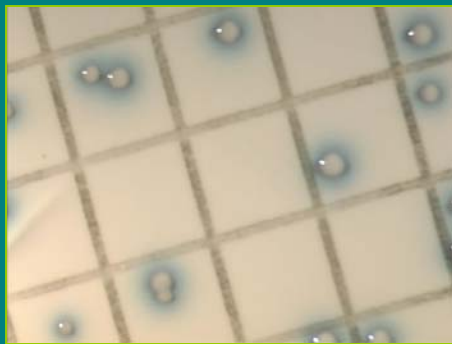
Test viability of null hypothesis

Ho: There is no difference between the size of *Aerococcus viridans* colonies and *Enterococcus faecalis* colonies.

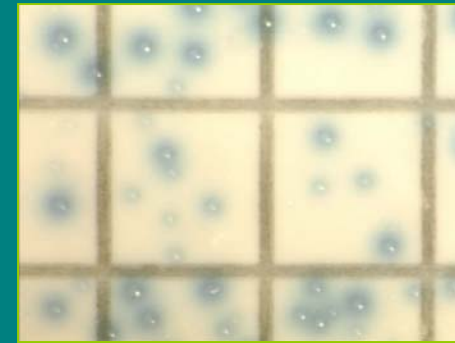


	n	Mean mm.	SD	95% CI of Mean
<i>E. faecalis</i>	139	0.732	0.1277	0.711 to 0.754
<i>A. viridans</i>	286	0.226	0.0504	0.220 to 0.231

Students t test based on ordinary means  
2-tailed p <0.0001 t statistic 42.36



*E. faecalis* ATCC



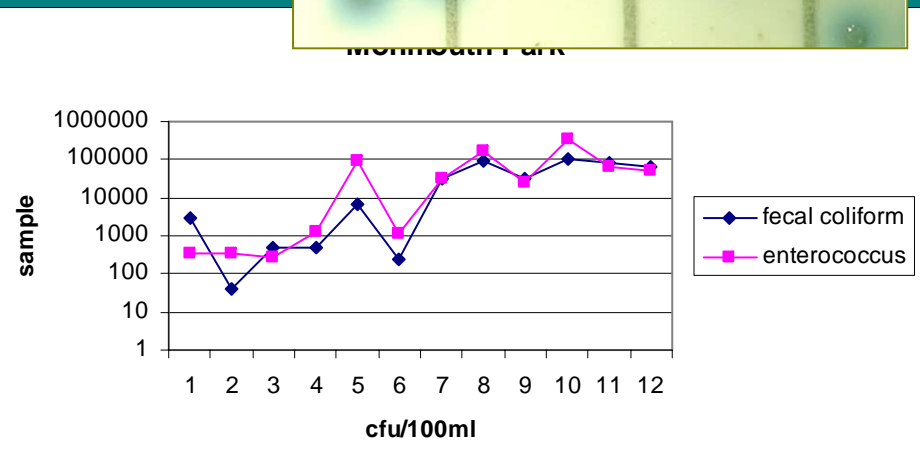
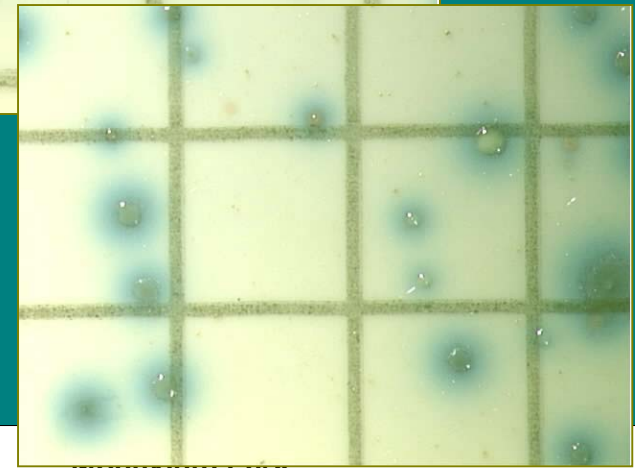
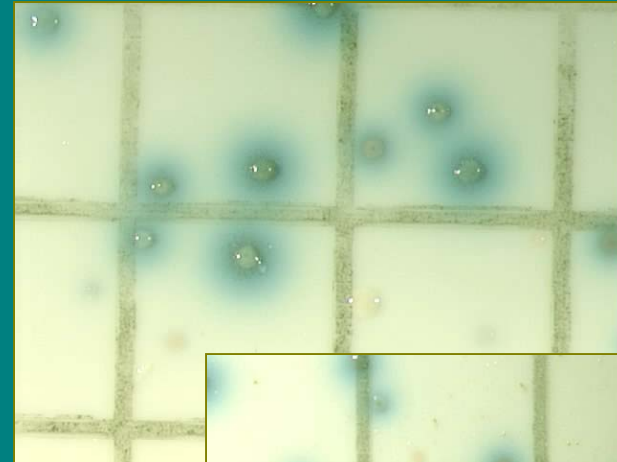
*A. viridans* ATCC

