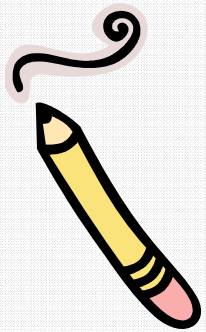
Notes From the Laboratory

Environmental Laboratory

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

Recreational Bathing

- Phytoplankton
- Microbiology



Presented at: NJ Water Monitoring Council Meeting

Jan 31, 2007

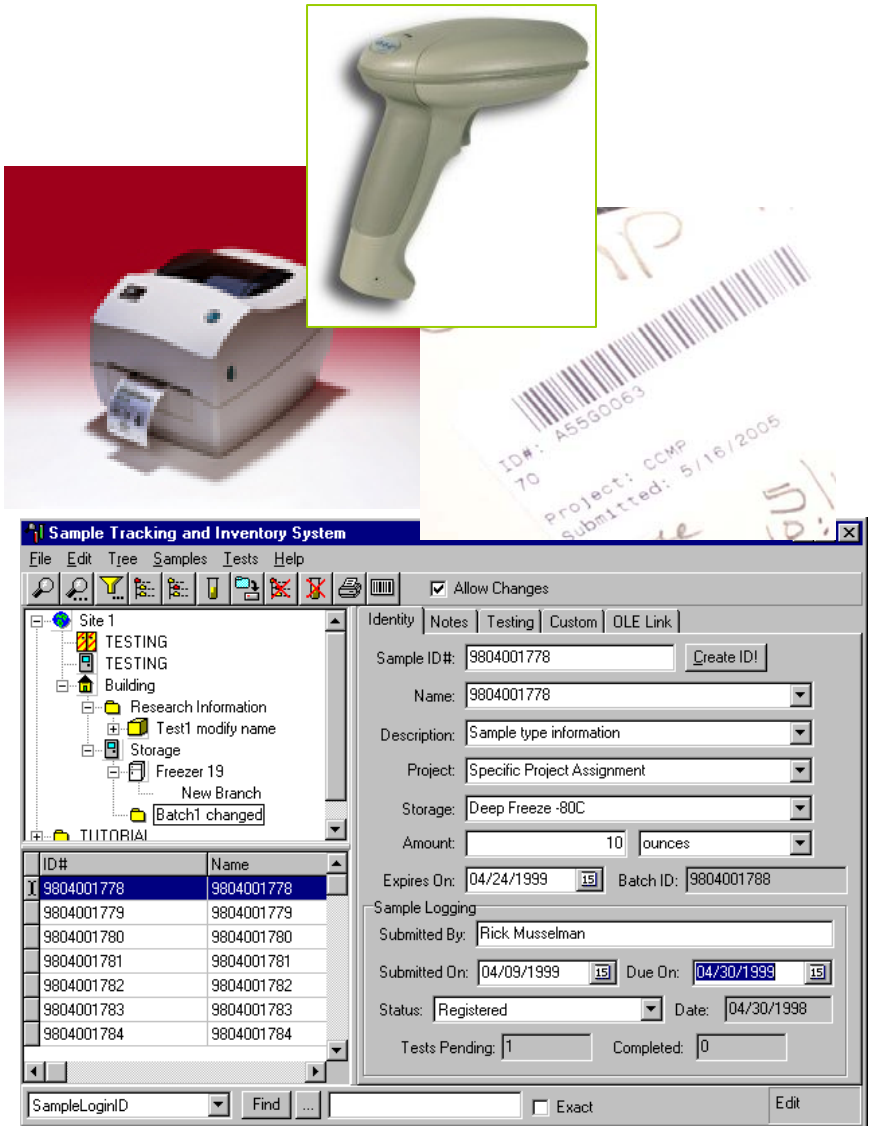
Presented by Becky Cosgrove, MCHD Environmental Laboratory Director

Monmouth County Dept of Health Environmental Laboratory

- Laboratory is Elective CEHA activity
  - Partial funding for equipment and disposables by NJDEP CEHA grant.
- Audited by NJDEP OQA
  - full audit Sept 2006
- Certified for limited list chem and micro parameters
  - EPA Method 1600 for Enterococcus, fc, HPC
  - DO, Sal, Temp, Cond, Turbidity, pH
  - TSS, Nitrate, Total – P, Ammonia

Laboratory Sample Tracking System

- Sample Tracking and Inventory System(STIS) by ChemSW
  - Windows database program bundled with handheld scanner
  - password protection
    - data only accessible to lab staff
  - drop down lists for fields
    - consistent descriptions
- generates automated sample ID reflecting current date
- Zebra barcode label printer
  - sample tags and bench sheets



Dyna uses barcode scanner

Phytoplankton

Enumeration and Identification

- Leica compound
  - Sedgewick Rafter cell count
  - 200X for count
  - Whipple grid in occular
- high resolution digital color 3 chip video camera
  - 1000X oil immersion for ID
  - Live window at monitor
- PAX-it! Ver 6.6 Windows based imaging system (frame grabber card)
- Tool for sharing images/second opinions/reports

Gyrodinium estuaralie

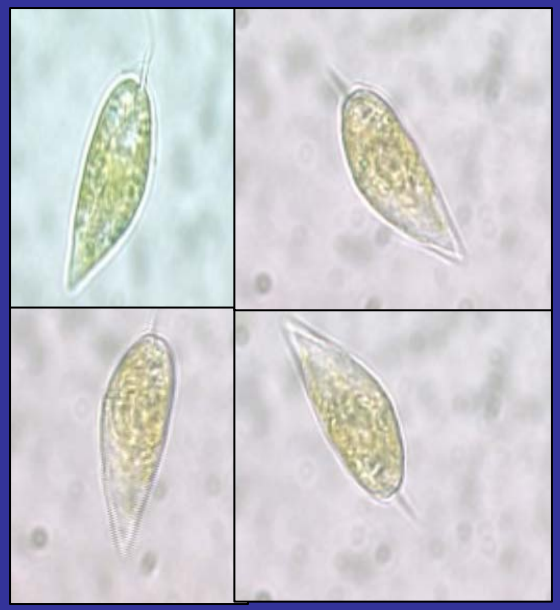
- Live View
  - Observation of natural motion is important in identification
- Unarmoured Dinoflagellate
  - L:W ratio is 1:3
  - Epicone and Hypocone about equal size
  - 2 chloroplasts
  - Girdle displacement is less than 1/3rd of total cell length
  - One transverse flagellum and one longitudinal

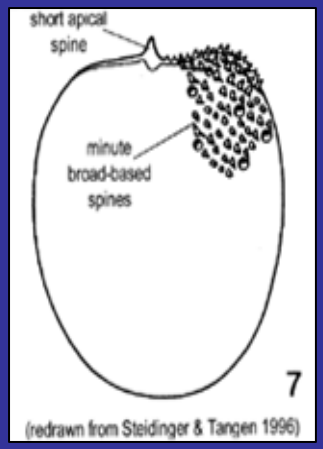
Heterocapsa spp.

Heterocapsa triquetra is a phototrophic marine dinoflagellate with wide coastal distribution. Often in high concentrations in low salinity areas. The cell is elongated, variable, and spindle shaped. The posterior hypotheca ends in a blunted oblique point. There is no apical spine. Girdle is equatorial, descending, displaced ½ girdle width with a short sulcus. *Heterocapsa triquetra* is 19-30 um long, 13-19 um wide.

