

LAB 14: ISOLATION AND IDENTIFICATION OF STREPTOCOCCI AND ENTEROCOCCI

GENERAL DISCUSSION

There are two genera of bacteria that can appear as a streptococcus arrangement that we will take up in the lab: **the genus *Streptococcus* and the genus *Enterococcus***. Both are **Gram-positive cocci** 0.5-1.0 μm in diameter, typically occurring **in pairs and chains of varying length when grown in a liquid medium**, and often **occurring singly, in pairs, short chains, and clusters when taken from an agar culture**. As learned in Lab 8, they are both **catalase-negative**.

A. The genus *Streptococcus*

Streptococcus species are usually classified clinically based on their **hemolytic properties on blood agar** and according to their **serologic groups**.

- Scanning electron micrograph *Streptococcus pyogenes*; courtesy of [Dennis Kunkel's Microscopy](#).
- [Transmission electron micrograph of *Streptococcus*](#) from the Rockefeller University web page.
- [Scanning electron micrograph of a *Streptococcus pneumoniae*](#).

The streptococci are usually isolated on Blood agar. **Blood agar** is one of the most commonly used media in a clinical lab. It consists of an enriched agar base (Tryptic Soy agar) to which **5% sheep red blood cells** have been added. Blood agar is commonly used to isolate not only streptococci, but also staphylococci and many other pathogens. Besides providing enrichments for the growth of fastidious pathogens, Blood agar can be used to detect hemolytic properties.

Hemolysis refers to is the lysis of the red blood cells in the agar surrounding bacterial colonies and is a result of bacterial enzymes called **hemolysins**. Although hemolysis can often be observed with the naked eye, ideally it should be examined microscopically using low power magnification, especially in cases of doubtful hemolysis. Reactions on blood agar are said to be beta, alpha, gamma, or double-zone:

1. **Beta hemolysis** (see [Fig. 1A](#) and [Fig. 1B](#)) refers to a **clear, red blood cell-free zone** surrounding the colony, where a **complete lysis of the red blood cells** by the bacterial hemolysins has occurred. This is best seen in subsurface colonies where the agar has been stabbed since some bacterial hemolysins, like streptolysin O, are inactivated by oxygen.
2. **Alpha hemolysis** (see [Fig. 5A](#) and [Fig. 5B](#)) appears as a zone of **partial hemolysis** surrounding the colony, often accompanied by a **greenish discoloration of the agar**. This is also best seen in subsurface colonies where the agar has been stabbed.
3. **Gamma reaction** (see [Fig. 6](#)) refers to **no hemolysis or discoloration of the agar** surrounding the colony.
4. **Double-zone hemolysis** (see [Fig. 10](#)) refers to **both a beta and an alpha zone of hemolysis** surrounding the colony.

See [Fig. 11](#) to view a photograph showing alpha, beta, and gamma hemolysis on blood agar.

See [Fig. 12](#) for a blood agar plate of a throat culture showing possible *Streptococcus pyogenes*.

Many of the streptococci can also be classified under the **Lancefield system**. In this case, they are divided into 19 different **serologic groups** on the basis of carbohydrate antigens in their cell wall. These antigenic groups are designated by the letters A to H, K to M, and O to V. Lancefield serologic groups A, B, C, D, F, and G are the ones that normally infect humans, however, not all pathogenic streptococci can be identified by Lancefield typing (e.g., *Streptococcus pneumoniae*). **Serologic typing** to identify microorganisms will be discussed in more detail later in **Lab 17**. Single-stranded DNA probes complementary to species-specific r-RNA sequences of streptococci and enterococci are also being used now to identify these organisms.

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1. The Beta Streptococci

DISCUSSION

Lancefield serologic groups A, B, C, D, F, and G are all streptococci that may show beta hemolysis on Blood agar. However, some group B streptococci are non-hemolytic and group D streptococci (discussed below) usually show alpha hemolysis or are non-hemolytic.

Streptococcus pyogenes, often referred to as **group A beta streptococci** or **GAS** because they belong

to Lancefield serologic group A and show beta hemolysis on blood agar, are responsible for most acute human streptococcal infections. *S. pyogenes* isolates are **Gram-positive cocci** 0.5-1.0 µm in diameter that typically form short chains in clinical specimens and longer chains in laboratory media. The most common infection is **pharyngitis** (streptococcal sore throat) with the organism usually being limited to the mucous membranes and lymphatic tissue of the upper respiratory tract. *S. pyogenes* is responsible for 15-30% of cases of acute pharyngitis in children and 5-10% of cases in adults. Between 5% and 20% of children are asymptomatic carriers. Pharyngitis is spread person to person primarily by respiratory droplets; skin infections are spread by direct contact with an infected person or through fomites.