# Biochemical Confirmations

- verify colonies by biochemical tests in method 1600
  - 48 to 96 hours
  - *Aerococcus viridans* usually will not grow in BHI at 45C

# Are all <0.5 mm colonies *Aerococci*?

- Estuary/freshwater samples and Storm drains of areas affected by Horse track/stable
  - 10 years
  - horse waste not managed properly it has a heavy impact on the surrounding waterbody
  - elevated fecal coliform
  - Method 1600 colonies are diameter <0.5 mm
- *Aerococcus* usually not associated with fecal waste
- Obvious sanitary issue overlooked if not counting smaller colonies



# <0.5 mm Fecal Streptococci?

Fecal streptococci

Enterococcus

*E. faecalis*

*E. faecium*

*E. avium*

*E. gallinarium*

*S. bovis*

*S. equinus*

- Assumptions that are accepted at the beaches, ie that streptococci have died off, does not work here
- close to the source and the plates are probably a mix of fecal streptococci
- Consider Streptococcus
  - *S equinus* is host specific and assoc w/ horses
  - *S equinus* assoc with runoff from feedlots and farmlands
  - Limited survival outside of animal intestinal tract
  - Indicates recent contamination

# Biochemical Confirmations

- verify colonies by biochemical tests
- Count colonies as presumptive for enterococcus
- What percent of 10 colonies confirm
- Use probability methods to adjust counts
- Non enterococcal group D streptococci such as *S. bovis* and *S. equinus*
  - Cannot grow in BHI with 6.5% NaCl

# Other researchers

(Ferguson et al. Sept 2005 Journal of Applied Microbiology)

- 11-26% false positive for environmental samples by method 1600
- 29.6% of the isolates from the mEI were *Streptococcus bovis* (non-enterococcus)
- Depends on where/what sample type (impacts)

# Further research on environmental samples

- Gaining better understanding of method 1600 outside of beaches
  - Freshwater / Estuary samples that impact swimming area, storm drains
  - Complaints and investigations
- If method 1600 grows fecal streptococci
  - Where/when does it die off in relation to bathing beaches
- May be important to id species
- Use fecal or *E. coli* and entero together