Heterocapsa rotundatum is 7.5-11 µm long and 6-7.5 um wide and resembles an arrowhead or mushroom. Synonym(s): *Amphidinium rotundatum* Lohmann 1908 , *Katodinium rotundatum* (Lohmann) Loeblich III 1965 The cell readily rounds up. The epicone longer and broader compared to the hypocone.

Prorocentrum spp.





Prorocentrum redfieldi Implicated in mild discomfort to bathers (NJDEP Annual Phytoplankton Reports) The production of DSP toxins has been confirmed in dinoflagellate *Prorocentrum redfieldi* (Viviani, 1992).

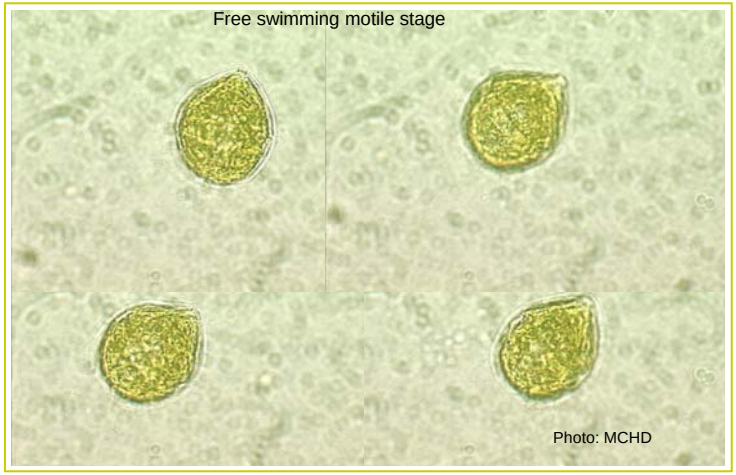
Prorocentrum minimum. Apical spine very small or lacking. Cell is triangular or heart shaped in valve view. Thecal surface covered with numerous short, broad spines and small scattered pores. *P. minimum* is an armoured, marine, planktonic, bloom-forming dinoflagellate. A toxic cosmopolitan species common in cold temperate brackish waters to tropical regions.

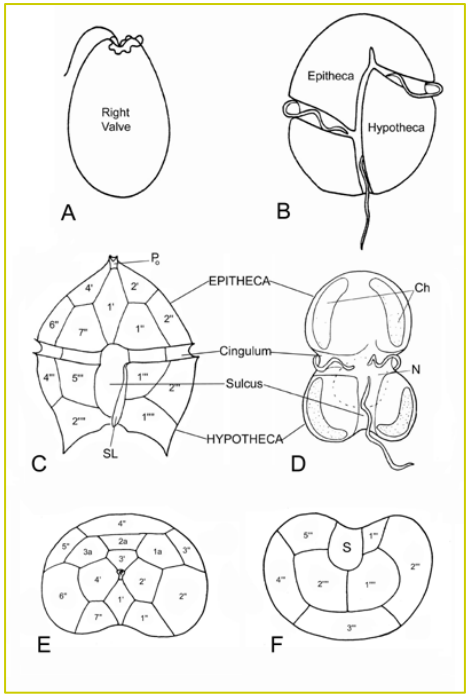
Dinoflagellate *Scrippsiella trochoidea*

Other Names: Peridinium trachoideum (Stein, Lemmermann 1910), Glenodinium trochoideum (Stein 1883).

The dinoflagellate *Scrippsiella trochoidea* was responsible for a low bloom in the Navesink River at Marine Park in July 25th 2005. Water discoloration was reported. The cell count of the sample was 360 cells/ml although it is suspected that the concentration may have reached greater levels in the river. MCHD personnel measured dissolved oxygen to be 4.7 mg/L and a temperature of 28.8C.

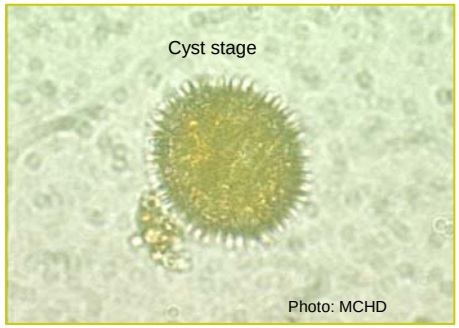
Dinoflagellates are unicellular eukaryotic microorganisms. They are free swimming protests with a forward spiraling motion propelled by two dimorphic flagella. *Scrippsella trochoidea* is a dinoflagellate that has been listed by UNESCO as a Harmful Algal Bloom forming species due to its ability to reach very high densities in stratified water bodies. The diel vertical migration by flagellated phytoplankton may result in increased growth rates over non-migrating phytoplankton by allowing access to light and nutrient resources in stratified water bodies where light and nutrients are vertically separated.





Identifying dinoflagellates: a. lateral view of a desmokont cell type (two dissimilar flagella apically inserted); b. ventral view of a dinokont cell type (two dissimilar flagella ventrally inserted); c. ventral view of a thecate peridinioid cell; d. ventral view of an athecate gymnodinoid cell; e. apical view of epithecal plates; f. antapical view of hypothecal plates. Ch = chloroplasts; N = nucleus; Po = apical pore plate; SL = sulcal list (Figs. a-b redrawn from Steidinger & Tangen 1996; Figs. c-f redrawn from Taylor 1987).

S. trochoidea is mesohaline. It is 18-34 um long, 17-28 um wide. Its chloroplasts are yellow-brown in color. Chloroplasts are membrane-bound organelle found in the cytoplasm that contain the chlorophyll pigments and the enzyme systems for photosynthesis. Two dissimilar flagella emerge from the anterior part of the cell.



The cyst stage is the dormant or resting non-motile cell stage. Some species produce resting cysts that can survive in sediments for an extended period of time, and then germinate to initiate blooms (Spector 1984).

Identification of Dinoflagellates in the Raritan Bay

The dinoflagellates *Heterocapsa triquetra, Katodynium rotundatum* and *Prorocentrum minimum* were responsible for a low bloom in the Raritan Bay, Atlantic Highlands Municipal Marina. Cloudy water was observed on June 5, 2006 in the morning of a gloomy day. The cell count for the dominant species in the sample was *Heterocapsa triquetra (*5,740 cells/ml), *Prorocentrum minimum* (5,420 cells/ml), *Heterocapsa rotundata* (1,620 cells/ml) and *Polykrikos* sp (280 cells/ml). Dinoflagellates are unicellular eukaryotic microorganisms. They are free swimming protests with a forward spiraling motion propelled by two dimorphic flagella. The diel vertical migration by flagellated phytoplankton may result in increased growth rates over non-migrating phytoplankton by allowing access to light and nutrient resources in stratified water bodies where light and nutrients are vertically separated.

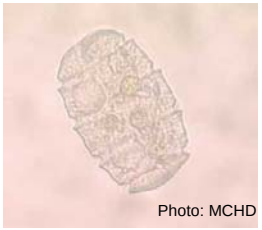


Photo: MCHD

Polykrikos kofoldi. A naked (not armoured) dinoflagellate. A permanent colony, usually of 4 or 8, sometimes 2 or 16 zooids. Each zooid with a girdle which may or may not be displaced. The sulcus is continuous on ventral side of the colony; each zooid with a transverse and a longitudinal flagellum. Nematocysts usually present.

