



The Monmouth County Department of Health

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Confirming Enterococcus Isolates From Bathing Beach Samples

by Becky Cosgrove, Environmental Laboratory Director

Genus Enterococcus

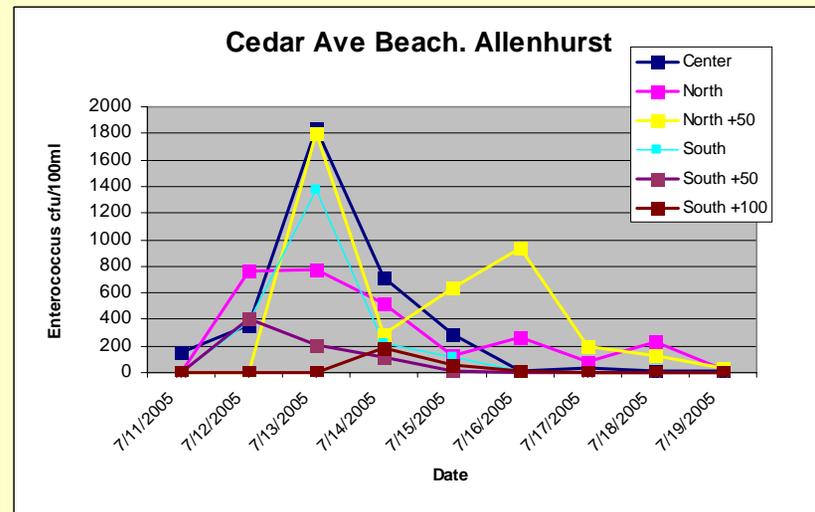
Why this group as indicator?

- enterococci in water
 - indicator of fecal pollution and possible presence of enteric pathogens
- Strong correlation with bather GI illness.
- 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

