

**Lab 14 Goals and Objectives:**

**Exercise 70: Staphylococci Identification**

Read and record results

**Exercise 71: Streptococci Identification**

Read and record results

Repeat Gram stains if necessary for sketch for report from old smears or plates (note: plates are not "fresh" so Gram results may be variable, but size, shape and arrangement will be fine).

If you have identified a particular assay you need to repeat to be able to solve your unknown, the media may be bought for ten points off your unknown report grade (per assay). Catalase (hydrogen peroxide) and Gram stain (staining reagents) assays are free.

Be sure you completely understand the lab report format: ask questions now!

**Mannitol Salt Agar**

Inoculation method: surface streak with loop

Contains: 7.5% NaCl, mannitol, Phenol red pH indicator: alkaline pH = red/pink, acidic pH = yellow

Selective and differential medium: selects for growth of organisms salt tolerant to 7.5% (usually Staphylococci). Of those that grow, differentiates organisms that have the ability to ferment mannitol to acid.

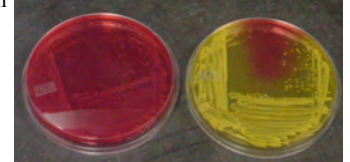
Results: Growth = salt tolerant to 7.5%

Yellow = positive for mannitol fermentation to acid

Pink = negative for mannitol fermentation to acid

No growth = negative for salt tolerance to 7.5%, inconclusive for mannitol fermentation

Growth = colonies don't count a heavy streak only as growth!



**Blood Agar**

Inoculation method: surface streak and stab with loop

Contains: BHIA, sheep blood

Discriminates organisms that have the ability to hemolyse red blood cells completely through production of hemolysins (streptolysins or alpha-toxin) or partially through ability to degrade hemoglobin pigment into green products (biliverdin)

Results:

Complete clearing of RBCs = Beta-hemolysis, positive for production of hemolysins

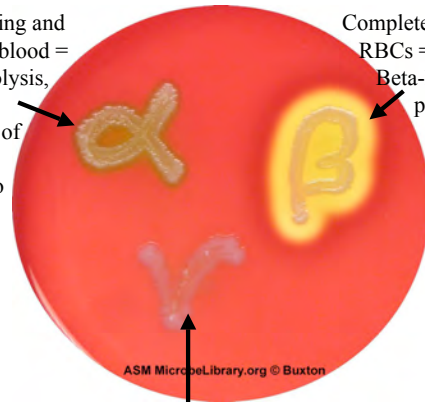
Partial clearing and greening of blood = Alpha-hemolysis, positive for degradation of hemoglobin pigment into biliverdin

No clearing with or without rusting = Gamma-hemolysis, negative for hemolysis



Partial clearing and greening of blood = Alpha-hemolysis, positive for degradation of hemoglobin pigment into biliverdin

Complete clearing of RBCs = Beta-hemolysis, positive for production of hemolysins



No clearing with or without rusting = Gamma-hemolysis, negative for hemolysis

**Rabbit Serum: Coagulase Test**

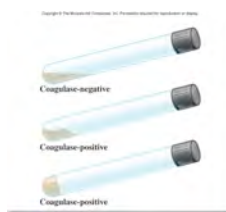
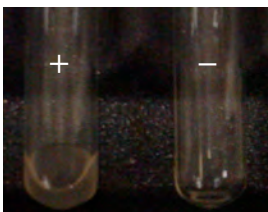
Inoculation method: loop transfer

Contains: rabbit serum (fibrinogen)

Discriminates organisms that can produce coagulase to catalyze the conversion of soluble fibrinogen into insoluble fibrin (clot)

Results: Solid = positive for fibrin formation, positive for coagulase production

Liquid = negative for coagulase production



**Blood Agar**

Inoculation method: surface streak and stab with loop

Contains: BHIA, sheep blood

Discriminates organisms that have the ability to hemolyse red blood cells completely through production of hemolysins (streptolysins or alpha-toxin) or partially through ability to degrade hemoglobin pigment into green products (biliverdin)