From the pharynx, however, the streptococci sometimes **spread to other areas of the respiratory tract** resulting in **laryngitis, bronchitis, pneumonia, and otitis media (ear infection)**. Occasionally, it may enter the **lymphatic vessels or the blood** and disseminate to other areas of the body, causing **septicemia, osteomyelitis, endocarditis, septic arthritis, and meningitis**. It may also **infect the skin**, causing **erysipelas, impetigo, or cellulitis**.

Group A beta streptococcus infections can result in two **autoimmune diseases, rheumatic fever and acute glomerulonephritis**, where antibodies made against streptococcal antigens cross react with joint membranes and heart valve tissue in the case of rheumatic fever, or glomerular cells and basement membranes of the kidneys in the case of acute glomerulonephritis.

Streptococcal pyrogenic exotoxin (Spe), produced by rare invasive strains and scarlet fever strains of *Streptococcus pyogenes* (the group A beta streptococci). *S. pyogenes* produces a number of SPEs that are cytotoxic, pyrogenic, enhance the lethal effects of endotoxins, and contribute to cytokine-induced inflammatory damage. **SPEs are responsible for causing streptococcal toxic shock syndrome (STSS)** whereby excessive cytokine production leads to fever, rash, and triggering the shock cascade. The **SPEs also appear to be responsible for inducing necrotizing fasciitis, a disease that can destroy the skin, fat, and tissue covering the muscle** (the fascia). SPE B is also a precursor for a cysteine protease that can destroy muscles tissue.

CDC reports that approximately 9,000-11,500 cases of invasive GAS disease occur each year in the U.S., with STSS and necrotizing fasciitis each accounted for approximately 6-7% of the cases. STSS has a mortality rate of around 35%. The mortality rate for necrotizing fasciitis is approximately 25%.

For further information on virulence factors for group A beta Streptococci, see the following Learning Objects in your Lecture Guide:

- [Teichoic Acids and Glycopeptides Cell Wall Fragments; Unit 3, Section C1c](#)
- [The Ability to Adhere to Host Cells: Pili and Adhesins; Unit 3, Section B2](#)
- [The Ability to Resist Phagocytic Engulfment; Unit 3, Section B5b](#)
- [The Ability to Resist Phagocytic Destruction and Serum Lysis; Unit 3, Section B5c](#)
- [The Ability to Evade Adaptive Immune Defenses; Unit 3, Section B6](#)
- [The Ability to Produce Harmful Superantigens; Unit 3, Section C2a1](#)
- [Toxins that Damage Cell Membranes; Unit 3, Section C2c](#)
- [The Ability to Induce Autoimmune Responses; Unit 3, Section C3](#)

The **group B streptococci** (GBS or *Streptococcus agalactiae*) usually show a small zone of beta hemolysis on Blood agar, although some strains are non-hemolytic. *S. agalactiae* isolates are **Gram-positive cocci** 0.6-1.2 µm in diameter that typically form short chains in clinical specimens and longer chains in laboratory media. They are **found in the gastrointestinal tract and genitourinary tract of 15%-45% healthy woman**. This reservoir, along with nosocomial transmission, provides the inoculum by which many infants are colonized at birth. The transmission rate from a mother colonized with GBS to her baby is thought to be around 50%. Most colonized infants (and adults) remain asymptomatic, however, an estimated **1-2% of neonates colonized will develop invasive GBS diseases, including pneumonia, septicemia, and/or meningitis**. Pregnant women should be tested to determine if they are GBS carriers and be given IV antibiotics if they are a carrier

Other infections associated with group B streptococci include **urinary tract infections, skin and soft tissue infections, osteomyelitis, endometritis, and infected ulcers (decubitus ulcers and ulcers associated with diabetes)**. In the immunocompromised patient it sometimes causes **pneumonia and meningitis**.

The **group C streptococci** (mainly *S. equi*, *S. equisimilis* and *S. zooepidemicus*) are beta hemolytic. They sometimes cause pharyngitis and, occasionally, bacteremia, endocarditis, meningitis, pneumonia, septic arthritis, and cellulitis. Group C streptococci are a common cause of infections in animals.