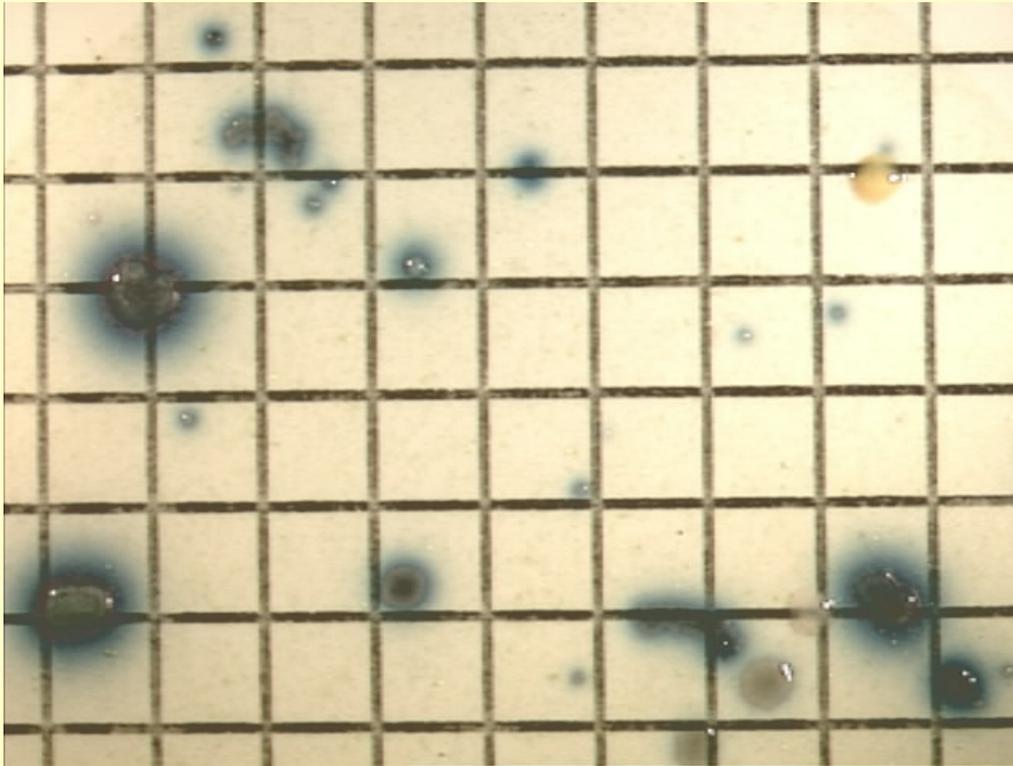
July 2005 Mussels and Seagulls



- 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



Range of colony sizes

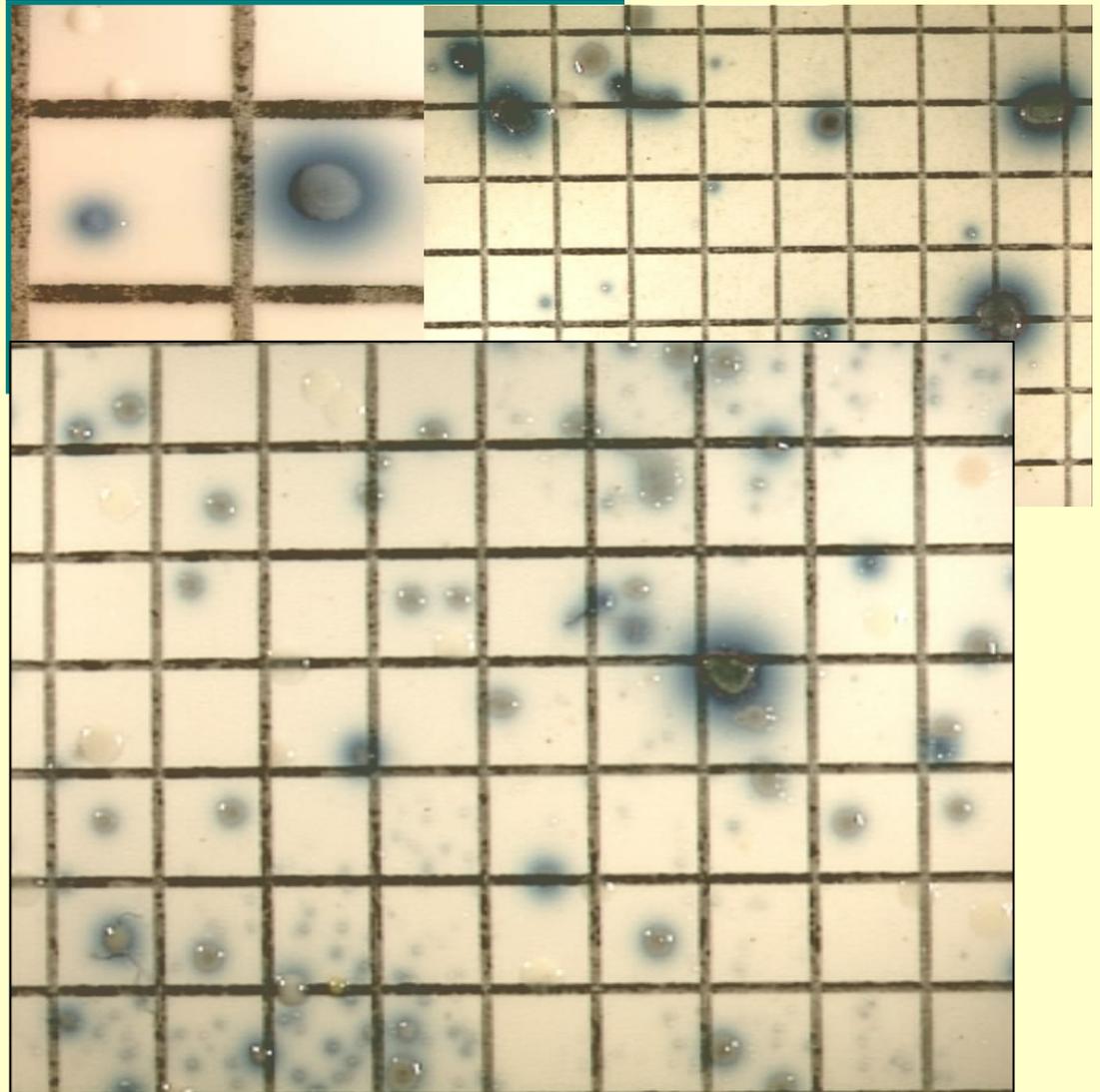


Bathing Beach at L street

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

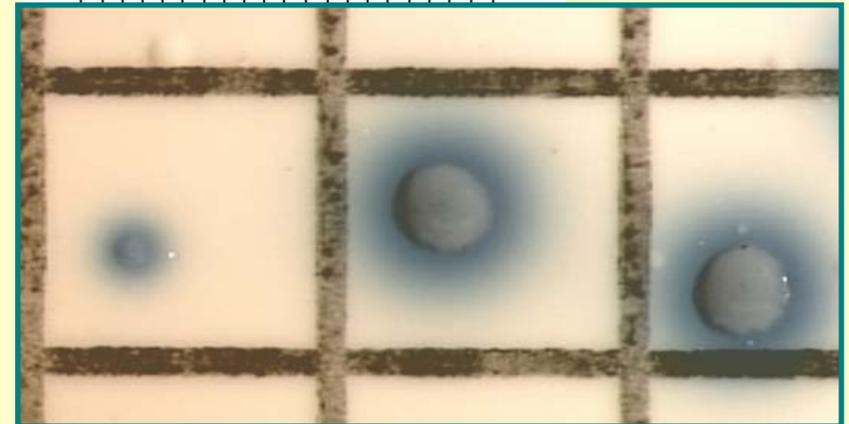
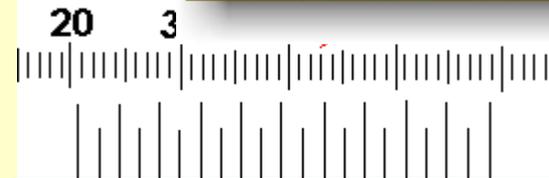
Method 1600 Enterococci: Change in definition of typical colony