Results:

Complete clearing of RBCs = Beta-hemolysis, positive for production of hemolysins

Partial clearing and greening of blood = Alpha-hemolysis, positive for degradation of hemoglobin pigment into biliverdin

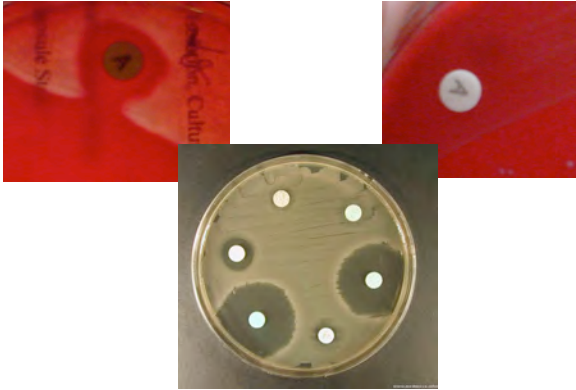
No clearing with or without rusting = Gamma-hemolysis, negative for hemolysis



**Bacitracin Susceptibility or Resistance**

Susceptible = organism killed  
 bacitracin: zone of no growth  
 around disk

Resistant = organism growth not  
 affected by bacitracin: organism  
 grows around and under disk



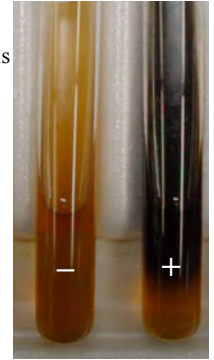
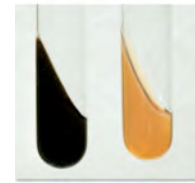
**Bile Esculin Hydrolysis Slant**

Inoculation method: surface streak with loop

Contains: esculin, ferric citrate (reacts with hydrolyzed esculin (esculetin) to produce black precipitate)

Discriminates organisms that can hydrolyze esculin into esculetin and dextrose

Results: Black = positive for esculin hydrolysis  
 Colorless = negative for esculin hydrolysis



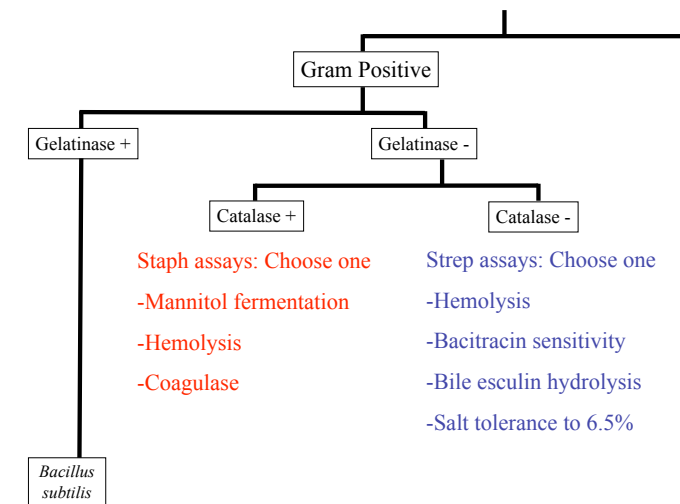
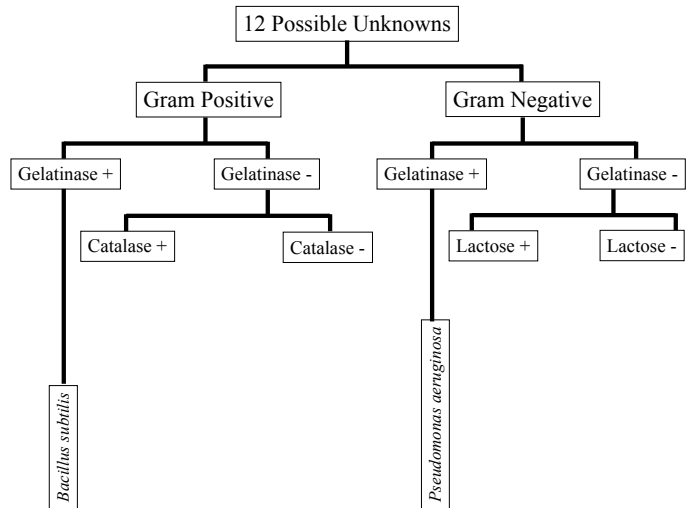
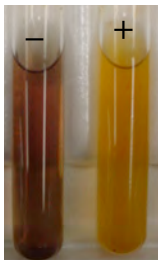
**Salt Tolerance Broth**