The **group F streptococci** (mainly *S. anginosus*) have been isolated from abscesses of the brain, mouth, and jaw. They also sometimes cause endocarditis.

The **group G streptococci** also show beta hemolysis. They sometimes cause pharyngitis and can also cause serious infections of the skin and soft tissues (mainly in the compromised host) as well as endocarditis, bacteremia, and peritonitis.

All of these beta hemolytic streptococci can be identified by biochemical testing and/or by serologic testing. Today you will look at the isolation and identification of group A beta streptococci (*Streptococcus pyogenes*) by biochemical testing. Serological identification will be performed in Lab 17.

[Concept map for Lab 14 - Organisms and Infections](#)

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ISOLATION AND IDENTIFICATION OF GROUP A BETA STREPTOCOCCI (*Streptococcus pyogenes*)

Group A beta streptococci are usually isolated on **Blood agar**. *Streptococcus pyogenes* produces

1. Very **small, white to grey colonies** approximately 1mm in diameter.
2. A zone of **beta hemolysis** (see Fig. 7) around 2-3mm in diameter surrounding each colony.

There are two streptococcal hemolysins, streptolysin S and streptolysin O. Streptolysin O can be **inactivated by oxygen** so more distinct hemolysis can be seen by **stabbing the agar several times**. In this way, some of the organisms form subsurface colonies growing away from oxygen. Since both streptolysin S and streptolysin O are active in the stabbed area, a more clear zone of beta hemolysis can be seen.

3. **Sensitivity to the antibiotic bacitracin found in a Taxo A® disc.**

Only the group A beta streptococci are sensitive to bacitracin, as shown by a **zone of inhibition around a Taxo A® disc** (see Fig. 7), a paper disc containing low levels of bacitracin. Other serologic groups of streptococci are resistant to bacitracin and show no inhibition around the disc. (The Lancefield group of a group A beta streptococcus can also be determined by direct serologic testing as will be demonstrated in Lab 17.)

See [Fig. 12](#) for a blood agar plate of a throat culture showing possible *Streptococcus pyogenes*.

[Concept map for Lab 14 - Identification](#)

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2. The Pneumococcus (*Streptococcus pneumoniae*)

DISCUSSION

Streptococcus pneumoniae, or the pneumococcus, is a lancet-shaped (pointed like a lance) Gram-positive coccus 0.6-1.2 μm in diameter. They typically appear as a **diplococcus**, but occasionally appear singularly or in short chains. Pneumococci are frequently found as normal flora of the **nasopharynx of healthy carriers**. Pharyngeal colonization occurs in 40%-50% of healthy children and 20%-30% of healthy adults.

Worldwide, as well as in the U.S., *S. pneumoniae* remains the most common cause of community-acquired pneumonia, otitis media, bacteremia, and bacterial meningitis. In the U.S., pneumococci are **the most common cause of community-acquired pneumonia requiring hospitalization**, causing an estimated 500,000 cases per year and usually occurring as a secondary infection in the debilitated or immunocompromised host. The pneumococci also cause between 6 and 7 million cases of **otitis media** per year, are the leading cause of **sinusitis** in people of all ages, are responsible for 55,000 cases of **bacteremia**, and 3000 cases of meningitis, being the most common cause of **meningitis** in adults and children over 4 years of age.

The capsule serves as the major virulence factor, enabling the pneumococcus to resist phagocytic engulfment, and glycopeptides from its Gram-positive cell wall can lead to excessive cytokine production and a massive inflammatory response.

Pneumococci show **alpha hemolysis** on Blood agar (see Fig. 5B).

For further information on virulence factors for *Streptococcus pneumoniae*, see the following Learning Objects in your Lecture Guide:

- [Teichoic Acids and Glycopeptides Cell Wall Fragments; Unit 3, Section C1c](#)
- [The Ability to Adhere to Host Cells: Pili and Adhesins; Unit 3, Section B2](#)
- [The Ability to Resist Phagocytic Engulfment; Unit 3, Section B5b](#)
- [The Ability to Evade Adaptive Immune Defenses; Unit 3, Section B6](#)
- [Toxins that Damage Cell Membranes; Unit 3, Section C2c](#)

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ISOLATION AND IDENTIFICATION OF PNEUMOCOCCI (*Streptococcus pneumoniae*)

1. Isolation on Blood agar

Pneumococci frequently require enriched media and increased CO₂ tension for initial isolation. They are usually isolated on **Blood agar** and incubated in a candle jar (a closed container in which a lit candle is placed to remove O₂ and increase CO₂) at 37C. On Blood agar, colonies appear small, shiny, and translucent. They are surrounded by a zone of **alpha hemolysis** (see [Fig. 5B](#)). Due to autolysis with age, the colonies may show a depressed center with an elevated rim.