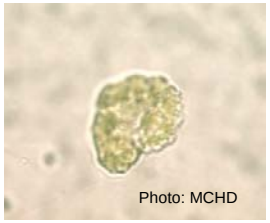



Photo: MCHD

The dinoflagellate *Heterosigma akashiwo* synonym *Olisthodiscus luteus.* Major Taxonomic Group: Raphidophytes 'Potato' shaped cells 11-25 X 8-13 µm. 2 sub-apically inserted flagella, one motile one trailing. Plastids are truly parietal, cell is only slightly compressed, recurrent flagellum lies in a ventral groove. Worldwide in brackish waters. Caused a large bloom in Bull's Bay SC in April of 2003. Toxic to fish, mechanism of toxicity is very poorly known.


Photo: MCHD

The dinoflagellate *Heterocapsa rotundatum* is 7.5-11 µm long and 6-7.5 um wide and resembles an arrowhead or mushroom. Synonym(s): *Amphidinium rotundatum* Lohmann 1908 , *Katodinium rotundatum* (Lohmann) Loeblich III 1965 The cell readily rounds up. The epicone longer and broader compared to the hypocone. Sometimes known as a mohogany tide, *H. rotundata* has caused large nuisance blooms in several portions of the world.

Photos and poster by B. Cosgrove 06/16/06

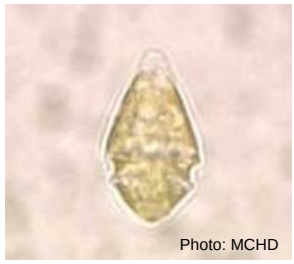

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Photo: MCHD

Gyrodinium grossestriatum The genus *Gyrodinium* is a group of naked, heterotrophic dinoflagellates. Girdle or cingulum displacement from one to more cingulum widths. Apical groove elliptical. The characteristic feature of Gyrodinium is not so much the cingulum displacement as the morphology of the apical groove system. The apical groove is an elliptical structure situated around the apical end, perpendicular to the longitudinal axis of the cell.


Photo: MCHD

Prorocentrum minimum is an armoured marine, bloom forming dinoflagellate species. It is potentially toxic; producer of venerupin (hepatotoxin) which caused shellfish poisoning in Japan,Gulf of Mexico and Florida The cell shape is triangular to oval-round. Cell size is 15-20 um long and 13-17 um wide. Valves are flattened and covered by minute spines and trichocyst pores. 2 dissimilar flagella emerge from V-shape depression at the anterior of the cell. One flagellum is transverse for providing propulsion and the longitudinal flagellum provides direction.

Identification of Flagellates of the Raritan Bay

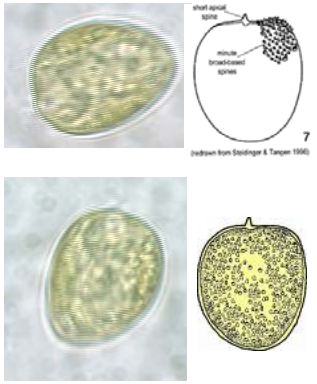
The following flagellate species were identified and captured digitally in a sample submitted to the Monmouth County DOH Environmental Laboratory. The sample was collected from Ideal Beach in Keansburg, early in August 2006.

Poster Created 08/28/06 by B. Cosgrove

Grass green. Frequently noted within estuaries and bay systems along the east coast and over the shelf.

Chlorella marina A member of the Chlorophyta (green algae). Cells ovoid, 4-6 x 7-10 um, cell walls thin and smooth. Chloroplast fills most of cell. Like other members of the Chlorococcales, reproduction takes place by internal cell division., in this instance forming non-motile autospores. (Picture shows cells with autospores.

Dinoflagellate ***Scrippsiella trochoidea*** is a mesohaline species. Listed by UNESCO as a harmful algal bloom forming species due to its ability to reach very high densities in stratified water bodies. 18-34 um long, 17-28 um wide. Chloroplasts are yellow brown. Two dissimilar flagella emerge from anterior.



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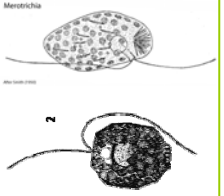
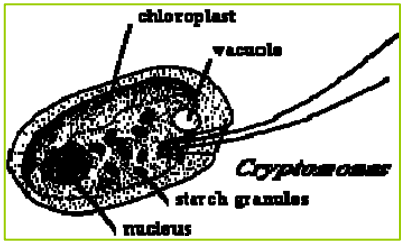


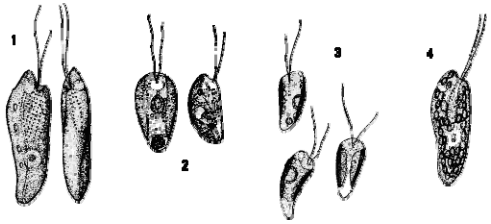
Merotrichia

Protoperidinium sp. Armoured dinoflagellate. Cells often have spines. Surface of cell is broken by grooves that hold 2 flagella. The cross-wise groove cuts the cell into an upper and lower half. In those cases where the groove goes up towards the upper half, it does so only a little bit.

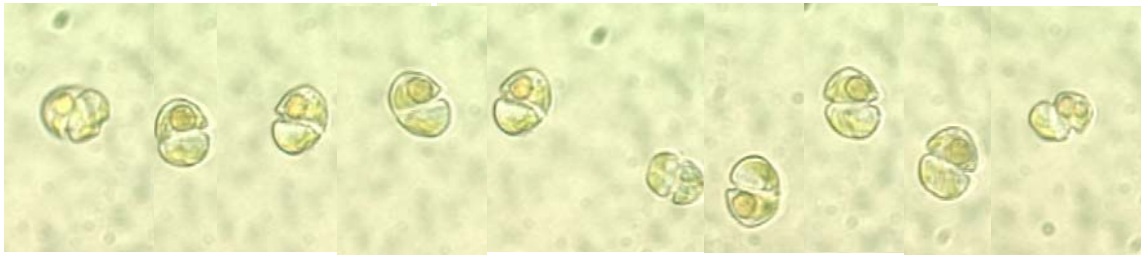
Protoperidinium



Cryptomonas sp. Cells oval or broadly rounded, or asymmetrical at the anterior end, with 2 (or 1) parietal, olive-green chloroplasts which are often red; flagella 2, attached within an apical gullet. Fast moving.