Inoculation method: loop transfer

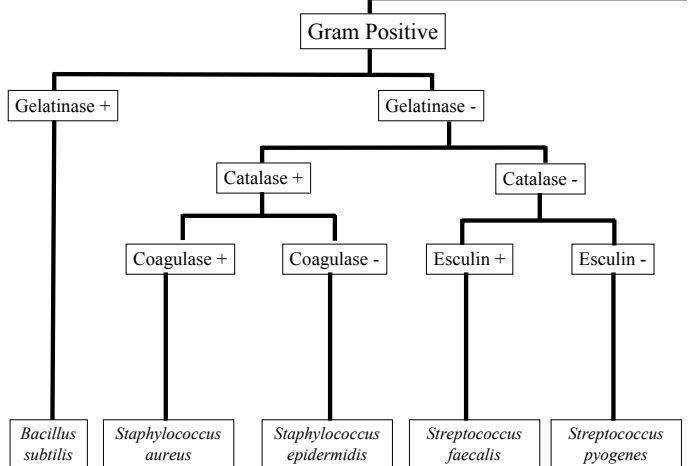
Contains: 6.5% NaCl, Bromocresol purple pH indicator: alkaline pH = purple, acidic pH = yellow (any growth should cause acid accumulation)

Discriminates organisms that display salt tolerance to 6.5%

Results: Yellow = positive for salt tolerance to 6.5%  
 Purple but turbid = positive for salt tolerance to 6.5%  
 Purple = negative for salt tolerance to 6.5%



**EXAMPLE!!!!**



Your Name \_\_\_\_\_  
 Your Unknown Number \_\_\_\_\_  
 Your Answer (Name species) \_\_\_\_\_

Sketch, draw a sketch here of your bacteria as it appeared with oil using appropriate colors for Gram stain and clearly show the shape and arrangement observed

Characteristic or Test	Media used	Other reagents used or indicators present in media	Biochemical or physical aspect(s) being assayed	Result
Size of cells	BHI broth	See Gram stain (1.)		measured in $\mu\text{m}^2$
Shape of cells				Cocci
Arrangement of cells				Staphylococcus (grape-like clusters)
Gram Stain	1. BHI broth 2. MacConkey agar	1. Crystal violet, Iodine, Alcohol, Safranin 2. Crystal violet and Staphin deoxycholate inhibit Gram positive growth	Bacterial wall structure: outer membrane over thin peptidoglycan or thick peptidoglycan with no outer membrane	Gram positive: 1. Purple by Gram stain 2. Did not grow on MacConkey agar
Optimal growth temperature				
Motility			Presence of flagella for movement	
Oxygen requirements		Resourse: pink in oxygen		
Lactose fermentation	1. Lactose broth 2. MacConkey agar 3. Russell's Double Sugar Agar slant	1. Durham tube 1 & 3. Phenol red pH indicator in media: yellow in acid 2. Neutral red pH indicator in media: pink in acid	Ability to ferment lactose to acid (1, 2, 3) with or without gas production (1.)	1. Positive for acid and positive for gas (yellow with bubble) 2. No growth on MacConkey: this inconclusive for lactose fermentation 3. Positive for acid (butt and slant yellow)