- Enterococcus is the target indicator bacteria for recreational marine bathing. USEPA Method 1600
- USEPA July 2004 issued clarification definition of typical colony to be not <0.5 mm
- Size criteria in July 2006 method



Accurate measurement of colonies

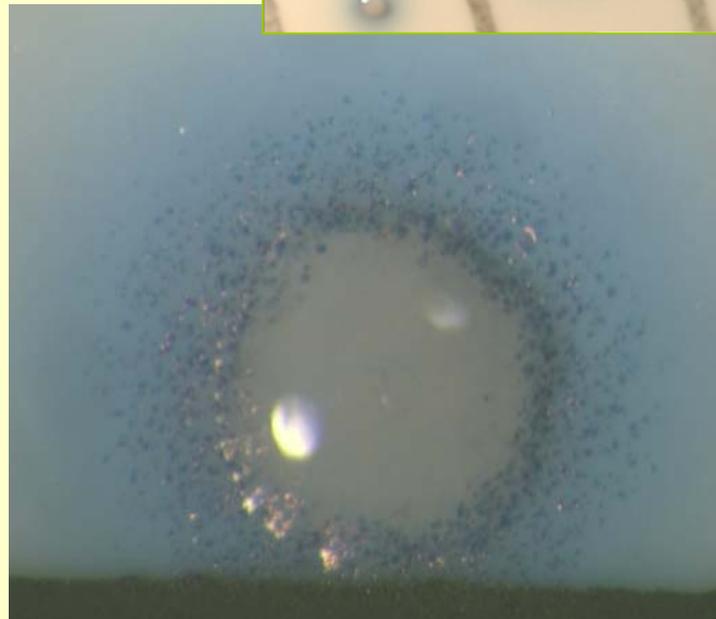
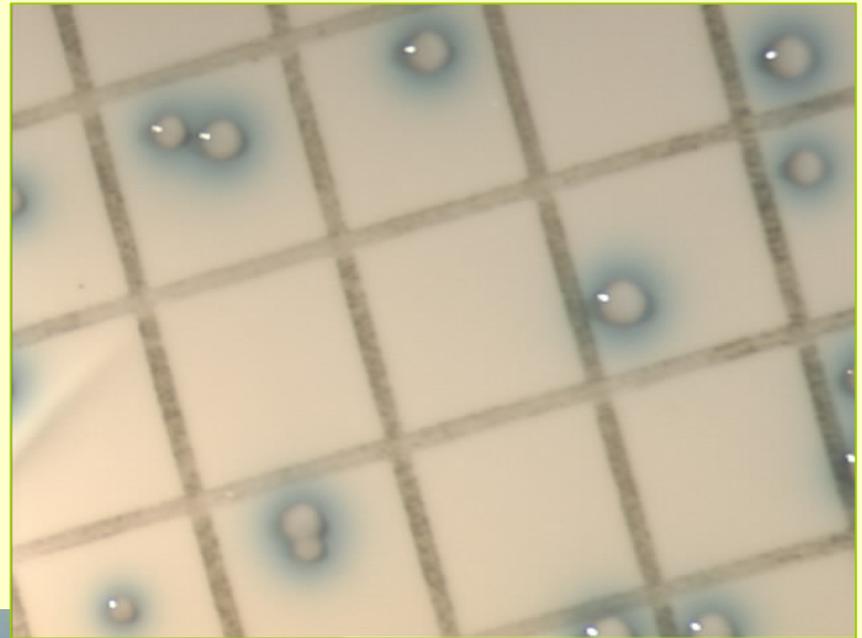
- Method 1600
 - mag of 2-5X or stereoscopic microscope
- Reticles or "eyepiece micrometers" in eyepiece microscope
- reticle is calibrated against a stage micrometer for each objective



