Unknown Identification

Overview

This lab should give you the background information and techniques you will need to successfully identify your case study specimens later. The micro lab website, your textbook, the web and assorted books available in lab will be the reference materials necessary for you to successfully complete the next several weeks of lab work.

- Each pair will receive one organism to identify…
  - You will be told if you have a staph, a strep or an enteric.
  - You will conduct tests appropriate for your organism to determine species identification.
- Each pair may have to present information on the specific organism they identified including…
  - Test results
  - Where it is part of the normal flora
  - When and where it becomes a pathogen
  - What diseases it causes
- Each pair may also be assigned a specific media test to present the following information on
  - What organism(s) does it differentiate?
  - What do positive results look like?
  - What is the biochemical basis of the test?

Lab Procedure

We have included the basic procedure for doing each biochemical test below. You will find more specific procedures for each biochemical test under the media section of the micro lab website. More complete information on selective & differential media can be obtained by consulting the Difco manuals in lab. You will need to look up the individual test for a more detailed description, including the biochemical basis of each test.

Test	Brief Instructions	Probable Results
TSA/BHI	Staphs/Enterics on TSA; Streps on BHI	Determine macromorphology
Gram Stain	To confirm culture purity	Staphs/Streps (Gram+), Enterics (Gram-)
Motility	Stab with a needle straight in and straight out of the center of the tube half way down. Incubate for 24 hours at 37°C. Staphs/Enterics in O_2; Streps in CO_2.	Motile organisms have obvious growth away from inoculation area; Non-motile organisms grow only in inoculation area.
McFarland Standard	Dilute your organism in a tube of sterile water to obtain a turbidity equivalent to a 0.5 McFarland test standard. Hold your diluted tube and the 0.5 McFarland test standard against the black-lined McFarland reference card to accurately rate the turbidity.	
FTM	Use a **sterile** transfer pipette to add 1 mL of your McFarland standard organism in the middle of the tube. Cap tightly; do not jostle. Incubate for 24 hours at 37°C.	Strict aerobes will grow near the top of the media; Facultative anaerobes will grow throughout; Strict anaerobes will grow near the bottom.
Catalase	Transfer a well-isolated colony to a clean slide & add 1 drop of 3% H_2O_2. Do not reverse the order & do not mix. Observe for immediate bubble formation.	Staphs/Enterics are catalase positive; bubble formation should occur. Note: do not take colony from a blood plate.
Oxidase	Add a few drops of oxidase reagent onto Whatman filter paper. Smear with a loop-full of organisms. Use colonies from low glucose, non-selective media.	Look for appearance of purple color within 10-15 seconds. See probable results table.

Table 1: Brief Description of general tests that every group will do.

Staphylococcus

The **Staph** groups will do these specific tests in addition to the general tests from Table 1.

- TGA (Tellurite Glycine Agar)
- Coagulase
- MSA (Mannitol Salt Agar)
- Novobiocin Antibiotic Disk Sensitivity
- Hemolysis (Blood Agar)

Test	Brief Instructions	Probable Results
TGA	Streak for Isolation. Incubate for 24 hours at 37°C.	See probable results table 3 below.
Coagulase	Add a loop-full or 0.5mL of a pure culture to 0.5mL rabbit plasma. Gently rotate tube to mix, do not shake. Incubate for 24 hours at 37°C.	Presence of clot indicates *S. aureus*
MSA	Streak for isolation. Incubate for 24 hours at 37°C.	See probable results table 3 below.
Novobiocin Antibiotic Disk Sensitivity	Dilute colonies from a pure culture into sterile saline to a 0.5 McFarland standard. Swab half the surface of a blood agar plate. Place a novobiocin disk lightly onto the surface. Incubate for 24 hrs at 37°C.	A zone of growth inhibition ≤16 mm in diameter in a coagulase(-) staph is indicative of *S. saprophyticus*. See probable results table 3 below.
Hemolysis	Streak the other half of the blood agar plate to check for hemolysis. Stab into the agar surface at the last part of your streak. Incubate24 hrs in O_2.	Beta hemolysis is indicative of *S. aureus*. See probable results table 3 below.

Table 2: Brief Description of Biochemical Tests for Staphylococcus Organisms.