Characteristic or Test	Media used	Other reagents used or indicators present in media	Biochemical or physical aspect(s) being assayed	Result
Mannitol fermentation	1. Mannitol broth 2. MSA plate	1. Durham tube 1 & 2. Phenol red pH indicator in media: yellow in acid	Ability to ferment mannitol to acid (1, 2.) with or without gas production (1.)	1. Positive for acid and negative for gas (yellow but no bubble) 2. Yellow on MSA = acid fermentation of mannitol
Glucose fermentation	1. Glucose broth 2. Russell's Double Sugar Agar slant 3. MR-VP broth	1. Durham tube 1 & 2. Phenol red pH indicator in media: yellow in acid 3A. Methyl Red test: Methyl red 3B. Voges-Proskauer test: Barritt's A + alpha naphthol Barritt's B + KOH	Ability to ferment glucose to acid (1, 2, 3A.) with or without gas production (1.) 3A. Ability to catabolize glucose into mixed acids (lactic, acetic and formic acids) in the mixed acid pathway 3B. Ability to catabolize glucose into the neutral end product 2,3-butanediol in the butyrene glycol pathway	1. Positive for acid and positive for gas (yellow with bubble) 2. Positive for acid but inconclusive for glucose fermentation (lactose positive result obscures the glucose result in this media) 3A. Positive for mixed acid (red floating layer) 3B. Negative for butanediol (amber media, no color change)
Citrate fermentation			Ability to produce catalase to catalyze $\text{H}_2\text{O}_2$ into $\text{H}_2\text{O}$ and $\text{O}_2$	
Oxidase production				
Nitrate reduction				

Characteristic or Test	Media used	Other reagents used or indicators present in media	Biochemical or physical aspect(s) being assayed	Result
Starch hydrolysis			Starch hydrolysis capabilities: ability to produce amylase to hydrolyze amylose and amylopectin (starches) into maltose, glucose and dextrins (sugars)	
Casein hydrolysis				
Fat hydrolysis		Spirit blue pH indicator in media: blue in acid		
Urea hydrolysis				
Gelatin hydrolysis				
Cytophan degradation				
Phenylalanine deamination				
Hydrogen sulfide production				
Blood agar hemolysis				
Bacitracin susceptibility				
Cocagulase production				
Stile esculin hydrolysis				

Characteristic or Test	Media used	Other reagents used or indicators present in media	Biochemical or physical aspect(s) being assayed	Result
Salt tolerance	1. Salt tolerance broth 2. MSA plate	1. Bromocresol purple pH indicator in media: yellow in acid 2. N/A	1. Salt tolerance broth assay for 6.5% NaCl tolerance 2. MSA assay for 7.5% NaCl tolerance	1. Tolerant of 6.5% NaCl (broth was yellow and turbid) 2. Tolerant of 7.5% NaCl (colonies grew on plate)

Computer Hints: from MS Word on Mac so yours may be different! Poke around.

1. Set page to landscape: File → Page Setup → Orientation
2. Type header info first (name, etc.) (if you insert the chart first you may not be able to put the header in later.) To center the text (alignment) or do italics (font) open the formatting palette: View → Formatting Palette → Font or Alignment
3. Insert your table: Table → Insert → Table → 5 columns, 28 rows (you can add more later using Table → Insert → Row)
4. Start typing info in the boxes.
5. To keep a row from splitting between pages: Table → Table Properties → Row, then de-select "Allow row to break across pages"
6. To resize columns to better fit the info, just click on and drag the border lines of the columns.
7. To have the column headings on each page, highlight the heading row then: Table → Heading Rows Repeat.

If you are still having trouble there is a MS Word template on the website: <http://www2.sunysuffolk.edu/courses> Download it and modify as necessary. A printed version of the document as posted will not have adequate space to serve as your paper data table for all the lab activities.

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