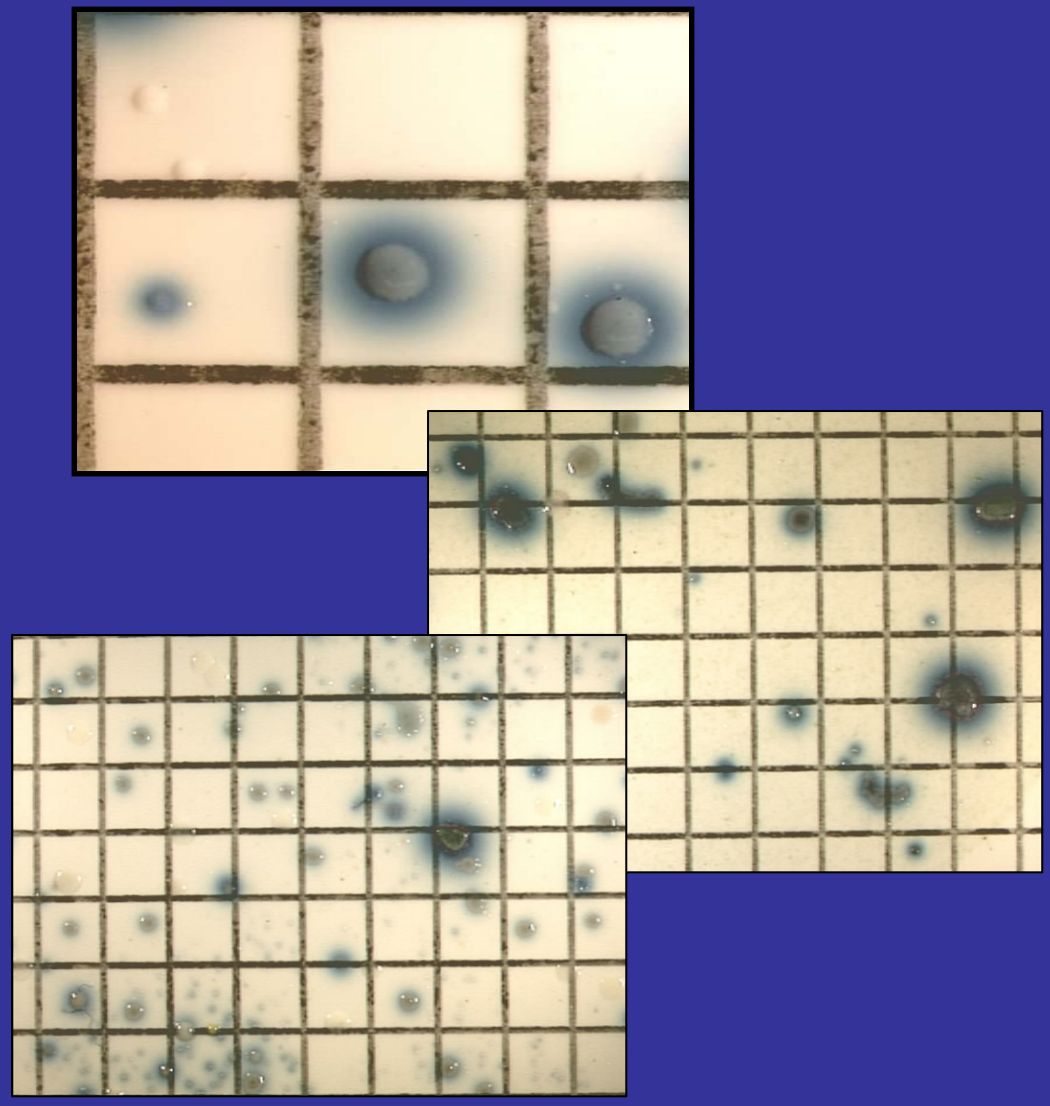
Chroomonas sp. Cells ellipsoid or oval, or slipper shaped, usually bilobed anteriorly. 2 flagella attached immediately below cell apex. 2 parietal, blue or bluish-brown chloroplasts.



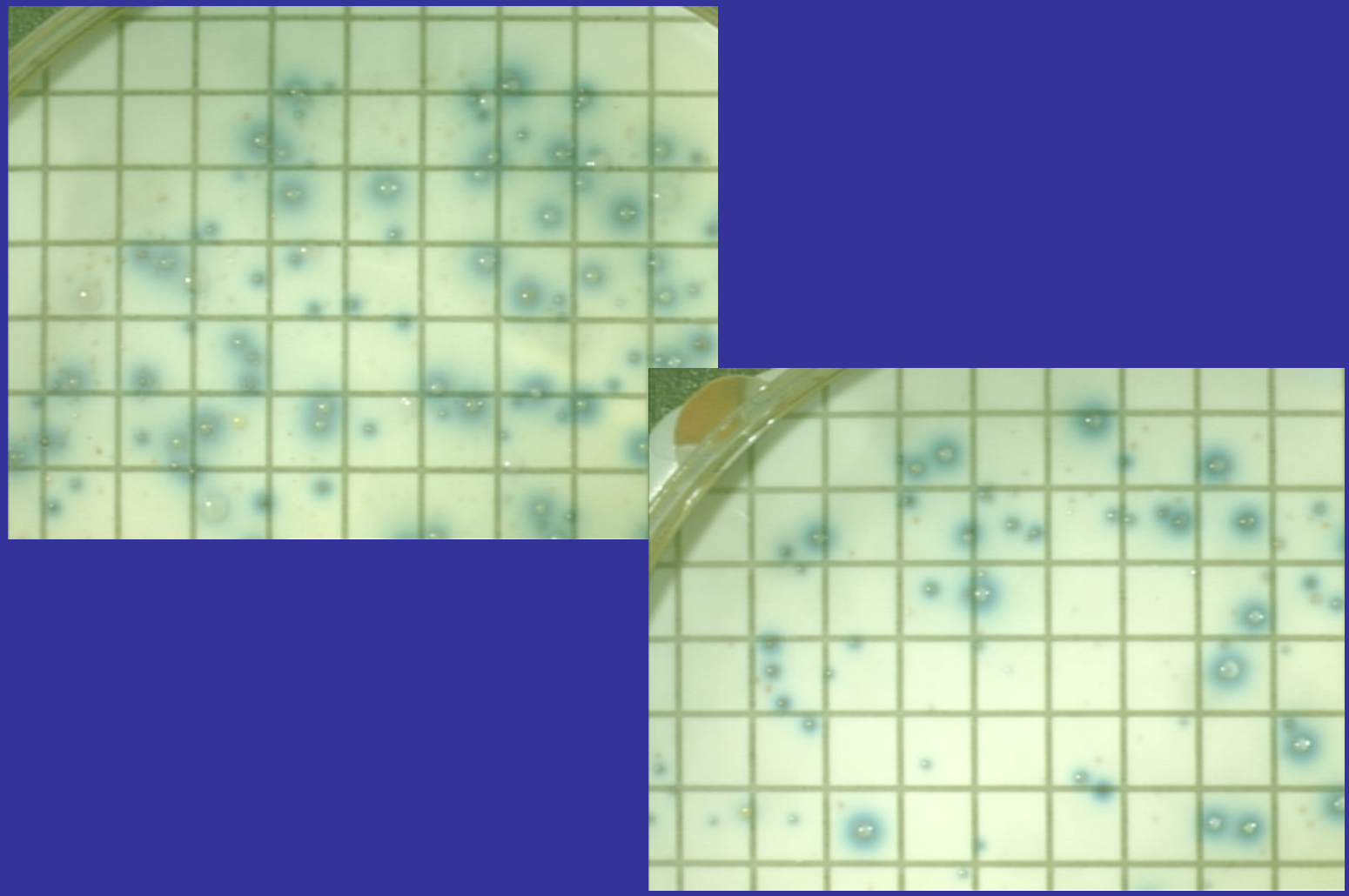
Gyrodinium estuariale. An unarmoured dinoflagellate. Cell length 11-16um. Cell width 9-12um. Length to width ratio 1:3. Epicone and hypocone are about equal in size. Nucleus is located centrally. Chloroplasts two, one in the epicone and one in the hypocone. Girdle displacement is less than 1/3 of the total cell length. One transverse flagellum and one longitudinal. Toxicity has not been detected in this species. It is common in the Raritan Bay.