2. Optochin sensitivity

Pneumococci are the only streptococci that are **sensitive to the drug optochin** (ethylhydrocupreine hydrochloride). This can be detected by a **zone of inhibition around a Taxo P® disc** (see [Fig. 10](#)), a paper disc containing the drug optochin, which is placed on the Blood agar plate prior to incubation.

3. Bile solubility test

Most colonies of *S. pneumoniae* will dissolve within a few minutes when a drop of bile is placed upon them. (This test will not be done in lab today.)

4. Gram stain of sputum

Streptococcus pneumoniae will usually appear as encapsulated, **Gram-positive, lancet-shaped diplococci**.

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3. The Viridans Streptococci

DISCUSSION

Ten species of streptococci are known as the **viridans streptococci**. They are the **dominant normal flora in the upper respiratory tract**. Species include *S. mutans*, *S. sanguis*, *S. mitis*, and *S. salivarius*. *S. mutans* is the primary cause of **dental caries**. Viridans streptococci are responsible for between 50% and 70% of the cases of **bacterial endocarditis**, especially in people with previously damaged heart valves. They are also frequently associated with **bacteremia, deep wound infections, dental abscesses, and abscesses of internal organs**. The [viridans streptococci](#) show **alpha hemolysis or no hemolysis** on Blood agar, do not possess Lancefield group antigens, and can be differentiated from other alpha streptococci by biochemical testing.

B. The Genus *Enterococcus*

DISCUSSION

Enterococci are **Gram-positive streptococci that are normal flora of the intestinal tract. They typically occur singly, in pairs, short chains, and clusters**, especially when taken off an agar culture for staining. Like the genus *Streptococcus*, the genus *Enterococcus* is **catalase-negative**. Enterococci **responsible for a variety of opportunistic infections** in humans, and serologically belong to **Lancefield group D streptococci**.

[Enterococcus faecalis](#) is the most common enterococcus causing human infections, representing 80-90% of human enterococcal clinical isolates. *E. faecalis* is **normal flora of the intestinal tract in humans** and is regularly isolated from **infections within the peritoneal cavity** (especially following penetrating trauma), **urinary tract infections, kidney infections, prostate infections, and infections of damaged or compromised skin** such as diabetic or decubitus ulcers, burns and surgical wounds. Other opportunistic enterococcal species include *E. faecium* and *E. durans*. **The enterococci have become the second most common bacterium isolated from nosocomial urinary and wound infections, and the third most common cause of nosocomial bacteremia.** Each year in the U.S., in fact, enterococci account for approximately 110,000 urinary tract infections, 40,000 wound infections, 25,000 cases of nosocomial bacteremia, and 1100 cases of endocarditis. Furthermore, the enterococci are **among the most antibiotic resistant** of all bacteria, with some isolates resistant to all known antibiotics.

- Scanning Electron Micrograph of [Enterococcus](#)
- [Diseases and Organisms in Healthcare Settings; from CDC](#)

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ISOLATION AND IDENTIFICATION OF ENTEROCOCCI

The enterococci may be isolated and identified using various selective and differential media. Two such media are:

1. SF broth

SF broth contains sodium azide, which inhibits most bacteria other than enterococci. The enterococci will grow in SF broth and **ferment the dextrose**, turning the pH indicator **from violet to a yellow-brown color** ([see Fig. 8](#)).

2. Bile Esculin agar

Unlike most bacteria, the enterococci will grow in the presence of the bile salts in the medium. They **hydrolyze the esculin**, producing esculetin which reacts with the iron salts in the medium turning the agar **black** ([see Fig. 9](#)).

On blood agar, **most strains of *Enterococcus faecalis* show gamma reaction on sheep blood agar**, however some strains exhibit beta hemolysis. Colonies are usually 1-2 millimeters in diameter. Enterococci are also being identified using chemiluminescent labelled DNA probes complementary to species-specific bacterial ribosomal RNA (rRNA) sequences.

