

Microbiology for the Health Sciences Laboratory

Special Projects Isolation and Identification of Normal Flora

Introduction

The respiratory and gastrointestinal tracts are composed of a diverse and complex normal flora. The purpose of this project is to use the techniques and skills you have developed over the semester to isolate and identify several microorganisms from these body compartments.

A. Review the following for reference:

1. Text: Chap 10, Section 10.2.
2. Carefully label all materials and keep excellent records of your work.

B. Do the following during lab:

Project 1 - Isolation and identification of microorganisms from your throat.

- Do a throat swab on yourself using a sterile swab.
- Inoculate a small portion of a blood agar (BA) plate. Dispose of the swab in the discard container.
- Do a streak plate (on the BA plate) to get isolated colonies. Incubate 24-48 hours at 37 C.
- Use a new BA plate to isolate (streak plate) 3-4 morphologically different colonies.
Repeat with a new BA plate if necessary to ensure that you have perfectly isolated colonies..
- Gram stain isolates and describe: Colony and microscopic morphology of isolates.
- Choose 2 to 4 that are different and transfer the isolates to brain heart infusion agar slants.
- Characterize the isolates: Gram stain, colony and microscopic morphology, metabolic reactions.
- Identify at least 2 different microorganisms, as close as possible, using the identification key provided.

Project 2 - Isolation and identification of a gram negative rod from your gi tract.

- Collect a very small fecal sample using a sterile swab.
- Carefully transfer the fecal matter to a tube of sterile water.
- Dispose of the swab in the discard container.
- Mix the water/fecal sample very well.
- Transfer a loopful of the material to a plate of EMB agar and a plate of T-Soy Agar.
- Streak for isolated colonies. Incubate for 24 hours at 37 C.
- Transfer 2 isolated colonies to T-soy agar slants. Choose 2 different mo's.
- Characterize the isolates: Gram stain, colony and microscopic morphology, metabolic reactions
- Identify, as closely as possible, using the identification key provided.
Note: If available, you may identify the mo using an Enterotube.
Note: You can eliminate *E. coli* by choosing one mo that is lactose negative.

Project 3 - Isolation of coagulase positive *Staphylococcus aureus* from your nose.

- Do a nasal swab and streak for isolated colonies on a plate of Tellurite Glycine Agar.
Incubate for 48+ hours at 37 C.
- Transfer a large, dark, shiny black colony (of gram positive cocci) to a tube of Trypticase Soy Broth.
Incubate for 24 hours at 37 C. Note: You must gram stain the isolate.
- Store the mo in TSB in the refrigerator until we later test the organism for coagulase production.
- To test for coagulase: Add two drops of the 24 hour culture to a tube with 0.25 ml of citrated rabbit plasma.
Incubate in a 37 C water bath checking every hour (up to 4 hours) for coagulation of the plasma. Incubate for 24 hours before calling the reaction negative.
Note: Coagulase is a virulence factor, and coagulase production correlates with virulence of *S. aureus*.

Project 4 - Isolation of yeasts from a mucous membrane.

- Collect a mucous membrane swab and streak for isolated colonies on Mycobiotic Agar.
Incubate for 48 hours at 37 C.
- Gram stain an isolate and transfer a yeast colony to a tube of Brain Heart Infusion Broth and incubate.
- Store the mo in BHIB in the refrigerator until we test the organism for germ tube production.
- To test for germ tube production: Add an inoculum of the isolated yeast to a tube of rabbit plasma and incubate for 2-3 hours at 37 C. Place a drop of the suspension on a microscope slide and observe for germ tubes. Germ tubes are appendages that are ½ the width and 3 - 4 times the length of the cell.
Note: Germ tubes enhance colonization of yeasts on mucous membranes.

C. Results:

1. You will write up a report on your projects in your notebooks.
2. You will turn in the following materials for Projects 1 and 2:
All plates (isolated colonies), all slants and all stained microscope slides.
3. You will **not** turn in the swabs, or any of the biochemical test media for Projects 1 and 2.
4. You will **not** turn in any of the materials used in Projects 3 and 4.
5. Note: I must be able to follow what you did, and how well you did it, using only your notebook, the report and the materials you give to me.

D. Techniques:

You will utilize techniques that you have already mastered as a part of this lab course.

All specimens should be treated as BSL-2 materials.

The following is a logical sequence for the isolation, characterization and identification of some common bacteria (Projects 1 and 2).

1. Isolation

- a. Streak for isolated colonies and incubate:
For throat swabs or fecal samples use Blood Agar (BA), Brain Heart Infusion Agar (BHIA), or a selective media like EMB and incubate at 37 C for 24-48 hours. Incubation in a candle jar (i.e. 5% CO₂), or at lower temperatures (e.g. 24 C) will enhance the growth of specific mo's.
- b. Re-isolate, if necessary, and maintain desired colony types:
Transfer to BA or BHIA, streak for isolated colonies and incubate. You may need to repeat this. When colonies are well isolated, transfer to a BA or BHIA slant, incubate and save in refrigerator.
- c. Streak for isolated colonies on selective media as necessary to isolate pathogenic fungi or bacteria. Re-isolation is not necessary, but it is necessary to gram stain isolates.