Enterococcus EPA Method 1600 "Mixed Growth Plates"

- September 2002 version typical enterococcus colony is **any** with blue halo
- Change in definition of typical colony
- July 2006 version typical enterococcus colony is **>0.5 mm** with blue halo
  - To exclude false positive *Aerococcus* sp. and other?
- July 2006 version has Additional QA/QC
  - BIOBALLS
- "Mixed growth plates" are difficult for tech to measure and count



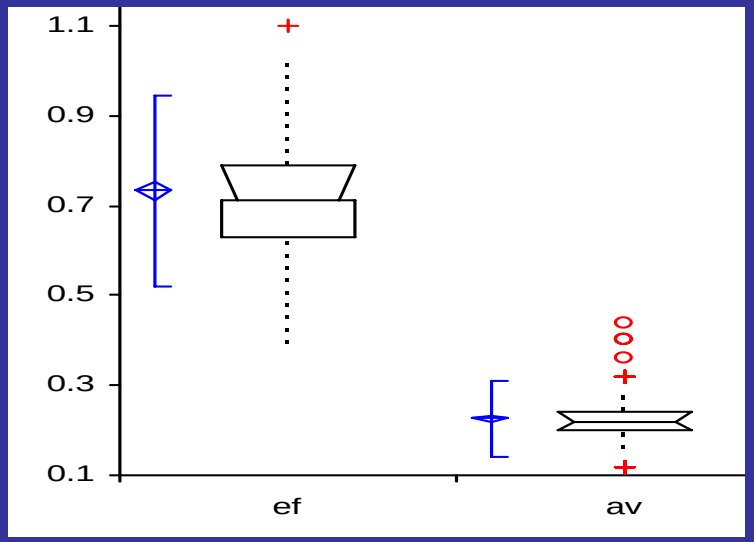
Range of Colony Sizes



American Type Culture Collection

Enterococcus faecalis *Aerococcus viridans*

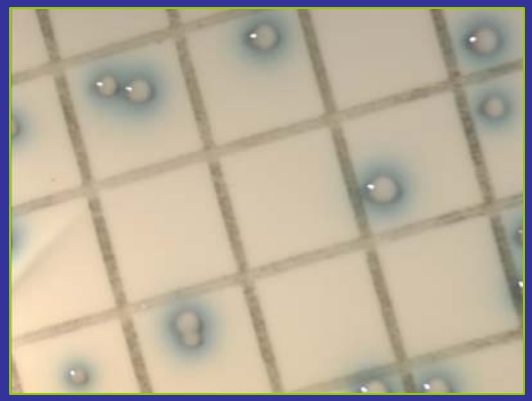
Test viability of null hypothesis
Ho: There is no difference between the size of *Aerococcus viridans* colonies and *Enterococcus faecalis* colonies



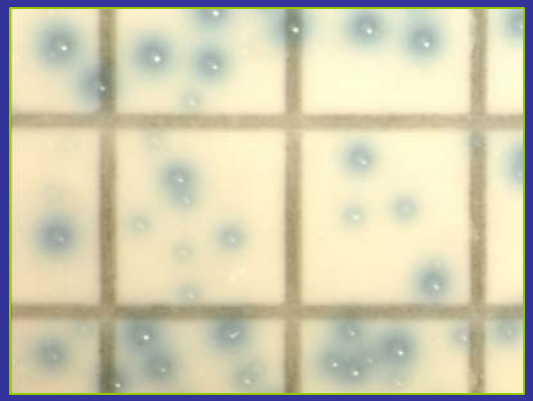
	n	Mean mm.	SD	95% CI of Mean	
E. faecalis	**139**	**0.732**	**0.1277**	**0.711**	**to 0.754**
A. viridans	**286**	**0.226**	**0.0504**	**0.220**	**to 0.231**

Students t test based on ordinary means

2-tailed p <0.0001 t statistic 42.36



E. faecalis ATCC



A. viridans ATCC

False negative/False positive study

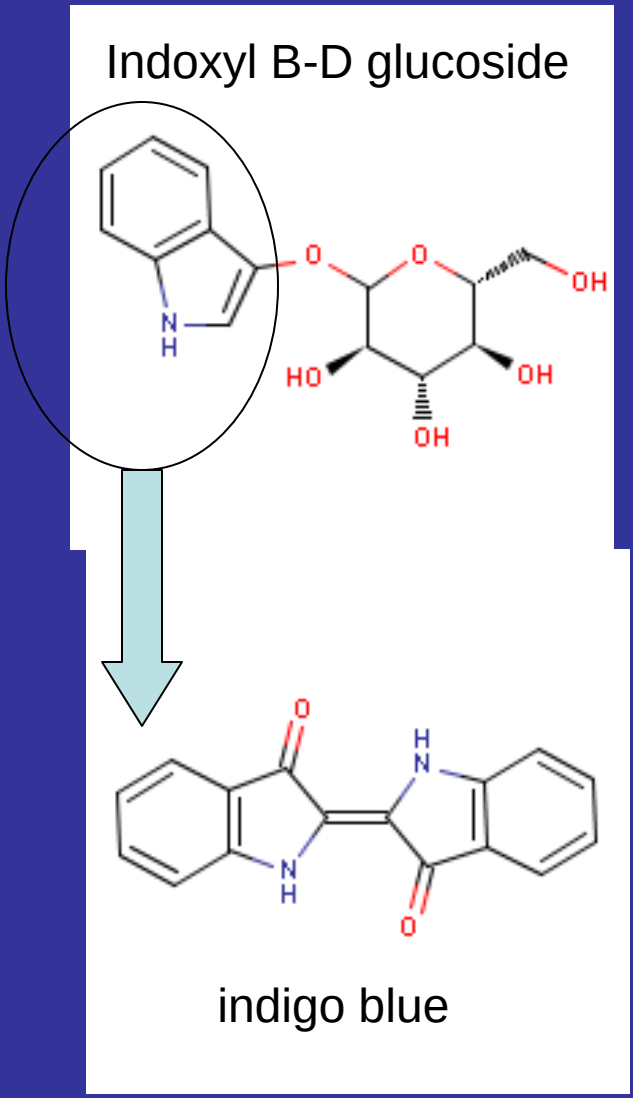
Chart updated January 2006	Blue w blue halo > 0.5 mm N=138	Blue w blue halo < 0.5 mm n=116	Messer and Dufour EPA Method
% confirmed	78%	58%	6.5 (false neg)
% not confirmed	22%	42%	6 (false pos)