SCENERIOS FOR TODAY'S LAB

Case Study #1

Choose **either unknown #1 or unknown #2** as your unknown for **Case Study #1**.

A 21 year old male complains of a sore throat and painful swallowing. A physical exam of the throat shows tonsillopharyngeal edema and erythema, a patchy exudate, petechiae on the soft palate, and a red, swollen uvula.

He has a temperature of 101.6 °F. He doesn't have a cough or a noticeably runny nose.

Assume that your unknown is a transport medium from a swab of this person's throat.

CAUTION: TREAT EACH UNKNOWN AS A PATHOGEN! Inform your instructor of any spills or accidents. **WASH AND SANITIZE YOUR HANDS WELL** before leaving the lab.

MATERIALS

1 plate of blood agar, 1 Taxo A ® disc, 1 sterile swab, inoculating loop

PROCEDURE (to be done in groups of 3)

1. Using a sterile inoculating loop, **streak your unknown for isolation** on a blood agar plate so as to get **single, isolated colonies** (see [Fig. 2, step 1](#), [Fig. 2, step 2](#), and [Fig. 2, step 3](#)). Before you streak your plate **draw an "X" on the bottom of the blood agar plate to indicate where you begin the streaking pattern.**

[Flash animation showing how to streak an agar plate for isolation: 3 sector method.](#)

[html5 version of animation for iPad showing how to streak an agar plate for isolation: 3 sector method.](#)

3. Using your inoculating loop, **stab** the agar several times in each of the growth areas in order to detect oxygen-sensitive hemolysins ([Fig. 2, step 4](#)).

4. **Place a Taxo A ® disc containing bacitracin in the center of the area of the plate that you first streaked (the area where you drew the "X")** where you expect to see heaviest growth ([Fig. 2, step 5](#)).

5. Incubate the blood agar plate **upside down and stacked in the petri plate holder on the shelf of the 37°C incubator corresponding to your lab section** until the next lab period.

Case Study #2

Choose **unknown #3** as your unknown for **Case Study #2**.

A hospitalized 57 year old female with an indwelling urinary catheter and multiple antibiotic therapies presents suprapubic discomfort, lower back pain, and a temperature of 101.1°F. A complete blood count (CBC) shows leukocytosis with a left shift. A urine dipstick shows a positive leukocyte esterase test, a negative nitrite test, 30mg of protein per deciliter, and red blood cells in the urine.

Assume that your unknown is a culture from this patient.

CAUTION: TREAT EACH UNKNOWN AS A PATHOGEN! Inform your instructor of any spills or accidents. **WASH AND SANITIZE YOUR HANDS WELL** before leaving the lab.

MATERIALS

1 bile esculin agar slant, 1 tube of SF broth, materials to perform a Gram stain, inoculating loop

PROCEDURE (to be done in groups of 3)

[Keep in mind that in a real clinical situation other lab tests and cultures for bacteria other than those upon which this lab is based would also be done.]

1. Do a **Gram stain** on the unknown (see Lab 6). Make sure you **review the instructions before you do the Gram stain**. Because Enterococci and Staphylococci can sometimes look similar in Gram stains done from a plate culture, you may wish to run the **catalase test** on your unknown (see Lab 8).

[Instructions for thje Gram Stain from Lab 6.](#)

[Instructions for Focusing a Microscope from Lab 1.](#)

2. Inoculate a **Bile Esculin agar slant** and a **SF broth tube** with your unknown. Incubate both tubes **in your test tube rack on the shelf of the 37°C incubator corresponding to your lab section** until the next lab period.

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[Medscape](#) articles on infections associated with organisms mentioned in this Lab Exercise. Registration to access this website is free.