2. Characterization

- a. Growth on blood agar. Note especially the following:
Hemolytic activity
Pigment formation
Size of colonies
Aerobic or anaerobic growth
Rate of growth
- b. Gram stain of isolated colonies. Note especially the following:
Gram positive, gram negative or gram variable (i.e. gram positive)
Rods, cocci or cocco-bacillary in shape
- c. For Gram Positive mo's, note especially the following items:
Morphology and arrangement
Spore formation
Catalase production
Glucose fermentation
- d. For Gram Negative mo's, note especially the following items:
Morphology and arrangement
Pigment production
Oxidase production
Glucose fermentation
Lactose fermentation

3. Identification

- a. Use the attached brief key to help identify your unknown microorganism.
The key is very abbreviated and many genera and species of normal microbiota are not included.
- b. Use the key as follows:
Answer questions I and II first, and then move on.
Answer questions A and B second, and then move on.
Answer questions 1, 2 etc next, and then move on.
Answer questions a, b etc and then move on.
Continue until you have identified (as close as possible) your mo's.
- c. Do biochemical tests only as necessary (and available) to help in your identification.
Your instructor will help you make decisions about what tests to do, the interpretation of the tests, and when you can do several tests all at once in an effort to save time.
- d. Do not do tests identified in []

Identification Key

- I. Cells are coccus in shape
- A. Gram positive (or gram variable)
- Catalase produced
 - Glucose not fermented, stained cells in tetrads, [coagulase (-)]
 - Yellow pigmented colonies, [hydrolyzes lipids]
 - Red pigmented colonies, [no lipid hydrolysis]
 - Glucose fermented, stained cells generally in clusters, [nitrate reduced]
 - Mannitol fermented, beta-hemolytic on blood agar, [coagulase (+)]
 - Mannitol not fermented, no hemolysis on blood agar
 - Mannose fermented [and usually fructose]
 - Mannose not fermented [or usually fructose]
 - Glucose fermented, stained cells in tetrads, [nitrate not reduced]
 - Catalase not produced
 - Growth in 6.5 % NaCl broth
 - No growth in 6.5% NaCl broth
 - Alpha-hemolysis on blood agar
 - Beta-hemolysis on blood agar
 - Gamma-hemolysis on blood agar
- B. Gram negative, catalase positive, oxidase positive,
- Glucose fermented, colonies on blood agar not pigmented
 - Glucose fermented, pigmented colonies on blood agar
 - Glucose not fermented, diplococci, pigmented colonies on blood agar
 - Glucose not fermented, cells arranged in clusters, colonies not pigmented
- II. Cells are rod or cocco-bacillary or irregular in shape
- A. Gram positive
- Endospores present in stained smears, catalase (+), aerobic, [gelatin (+)]
 - Aerobic, catalase (+), [gelatin (+)]
 - Strict anaerobe, catalase (-)
 - No endospores present
 - Aerobic, catalase (+), [gelatin (-)]
 - Aerobic, catalase (+), [acid-fast (+)], very slow (7+ days) growth
 - Strict anaerobe
 - Microaerophilic, catalase (-), (easily con. w/ Strep sp)
- B. Gram negative, aerobic or facultatively anaerobic
- Glucose not fermented, oxidase positive
 - Glucose oxidized, gelatin hydrolyzed, Caseinase produced, diffusable pigments
 - Glucose not oxidized, white or yellow colonies
 - Glucose fermented, oxidase negative: Use Enterotube, or:
 - Lactose fermented
 - Citrate utilized
 - MR(-), VP(+)
 - MR(-), VP(+), Encapsulated
 - MR(+), VP(-)
 - Citrate not utilized
 - Lactose not fermented
 - Red pigment at 25 C
 - No red pigment
 - Indole produced
 - H₂S produced
 - No H₂S
 - Indole not produced, mannitol not fermented
 - Indole not produced, H₂S(+), mannitol fermented
 - Citrate (-), H₂S (-), MR(+)
- Micrococcus sp
Micrococcus luteus
Micrococcus roseus
Staphylococcus sp
Staph. aureus

Staph. epidermidis
Staph. saprophyticus
Gaffkya sp.

Enterococcus sp.
Streptococcus sp
Strep. pneumonia
Strep. pyogenes
Streptococcus sp.

Neisseria & Moraxella sp
Neisseria sicca, mucosa et al
Neisseria subflava
Neisseria flavescens
Moraxella catarhalis
- Bacillus sp.
Clostridium sp.

Corynebacterium sp.
Mycobacterium sp.
Propioibacterium sp, Others
Lactobacillus sp.
- Pseudomonas sp.
Alkaligenes sp.

Enterobacter aerogenes
Klebsiella pneumoniae
Citrobacter freundii
Escherichia coli

Serratia marcescens

Proteus vulgaris
Morganella morganii
Proteus mirabilis
Salmonella sp.
Shigella sp.

Notes: 1) For gram (+), catalase (+), cocci, inoculate glucose, mannitol and mannose fermentation broths all at once.
2) For gram (-) cocci, oxidase is a confirmatory test only, but may help distinguish gram (+) from gram (-) cocci.
3) Pigment production is a macroscopic observation of whole colonies and is noted on blood agar.

E. Results and Discussion