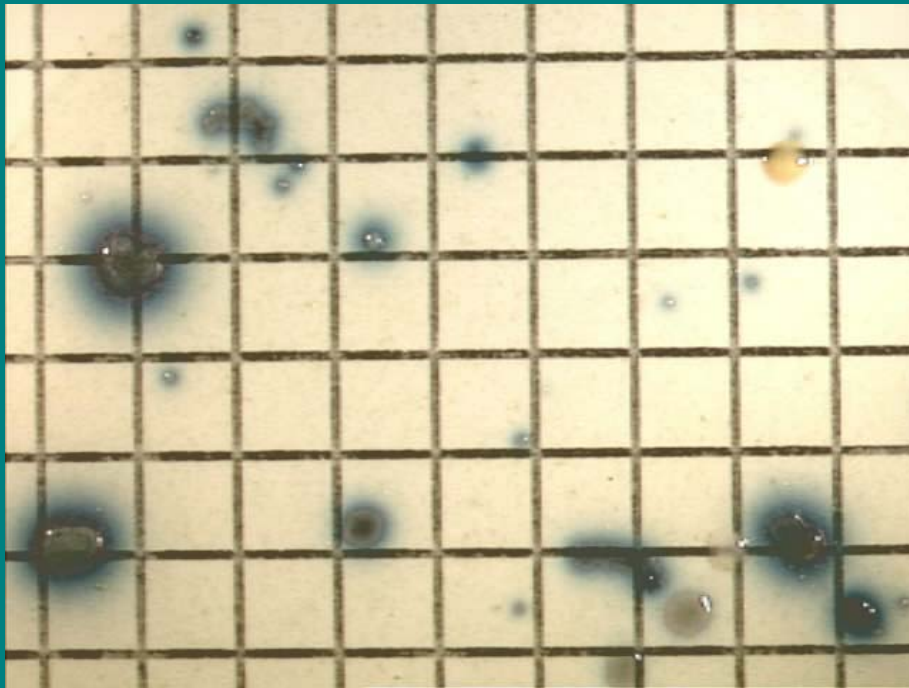


# Objective 2

- Collect data, for various environmental samples, on the specificity of mEI agar