BIO BALL btfbio.com

reference material

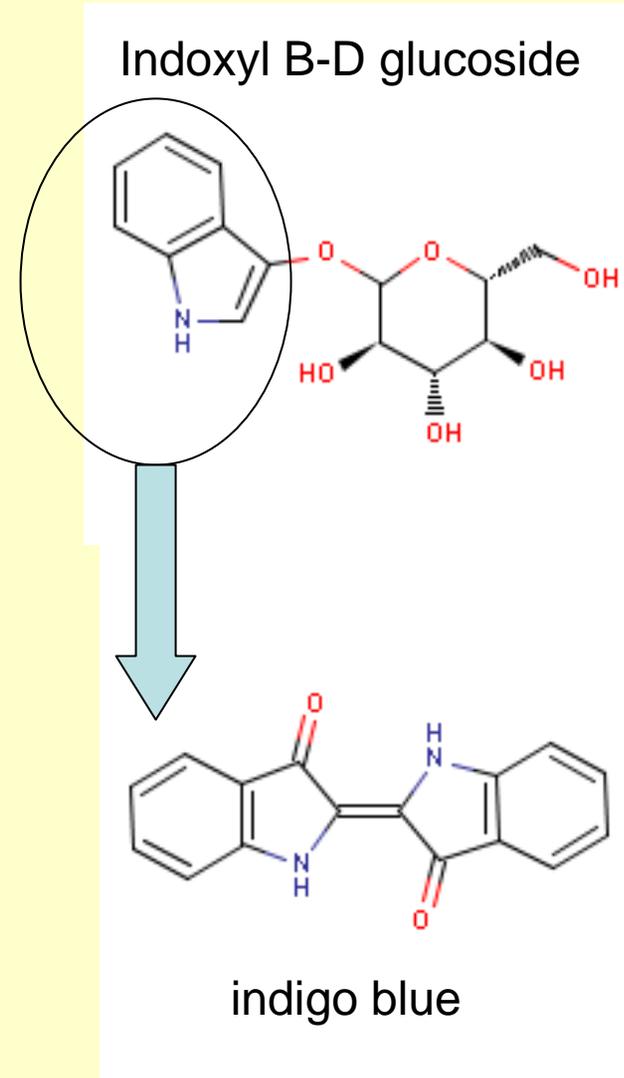
- *Enterococcus faecalis* BIOBALL
 - Precise # of cells
 - about \$17.00 each ball
 - use for OPR
 - colony has indigo precipitate which appear as crystals.



Blue Halos

Enzyme Action

- All major enterococci sp. produce enzyme, B glucosidase
- So do some *Streptococci* sp.
- So do *Aerococcus* sp.
- Indoxyl B-D glucoside in mEI medium is cleaved
 - Blue halo is formed when Indigo blue complex precipitates and diffuses into surrounding media



Aerococcus viridans
(non-enterococcal bacteria)

- A NJ Department of Environmental Protection(NJDEP) Division of Science, Research, and Technology white paper reported a high-rate false positive organism when using the September 2002 version of Method 1600 for marine bathing water samples(1).

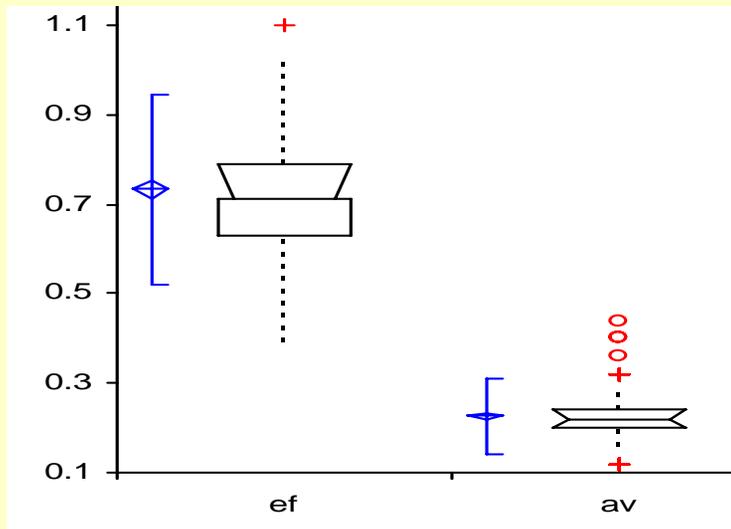
- The NJDEP white paper concluded the false positive was *Aerococcus viridans*.

1. Feerst, E., Hovendon, B., Atherholt, T. (2005) NJDEP Division of Science, Research and Technology. The September 2002 Version of the Method 1600 Enterococcus Test: *Aerococcus viridans* as a false positive organism.

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.

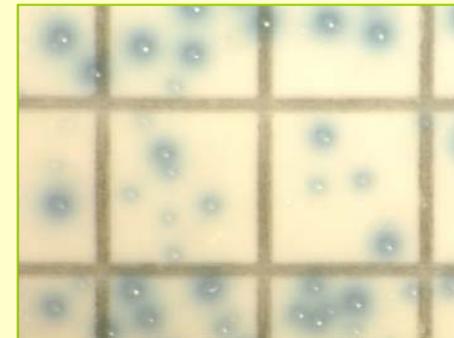


| | n | Mean mm. | SD | 95% CI of Mean | |
|--------------------|-----|----------|--------|----------------|----------|
| <i>E. faecalis</i> | 139 | 0.732 | 0.1277 | 0.711 | to 0.754 |
| <i>A. viridans</i> | 286 | 0.226 | 0.0504 | 0.220 | to 0.231 |

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



E. faecalis ATCC



A. viridans ATCC

Range of colony sizes

False negative/False positive study

Number of isolates from mEI and per cent that confirmed as Enterococcus using biochemical tests*

| Year | typical ≥0.5 mm | non-typical < 0.5 mm |
|------|--------------------|-------------------------|
| 2005 | 101(54%) | 34(68%) |
| 2006 | 138(94%) | 63(62%) |
| 2007 | 115(99%) | 49(51%) |