Confirmed for Enterococcus by classical biochemical techniques

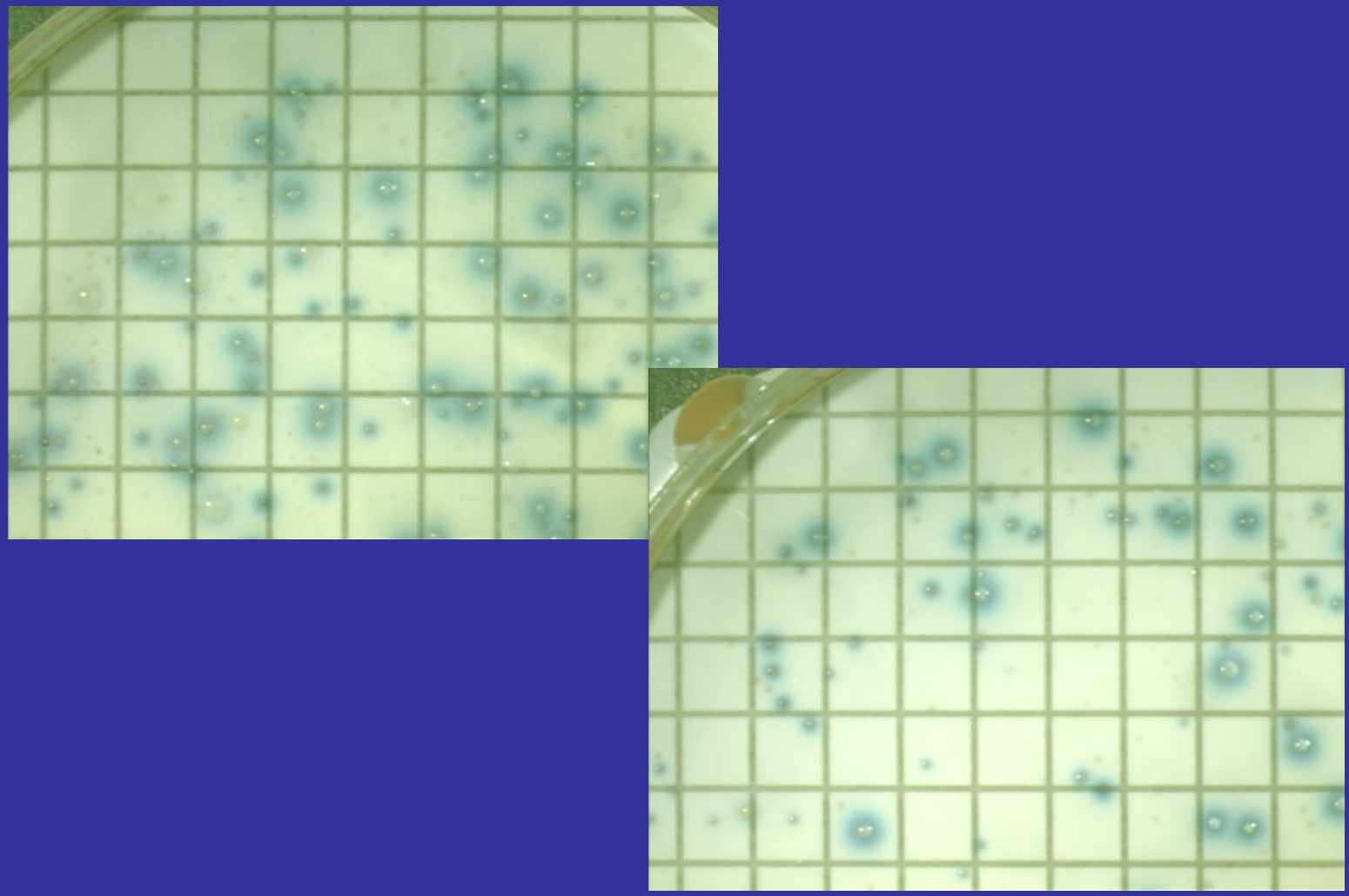
- BHI at 45C
- BHI with NaCl
- Hydrolysis of Esculin

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 (indole cleaved off)
  - Blue halo is formed when Indigo blue complex precipitates and diffuses into surrounding media

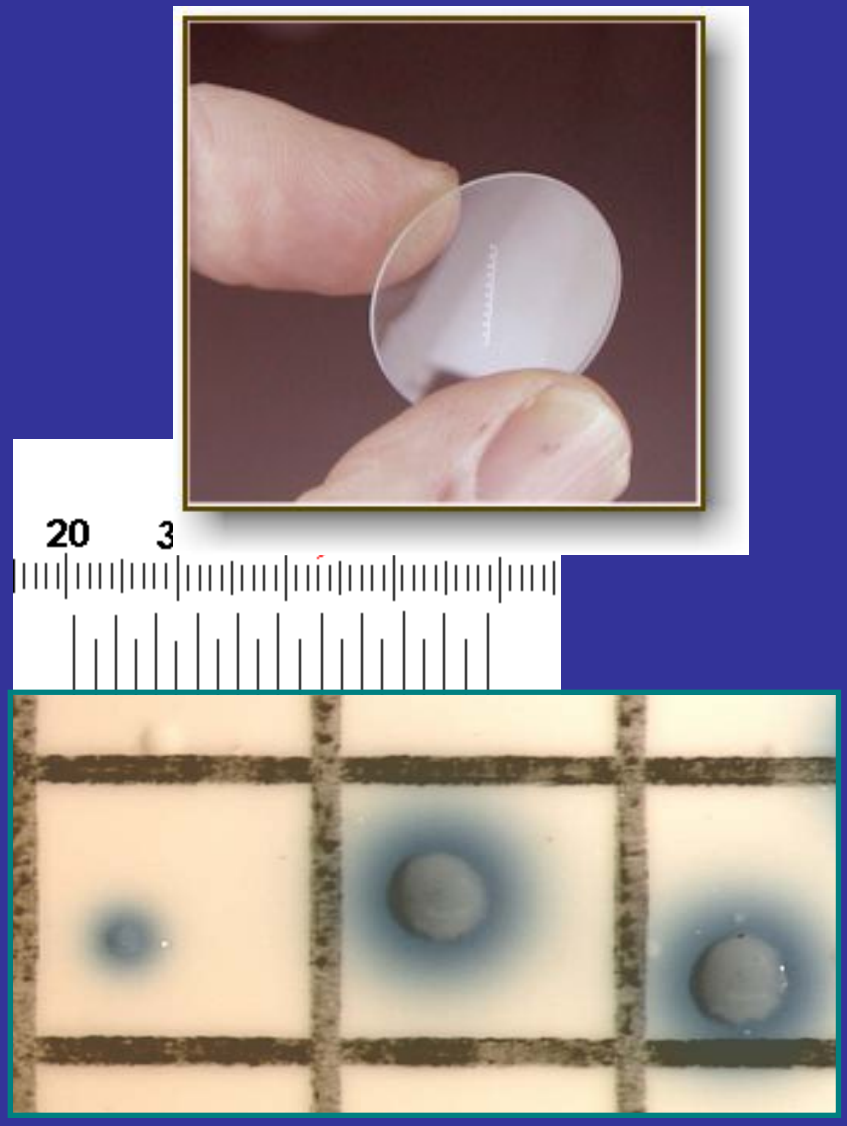


Range of Colony Sizes



Accurate measurement of colonies

- Method 1600
  - mag of 2-5X or stereoscopic microscope
- Reticules or "eyepiece micrometers“
- reticule is calibrated against a stage micrometer for each objective

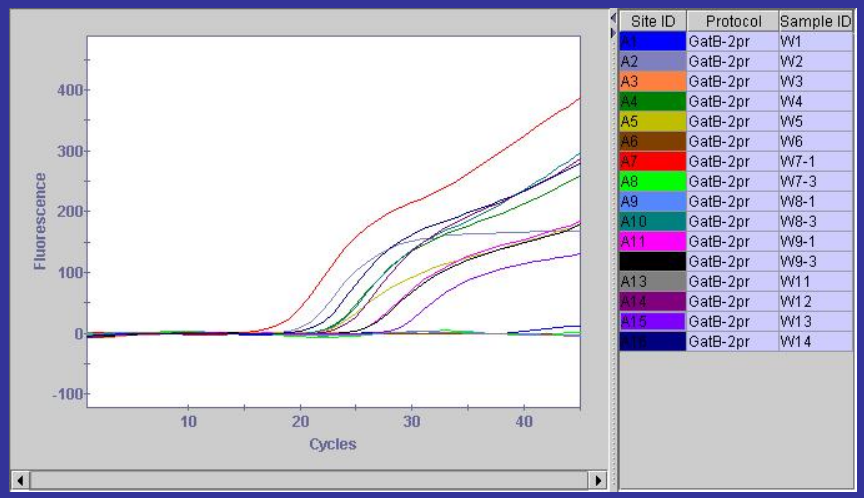


Strep API (BioMerieux) kits

- Colonies that grow on mEI agar
  - gram positive and catalase negative
    - *Enterococcus faecium*
    - *E. faecalis*
    - *E. durans*
    - *E. avium*
    - *E. gallinarium*
    - *Streptococcus uberis*
    - *Aerococcus viridans*
- Kit codes for only 5 species of enterococcus
- Accuracy of API kits varies by species
- Literature says not so good for environmental isolates