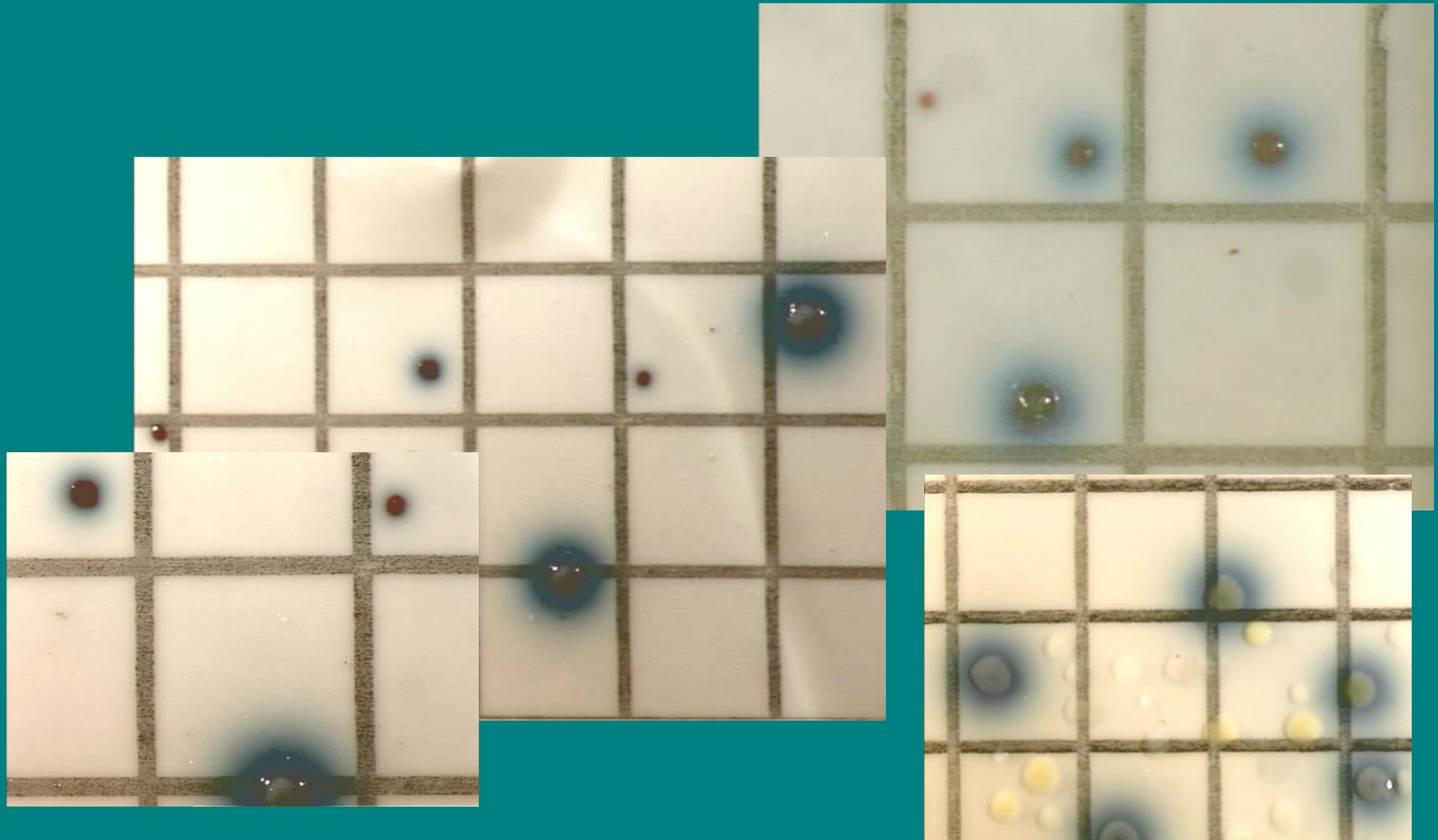
# Specificity



Beach at L street

- Mixed growth plates are difficult to count
  - detritus causes colonies to grow together
  - Range of sizes
  - Range of halo intensity
  - Grey colonies

Small (0.2-0.3mm) red colonies with blue halos



# Method 1600 Biochemical confirmation of isolates

- enterococci
  - Gram + cocci
  - grow in BHI @ 45C
  - BHI with 6% NaCl
  - hydrolyse esculin
- Use various environmental samples
- compare MCHD false neg/false pos rate to EPA reported rate for method



# MCHD

## False negative/False positive rate

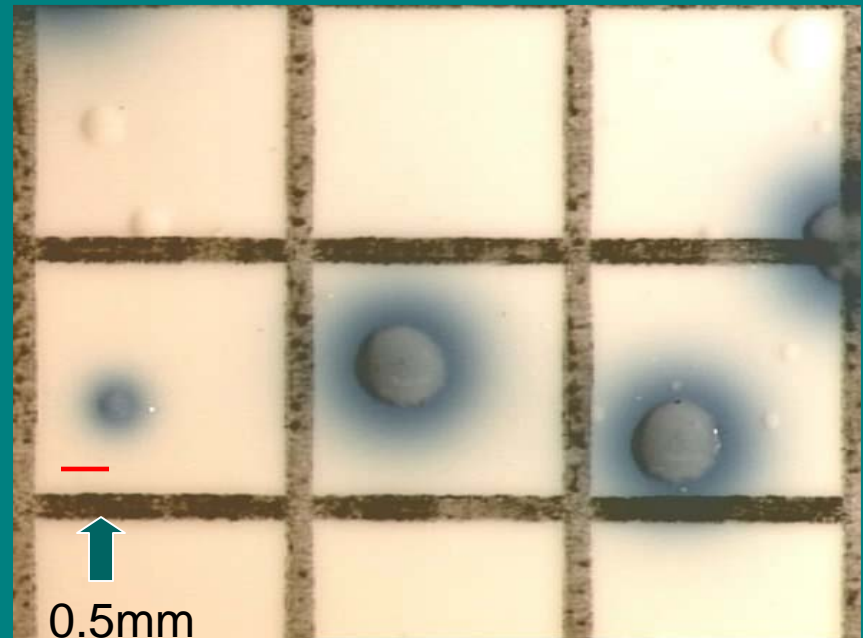
Blue w blue halo > 0.5 mm N=138	Blue w blue halo < 0.5 mm n=116
78% confirmed (22% false neg)	58% confirmed (42% false pos)