	Staphylococcus aureus	*Staphylococcus epidermidis*	*Staphylococcus haemolyticus*	*Staphylococcus saprophyticus*	*Staphylococcus xylosus*
Macromorphology	Creamy/Tan Medium	Creamy/Tan Pinpoint	White Small	Creamy/Tan Wavy Margin	Yellow/Orange Medium
FTM	Facultative Anaerobe	Facultative Anaerobe	Facultative Anaerobe	Facultative Anaerobe	Facultative Anaerobe
Motility	Non Motile	Non Motile	Non Motile	Non Motile	Non Motile
Catalase	Positive	Positive	Positive	Positive	Positive
Oxidase	Negative	Negative	Negative	Negative	Negative
TGA	Black Colonies	Gray Colonies Minimal Growth	Gray Colonies Minimal Growth	Gray Colonies	Gray/Black Colonies
Coagulase	Positive	Negative	Negative	Negative	Negative
MSA	Colorless Colonies Yellow Media	Colorless/Pink Colonies Pink Media	Colorless/Pink Colonies Pink Media	Colorless Colonies Yellow Media	Colorless Colonies Yellow Media
Novobiocin	Susceptible	Susceptible	Susceptible	Resistant	Resistant
Hemolysis	Alpha Prime or Beta Hemolysis	Alpha or Alpha Prime Hemolysis	Alpha Prime or Beta Hemolysis	Alpha Hemolysis	Alpha Hemolysis

Table 3: Probable Results for Staphylococcus Organisms.

Click on a link to an organism to learn more about that specific organism. Click on a link to a media test to learn more about that specific media test. Click on a link to a specific result to see a picture and a more elaborate description of the reaction.

Streptococcus

The **Strep** groups will do these specific tests in addition to the general tests from Table 1.

- Optochin, Bacitracin, and SXT antibiotic disks
- Hemolysis (Blood Agar)
- Hippurate hydrolysis
- Salt tolerance broth
- Bile Esculin

Test	Operating instructions	Probable Results
Optochin Bacitracin SXT	Use your 0.5 McFarland standard to swab half the surface of a blood agar plate. Evenly place one of each disk on the swabbed agar surface.	Any zone of inhibition around the Bacitracin disk is indicative of *S. pyogenes*. See probable results table 5 below.
Hemolysis	Streak the other half of the plate to check for hemolysis. Stab into the agar surface at the last part of your streak. Incubate for 24 hrs in CO_2.	Beta hemolysis is indicative of *S. pyogenes* and *S. agalactiae* (sometimes). See probable results table 5 below.
Hippurate Hydrolysis	Add 5 drops of sterile water to a vial of sodium hippurate. Add enough colonies to this solution to give a turbid suspension. Incubate 24hrs in CO_2. Add 5 drops of ninhydrin solution.	Appearance of a purple color is indicative of *S. agalactiae* and *Strep faecalis*. See probable results table 5 below.
Salt Tolerance	Lightly inoculate broth. Loosely cap and incubate for 24-48 hours in CO_2.	Yellow color change indicative of *Enterococcus faecalis*. See probable results table 5 below.
Bile Esculin	Streak the surface of the slant. Leave the cap loose. Incubate for 24-48 hours in CO_2.	Blackening of the agar is indicative of *S. bovis* and *S. faecalis*. See probable results table 5 below.

Table 4: Brief Description of Biochemical Tests for Streptococcus Organisms.

	Streptococcus agalactiae	***Streptococcus bovis***	***Streptococcus faecalis***	***Streptococcus mutans***	***Streptococcus pyogenes***
Macromorphology	Medium	Pinpoint	Medium	Pinpoint	Small
FTM	Facultative Anaerobe	Facultative Anaerobe	Facultative Anaerobe	Facultative Anaerobe	Facultative Anaerobe
Motility	Non Motile	Non Motile	Non Motile	Non Motile	Non Motile
Catalase	Negative	Negative	Negative	Negative	Negative
Oxidase	Negative	Negative	Negative	Negative	Negative
Optochin	Resistant	Resistant	Variable	Resistant	Resistant
Bacitracin	Variable	Resistant	Resistant	Resistant	Susceptible
SXT	Resistant	Variable	Variable	Variable	Resistant
Hemolysis	Gamma Hemolysis	Alpha Hemolysis	Alpha Hemolysis	Gamma Hemolysis	Beta Hemolysis
Hippurate	Positive	Negative	Positive	Negative	Negative
Salt Tolerance	Variable	Negative	Positive	Negative	Negative
Bile Esculin	Negative	Variable	Positive	Positive	Negative

Table 5: Probable Results for Streptococcus Organisms

Click on a link to an organism to learn more about that specific organism. Click on a link to a media test to learn more about that specific media test. Click on a link to a specific result to see a picture and a more elaborate description of the reaction.