*number in parentheses indicates percent that confirmed

Confirmed for Enterococcus by classical biochemical techniques

- BHI at 45C
- BHI with NaCl
- Hydrolysis of Esculin

Attempt to determine species using API kits

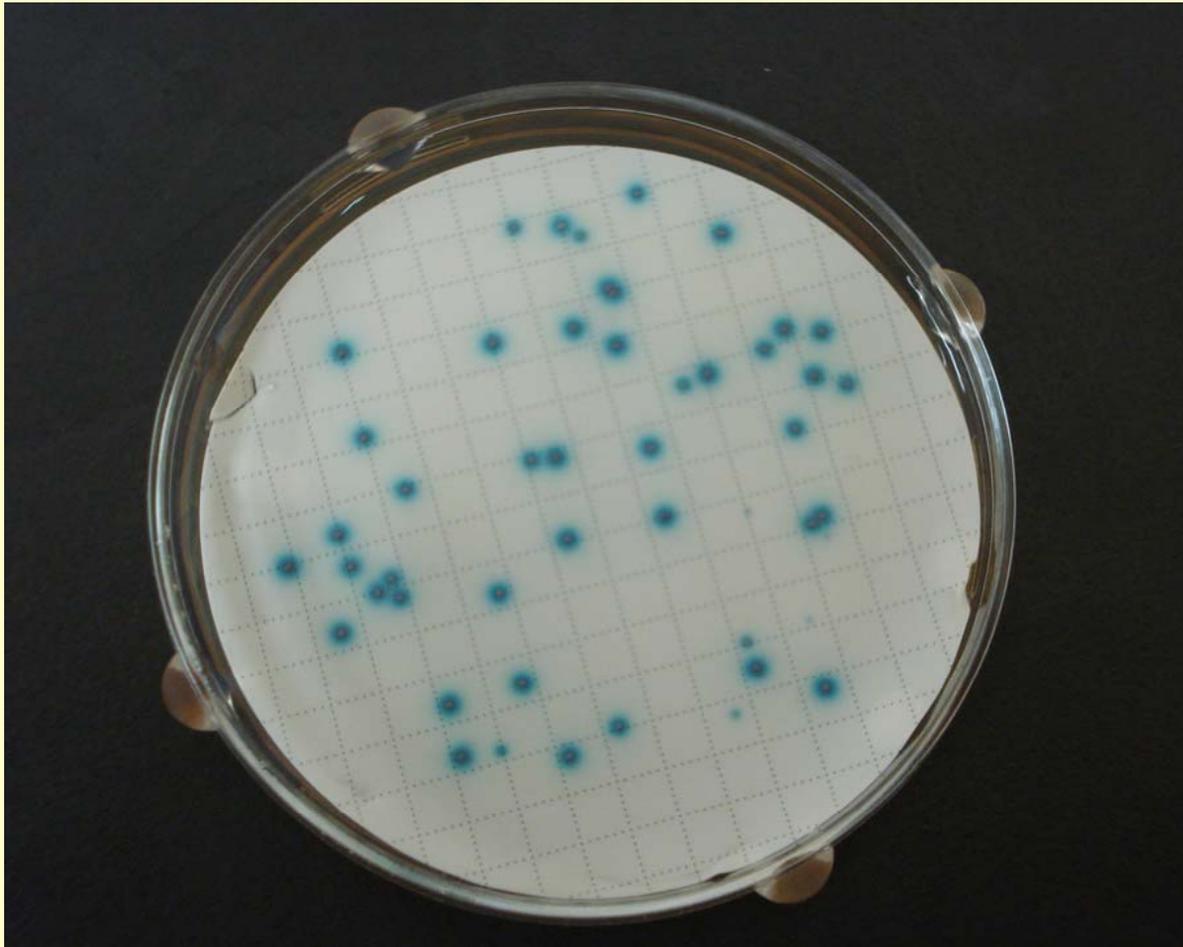


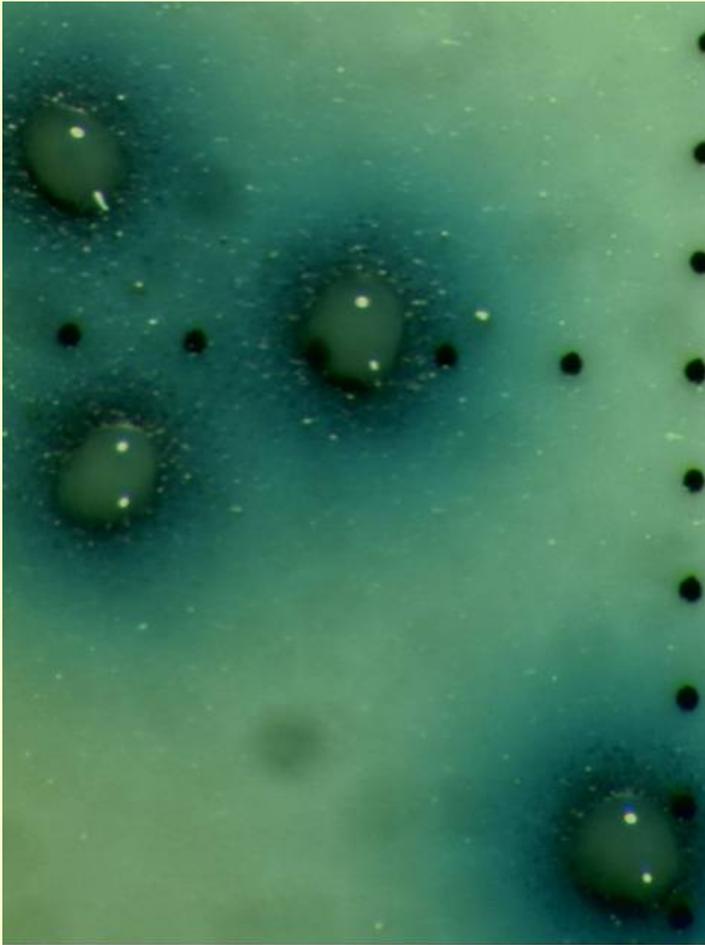
- Use API(BioMerieux) on isolates from coastal samples. What gram positive, catalase negative, bacteria grow on mEI agar
 - *Enterococcus faecium*
 - *E. faecalis*
 - *E. durans*
 - *E. avium*