# USEPA Method 1600 specificity of the medium

- USEPA METHOD
  - false negative rate is 6%
  - false positive rate is 6.5%
- Based on various environmental samples
- Assumptions are that EPA data based on random selection of typical colonies, regardless of size, that grew on mEI.
  - April 2002 definition of a typical colony is “all colonies (regardless of color) with a blue halo”

# Document Colonies

- Typical colony are those w diameter  $>0.5$  mm and have a blue halo
- Measure colony only, not including halo
- Record all colony sizes on back of benchsheet
- Record any unusual or interfering growth
- Digital Images



# Objective 3

- Determine species

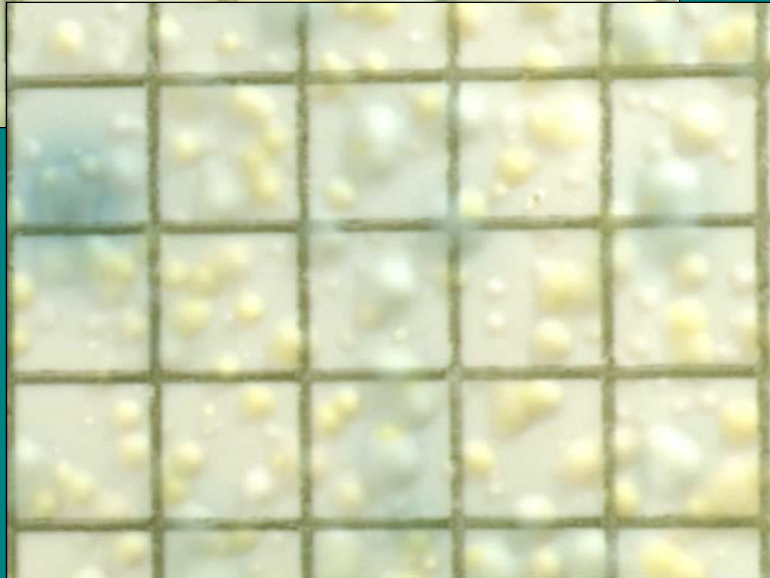
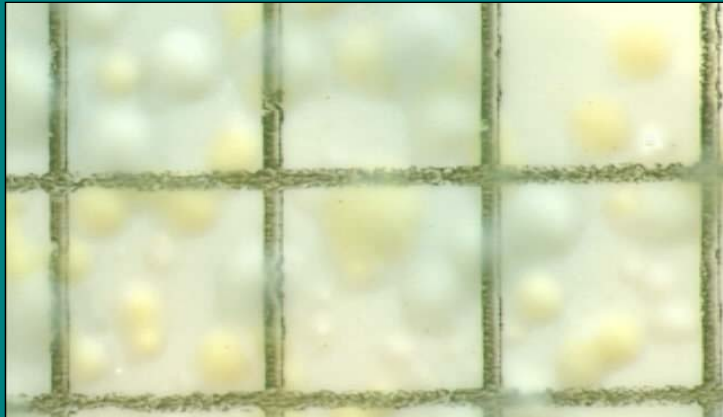
# API kits



- Use API(BioMerieux) on isolates from coastal samples. What gram positive, catalase negative, bacteria grow on mEI agar
  - *Enterococcus faecium*
  - *E. faecalis*
  - *E. durans*
  - *E. avium*
  - *E. gallinarium*
  - *Streptococcus uberis*
  - *Aerococcus viridans*
- Accuracy varies by species
- Exercise caution when using rapid biochemical kits for environmental entero strains. (DF Moore 2005 ASM meeting)
- Expensive