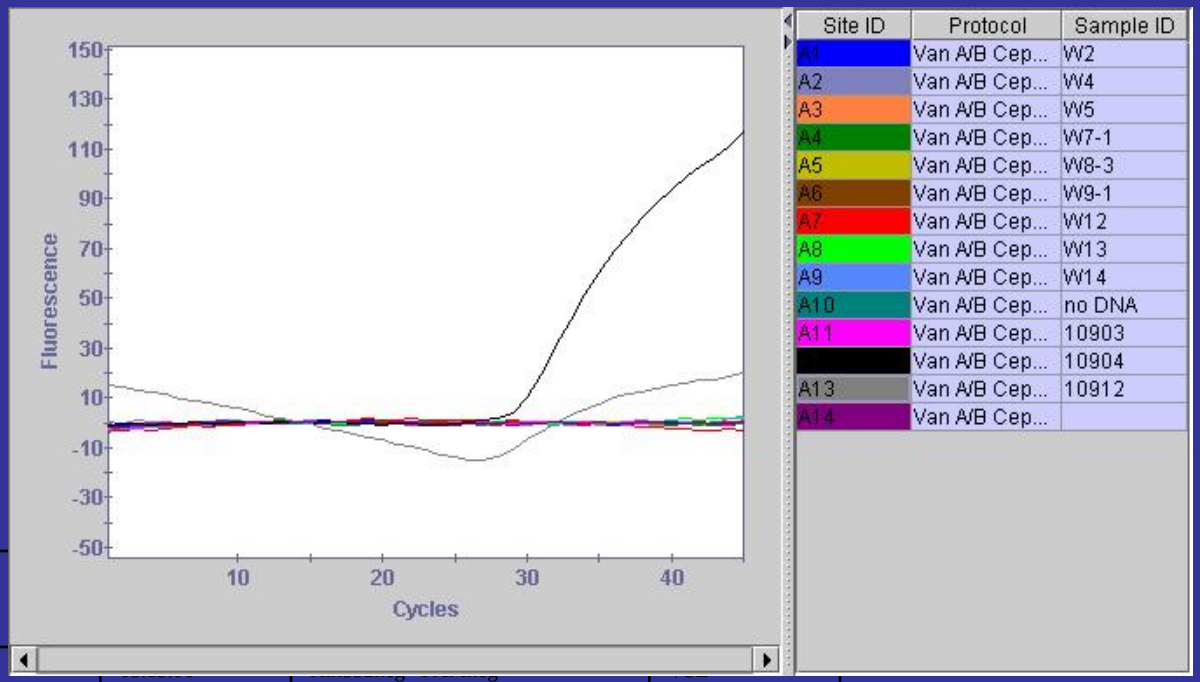
Gat B gene area

E. faecalis and *E. facium* probes



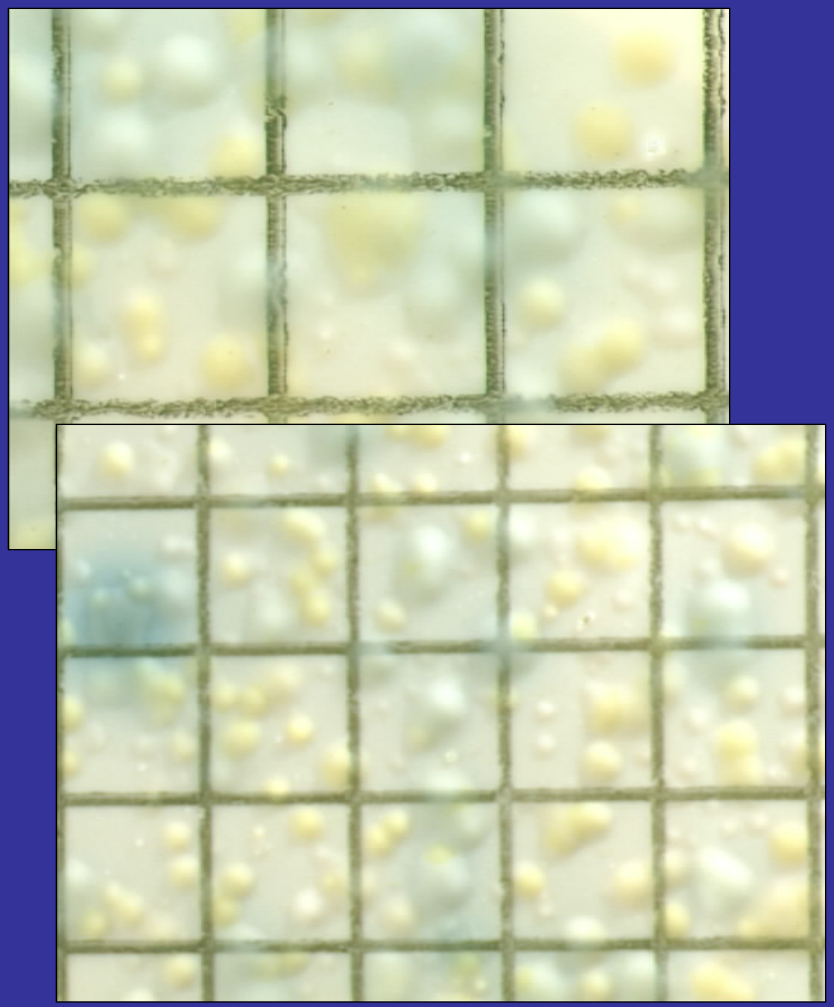
PHRI THE PUBLIC HEALTH RESEARCH INSTITUTE at the International Center for Public Health

#	Strain	ID	Date of DNA isolation	Results of run gatB(TT)	Reported phenotype
A1	W1	A5BU0003	03.13.06	FAMneg	-
A2	W2	A5BU0005	03.13.06	FAMpos	+
A3	W3	A5BU0008	03.13.06	FAMneg	-
A4	W4	A5BU0010	03.13.06	FAMpos	+
A5	W5	A5BU0017	03.13.06	FAMpos	+
A6	W6	A5BU0020	03.13.06	FAMneg	-
A7	W7-1	A61N0002(BHI)	03.13.06	FAMpos	+
A8	W7-3	A61N0002(Nut a)	03.13.06	FAMneg	-
A9	W8-1	A61N0004(BHI)	03.13.06	FAMneg	-
A10	W8-3	A61N0004(Nut a)	03.13.06	FAMpos	+
A11	W9-1	A61N0005(BHI)	03.13.06	FAMpos	+
A12	W9-3	A61N0005(Nut a)	03.13.06	FAMpos	+
A13	W11	Single col., blue	03.13.06	FAMneg	-
A14	W12	Single col., brown	03.13.06	FAMpos	+
A15	W13	Single col., blue	03.13.06	FAMpos	+
A16	W14	Single col., brown	03.13.06	FAMpos	+