 - *E. gallinarium*
 - *Streptococcus uberis*
 - *Aerococcus viridans*
- Accuracy varies by species
- Exercise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

Optical Illusion

Are smaller than 0.5 mm

Look like enterococci colonies but are actually <0.5 mm



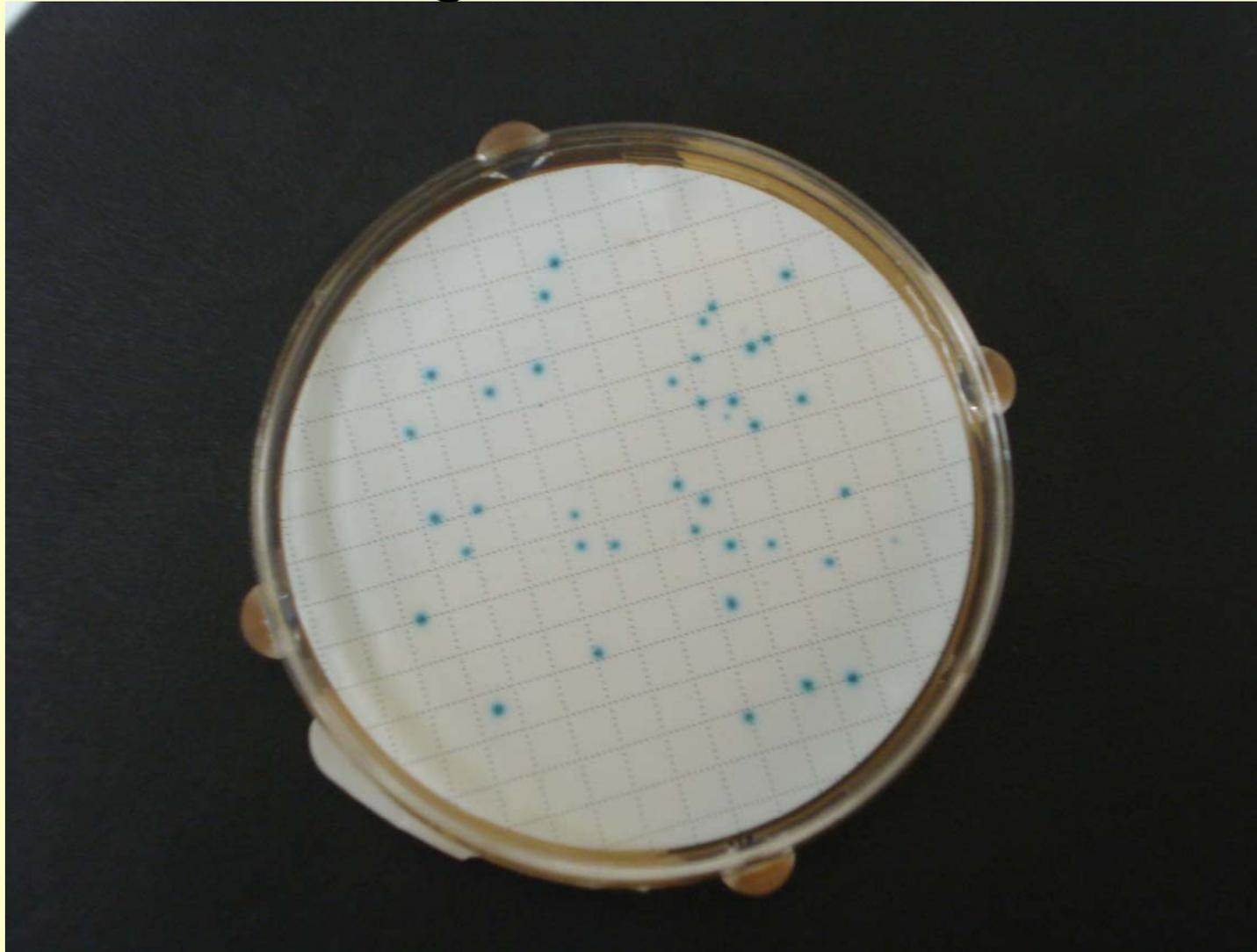


Mag. 30X Enterococcus colonies of this type appear wet, reflecting light, raised gum drop shaped, and the blue halo sparkles with indigo crystals. (Can tell by the grid spots that are <0.5 mm.)