# Confluent Staphylococcus on CCMP Samples (Coagulase Negative Staph)



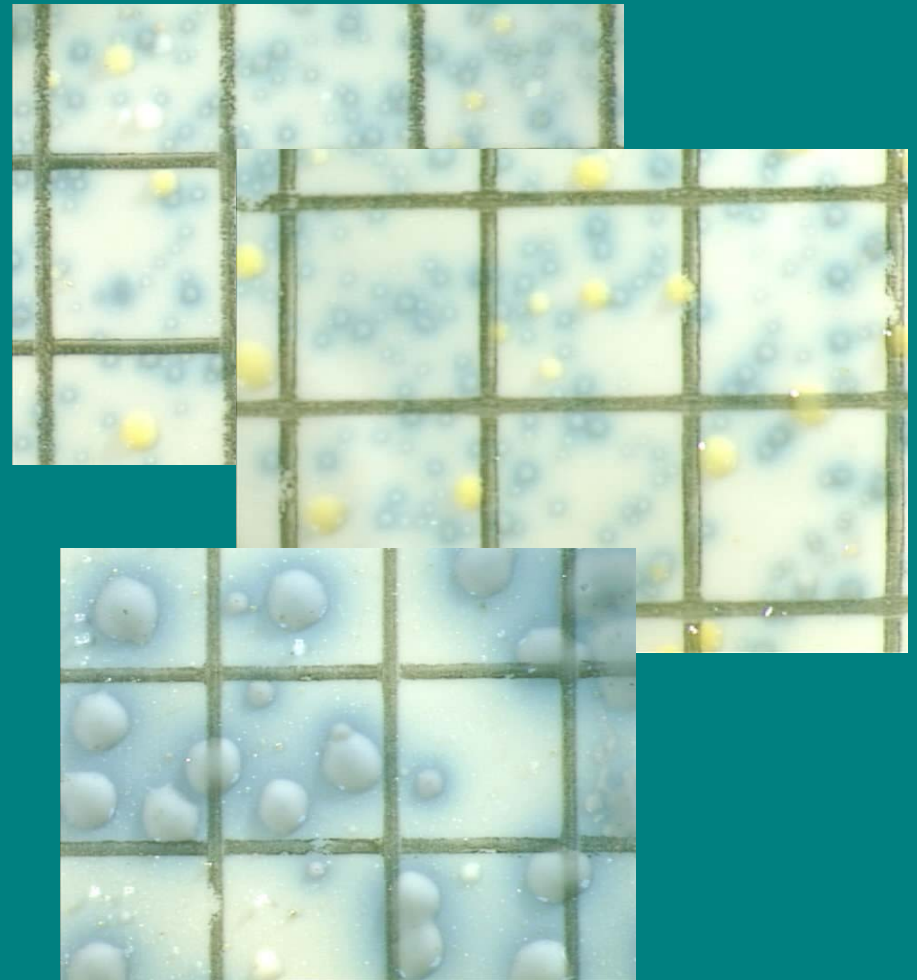
- confluent growth later determined to be *Staphylococcus sp.*
  - June 2005
  - occasionally, although to a lesser degree, throughout the summer.
  - September 2005
- interfering confluent growth
- *Staphylococcus*
  - temperature range of 15 to 45C
  - 15% NaCl concentrations (likes salt)
  - not considered to be a natural inhabitant of environmental waters

# Confirm Staphylococcus

- Catalase test to differentiate strep from staph
- Coagulase negative staph
- API Staph Kits
  - Species ID Not successful
  - CNS difficult to determine because many CNS isolates show indeterminate traits
  - species is not one the kit codes for
  - Kits for most prevalent clinical pathogens
- Outside lab (PHRI) confirmation

# Growth on mEI has variable appearance

- Recognize and differentiate the false positive *Aerococcus*
- Investigate non-typical growth of various environmental samples
- PCR analysis on colonies



Any questions?