Gram Negative Enterics

The **Enteric** groups will do these specific tests in addition to the general tests from Table 1.

- Mac (MacConkey's Agar)
- EMB (Eosin Methylene Blue)
- HEA (Hekton Enteric Agar)
- MR-VP
- Citrate
- TSI (Triple Sugar Iron)
- Urea broth

Test	Operating instructions	Probable Results
Mac	Streak for isolation. Incubate 24-48 hrs at 37°C.	See probable results table 7 below.
EMB	Streak for isolation. Incubate 24-48 hrs at 37°C.	See probable results table 7 below.
HEA	Streak for isolation. Incubate 24-48 hrs at 37°C.	See probable results table 7 below.
MR-VP	Inoculate with a single colony. Incubate 48hrs at 37°C. See media tests on website for further procedures after incubation.	See probable results table 7 below.
Citrate	Streak surface only. Incubate loosely-capped 24-48hrs at 37°C.	See probable results table 7 below.
TSI	With a needle pick the center of a well isolated colony. Stab the center of the tube to within 3-5 mm of the bottom. Withdraw the needle and lightly streak the surface of the slant. Incubate for 24 hrs at 37°C.	See probable results table 7 below.
Urea	Heavily inoculate a tube of urea broth. Shake tube to distribute organisms. Incubate for 24-48 hrs at 37°C.	See probable results table 7 below.

Table 6: Brief Description of Biochemical Tests for Enteric Organisms.

	Escherichia coli	*Klebsiella pneumoniae*	*Proteus vulgaris*	*Pseudomonas aeruginosa*	*Salmonella typhimurium*	*Shigella flexneri*
Macro morphology	Creamy/Tan Medium	Mucoid/Tan Medium	Translucent Diffusible	Translucent Diffusible	Creamy/Tan Medium	Creamy/Tan Medium
FTM	Facultative Anaerobe	Facultative Anaerobe	Facultative Anaerobe	Strict Aerobe	Facultative Anaerobe	Facultative Anaerobe
Motility	Motile	Non Motile	Motile	Motile	Motile	Non Motile
Catalase	Positive	Positive	Positive	Positive	Positive	Positive
Oxidase	Negative	Negative	Negative	Positive	Negative	Negative
Mac	Pink/Purple w/ precipitate	Purple/Yellow w/ precipitate	Colorless Yellow Media	Colorless Yellow Media	Colorless Yellow Media	Colorless Yellow Media
EMB	Black w/Green Metallic Sheen	Purple maybe Green Metallic Sheen	Colorless or Pink	Colorless or Pink	Colorless or Pink	Colorless or Pink
HEA	Yellow/Orange w/precipitate Poor Growth	Yellow/Orange w/precipitate Poor Growth	Yellow/Orange w/Black centers precipitate	Clear Colonies Blue Media	Blue/Green w/Black Centers Blue Media	Clear Colonies Blue Media
MR/VP	+/-	+/-	+/-	-/-	+/-	+/-
Citrate	Negative	Variable	Negative	Positive	Positive	Negative
TSI	Yellow Slant Yellow Butt Gas	Yellow Slant Yellow Butt Gas	Yellow Slant Yellow Butt Gas, H_2S	Unchanged Slant & Butt	Red Slant Yellow Butt Gas, H_2S	Unchanged Slant Yellow Butt
Urea	Negative	Variable	Positive	Negative	Negative	Negative

Table 7: Probable Results for Gram Negative Enteric Organisms

Click on a link to an organism to learn more about that specific organism. Click on a link to a media test to learn more about that specific media test. Click on a link to a specific result to see a picture and a more elaborate description of the reaction.