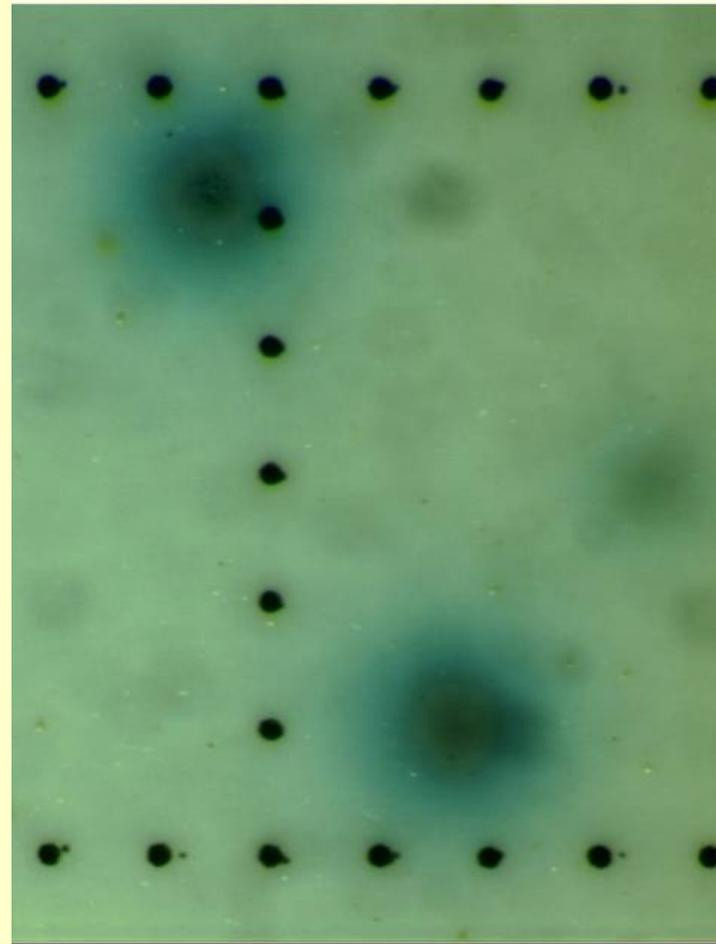
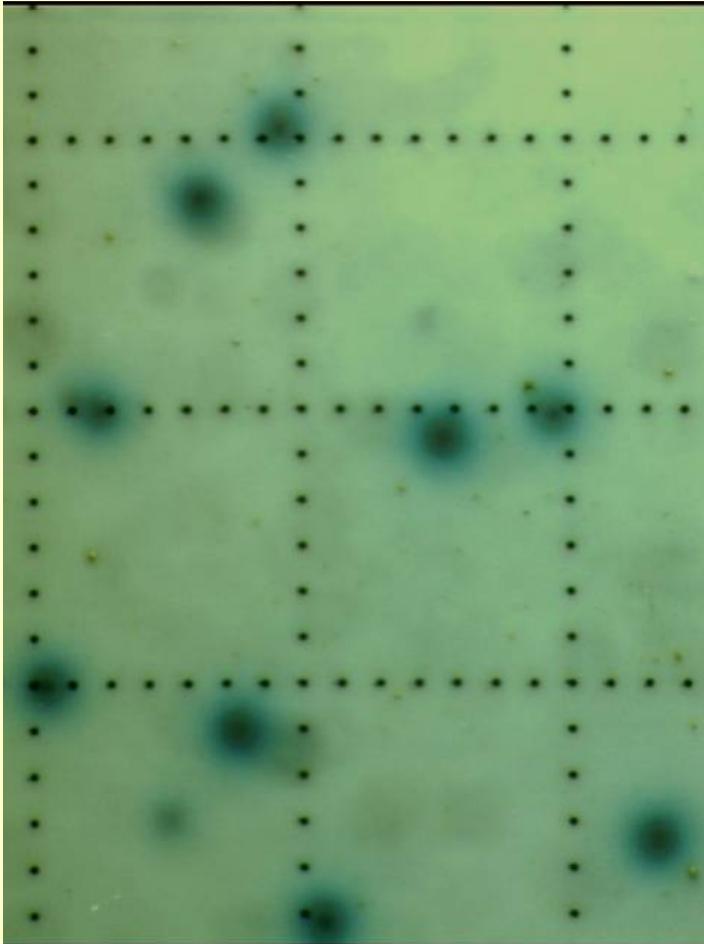
PCR/DNA Sequencing 16S ribosomal RNA by EMSL Analytical, Westmont, NJ.