#	**Strain**	**DNA isolation method**			
A1	W2	A5BU0005			
A2	W4	A5BU0010	03.13.06	Alx532neg FAMneg	VSE
A3	W5	A5BU0017	03.13.06	Alx532neg FAMneg	VSE
A4	W7-1	A61N0002(BHI)	03.13.06	Alx532pos FAMneg	VRE (VanA+)
A5	W8-3	A61N0004(Nut a)	03.13.06	Alx532neg FAMneg	VSE
A6	W9-1	A61N0005(BHI)	03.13.06	Alx532neg FAMneg	VSE
A7	W12	Single col., brown	03.13.06	Alx532pos FAMneg	VRE (VanA+)
A8	W13	Single col., blue	03.13.06	Alx532neg FAMneg	VSE
A9	W14	Single col., brown	03.13.06	Alx532neg FAMneg	VSE
A10	No DNA			Alx532neg FAMneg	
A11	10903	control	01.03.06	Alx532pos FAMneg	VRE
A12	10904	control	10.03.06	Alx532neg FAMpos	VRE
A13	10912	control	10.03.06	Alx532pos FAMpos	VRE
A14	W7-3	A61N0002(Nut a)	03.13.06	Alx532neg FAMneg	VSE

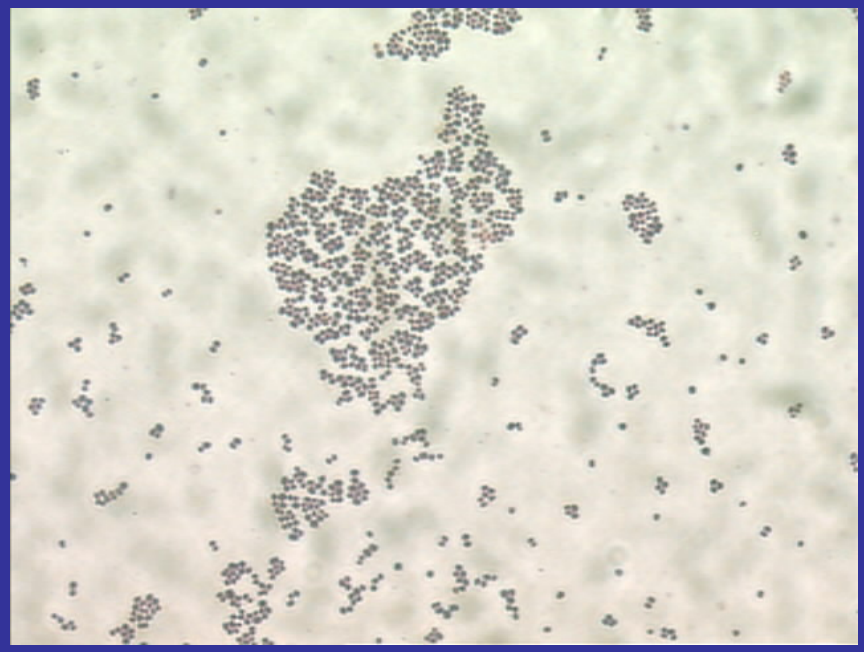
Confluent *Staphylococcus* sp. on Rec bathing Enterococcus plates



- Staph is also gram +
- salt (which the staph will tolerate)
- interfering confluent (flowing together) or TNTC growth
  - Staphylococcus is competition for nutrients and space on mEI plates
    - Evidenced by bluish “undergrowth”
    - Resample
- Raritan/Sandy Hook Bay

Staphylococcus ID

- Gram positive/catalase positive
  - Catalase simple test to separate Strep from Staph
- Coagulase negative (CNS) Less pathogenic for humans
  - Probably not significant in its interactions with humans
- API Staph ID Kits (biochemical profile)
  - For species ID
  - Limited success
    - 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 ie they can alert clinician that is *Staph aureus*



Biochemical Characteristics of Staphylococcus isolates

GLU	FRU	MNE	MAL	LAC	TRE	MAN	XLT	MEL	NIT	PAL	VP	RAF	XYL	SAC	MDG	NAG	ADH	URE
POS	POS	POS	POS	POS	POS	NEG	NEG	NEG	POS	NEG	POS	NEG	NEG	POS	NEG	POS	NEG	POS
POS	POS	NEG	POS	NEG	POS	NEG	NEG	NEG	NEG	POS	POS	NEG	NEG	POS	NEG	NEG	POS	POS
POS	POS	NEG	POS	NEG	POS	NEG	NEG	NEG	NEG	POS	POS	NEG	NEG	POS		POS	POS	POS
POS	POS	NEG	POS	POS	POS	NEG	NEG	NEG	NEG	POS	POS	NEG	NEG	POS	NEG	NEG	POS	POS
POS	POS	NEG	POS	NEG	POS	NEG	NEG	NEG	NEG	POS	POS	NEG	NEG	POS	NEG		NEG	NEG
POS	POS	NEG	POS	NEG	POS	NEG	NEG	NEG	NEG	POS	POS	NEG	NEG	POS	NEG		NEG	NEG
POS	POS	NEG	POS	NEG	POS	NEG	NEG	NEG	NEG	POS	POS	NEG	NEG	POS	NEG	NEG	POS	POS
* POS	POS	NEG	POS	NEG	POS	NEG	NEG	NEG	NEG	POS	POS	NEG	NEG	POS	NEG	NEG	POS	POS
* POS	POS	NEG	POS	NEG	POS	NEG	NEG	NEG	NEG	POS	POS	NEG	NEG	POS	NEG	NEG	POS	POS
* POS	POS	NEG	POS	POS	POS	NEG	NEG	NEG	NEG	POS	POS	NEG	NEG	POS	NEG	NEG	NEG	POS
POS	POS	NEG	POS	POS	POS	NEG	NEG	NEG	NEG	POS	POS	NEG	NEG	POS	NEG	NEG	NEG	POS
POS	POS	NEG	POS	NEG	POS	NEG	NEG	NEG	POS	POS	POS	NEG	NEG	POS	NEG	NEG	POS	POS
POS	POS	NEG	POS	NEG	POS	NEG	NEG	NEG	POS	POS	POS	NEG	NEG	POS	NEG	NEG	POS	POS
POS	POS	NEG	POS	NEG	POS	NEG	NEG	NEG	POS	POS	POS	NEG	NEG	POS	NEG	NEG	POS	POS
POS	POS	NEG	POS	POS	POS	NEG	NEG	NEG	POS	NEG	POS	NEG	NEG	POS	NEG	NEG	POS	POS

* Near to *Staphylococcus warneri*

PCR and DNA sequence analysis (EMSL)

Staphylococcus pasteuri

- *Staph. pasteuri* can be phenotypically distinguished from all other novobiocin susceptible *Staph.* spp EXCEPT *Staph. warneri* from which it can only be differentiated by genotyping! (done in1993)
- Three isolates
  - Horseshoe Cove 10/03/05
  - Sandy Hook Light 06/06/06
  - Spermacetti Cove 07/17/06
- GOOGLE not much except for “Space bugs”

Conclusions

- Enterococcus colonies can have variable appearance and be difficult to enumerate
  - Rapid PCR based methods using molecular probes may be useful for quick confirmations.
- Staphylococcus sp can grow confluently
  - May/maynot be important to identify by PCR non-entero growth when it interferes
- Is the species of Enterococcus different in particular places? Can species ID assist in source tracking?