| | |
|-------------------------------------|--------------------------------------------------------------------|
| The Terrace Bathing | <i>Enterococcus hirae</i> |
| The Terrace Bathing | <i>Enterococcus faecalis</i> |
| Horseshoe Cove | No match in database |
| Horseshoe Cove | <i>Staphylococcus pasteurii</i> |
| Horseshoe Cove | <i>Enterococcus hirae</i> |
| Navesink River Rec Center Highlands | No match in database |
| Navesink River Rec Center Highlands | <i>Enterococcus gallinarum</i> / <i>Enterococcus casseliflavus</i> |
| Navesink River Rec Center Highlands | <i>Enterococcus gallinarum</i> |
| Raritan Bay Thompson Ave | <i>Enterococcus faecalis</i> |

Streptococcus alactolyticus @ a
bathing beach 390/cfu's



Streptococcus alactolyticus
Higher magnification



Colonies are smaller than 0.5 mm diameter, appear to be poor growers, are dry looking, not raised, and the blue halo does not sparkle with indigo blue crystals but seems “stained” instead.





PROBABLE SOURCES: mussels + birds + coves:

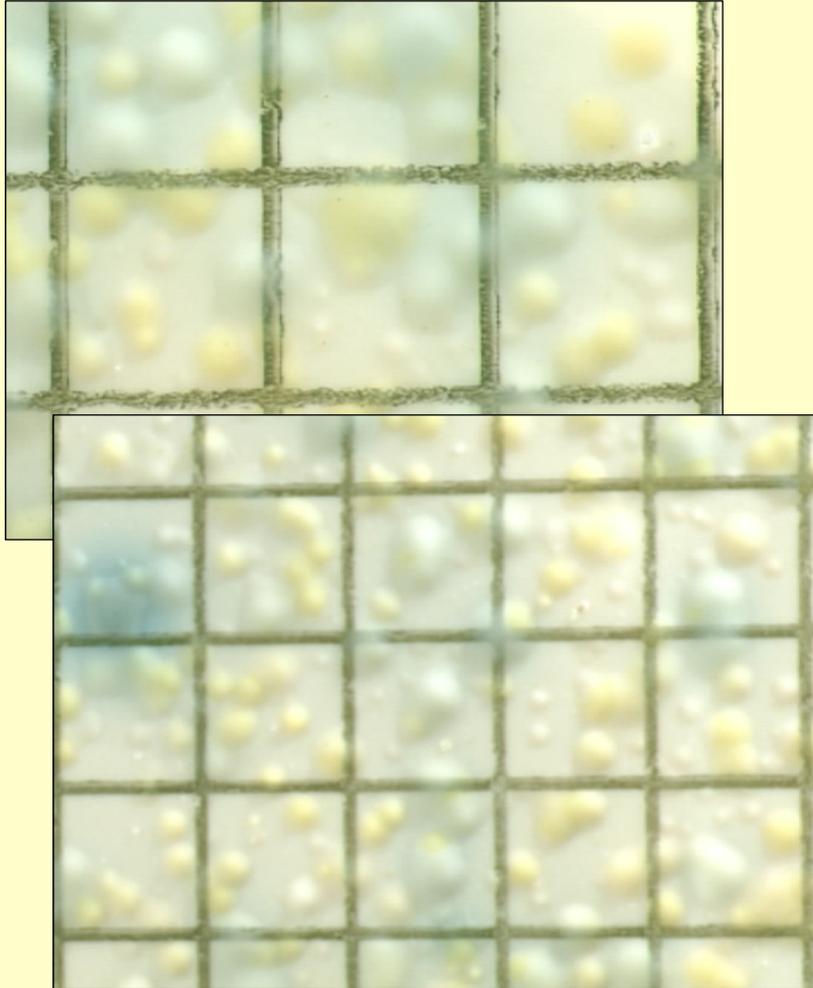
Remember Enterococcus differ from streptococcus by being resilient/tolerant organisms Target Indicator is more resistant to harsh salt water environment. Presence of Streptococcus may indicate more recent pollution

OTHER POTENTIAL SOURCES:

Runoff from pool, parking lot, tennis court, dumpster area and potentially cabana:

Confluent *Staphylococcus* on CCMP Enterococcus plates
PCR and DNA sequencing (EMSL Analytical Westmont, NJ)

Staphylococcus pasteurii



- interfering confluent (flowing together) or TNTC growth
 - *Staphylococcus* is competition for nutrients and space
- Raritan/Sandy Hook Bay
 - Three isolates over three years
 - Horseshoe Cove 10/03/05
 - Sandy Hook Light 06/06/06
 - Spermacetti Cove 07/17/06
- 1993 - *S. pasteurii* differentiated from *S. warneri* by molecular methods