- [Streptococcus pyogenes](#)
- [Bacterial Pharyngitis](#)
- [Viral Pharyngitis](#)
- [Group B Streptococcus](#)
- [Enterococcus species](#)
- [Streptococcus pneumoniae](#)

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RESULTS

1. Demonstrations of Pneumococci (*Streptococcus pneumoniae*)

Blood agar with Taxo P® (optochin) disc

description of colony	
type of hemolysis (alpha, beta, or gamma)	
Taxo P® disk (optochin) results (inhibition or no inhibition)	

Case Study Lab Report for Lab 14: *Streptococcus* and *Enterococcus*

The concept behind the case studies presented in Lab 14 used to illustrate the genus *Streptococcus* and the genus *Enterococcus* is for you and your lab partners as a group to:

1. **Determine whether or not the patient in case study #1 has streptococcal pharyngitis.**
2. **Come up with a valid diagnosis of the infectious disease in case study #2 and identify the bacterium causing that infection.**
3. **Support your group's diagnose** based on:
 - a. Any relevant facts in the **patient's history**. (A reliable on-line source will be used to support this.)
 - b. The patient's **signs and symptoms**. (A reliable on-line source will be used to support this.)
 - c. **Each of the individual lab tests** given in your case study.
 - d. All **microbiological lab tests you performed** as part of the project.

The due date for this report can be found on the class calendar. Remember, you are **working as a group to solve a problem**. Your grade for this lab is based on the **completeness of your report and written evidence of the critical thinking process that went into making and supporting your diagnosis**, therefore, it is critical that all members of the group participate, question any conclusions being made by the group, and contribute to the report. Remember, you are trying to convince your instructor that you understand how the diagnosis was made by **supporting that diagnosis with data**. Your group will work together to write the report and **submit one hard copy of that report for your group**. Part of your grade will be based on evaluation of your work by your team members.

Be sure to handle all the bacterial cultures you are using in lab today as if they are pathogens! Be sure to wash and sanitize your hands well at the completion of today's lab.

Also, make sure you **observe the results of of someone in your lab who had an unknown different from yours in case study #1.** The Performance Objectives for Lab 14 tell you what you are expected to be able to do on the practical.

[Rubric used for grading your Case Study #1 Lab Report.](#)

[Rubric used for grading your Case Study #2 Lab Report.](#)

[Team Member Evaluation Form used for grading your Lab 14 Lab Report.](#)

Each member of the group must:

- 1. Print a copy of each of the two rubrics from the links above.***
- 2. Print and fill out a copy of the Team Member Evaluation Form from the link above.***
- 3. Staple them together and hand them in to me the day your Lab 14 Case Study Lab Report is due.***

For more information on writing your Lab Report, see [Course evaluation for Lab \(Core labs, case studies, lab quizzes\)](#) under Course Info in the menu of the BIOL 230 website.

[Click here to print a PDF copy of this lab report.](#)

Your Name:

Others in your group:

Lab section:

Date:

A. Case Study #1 from Lab 14: Unknown #1

Each member of the group must:

- 1. Print a copy of each of the two rubrics from the links above.***
- 2. Print and fill out a copy of the Team Member Evaluation Form from the link above.***
- 3. Staple them together and hand them in to me the day your Lab 14 Case Study Lab Report is due.***

A 21 year old male complains of a sore throat and painful swallowing. A physical exam of the throat shows tonsillopharyngeal edema and erythema, a patchy exudate, petechiae on the soft palate, and a red, swollen uvula. He has a temperature of 101.6 °F. He doesn't have a cough or a noticeably runny nose.

1. Patient's signs and symptoms

Read the case study. Explain how the patient's signs and symptoms contributed to your diagnosis of the type of infectious disease seen here. You are urged to use the computers in lab to search reliable medically oriented Internet sources to support this. Reliable sources you might consider are *Medscape* (http://emedicine.medscape.com/infectious_diseases) and The Centers for Disease Control and Prevention

(CDC) at <http://www.cdc.gov/>. Cite any sources you use at the end of this Patient's Symptoms section in APA style (<http://www.apastyle.org/>).

The **patient's signs and symptoms should suggest a general type of infectious disease** that is present, such as a urinary tract infection, a wound infection, gastroenteritis, pharyngitis, pneumonia, septicemia, etc. **You need to determine the general type of infection in order to determine what microbiological tests to perform to identify the bacterium causing the infection.** Search at least one medically-oriented reference article from a reliable site such as *Medscape* and **use this article to support your diagnosis of the type of infectious disease seen here.** Don't forget to **cite any sources you used in APA style directly under this Patient's Signs and Symptoms sections** of this Lab Report.

2. Microbiological lab tests you performed in Lab 14

a. Blood agar with Taxo A® (bacitracin) disc: Unknown #1

Give the results of the Blood agar with Taxo A® (bacitracin) disc you performed on the unknown you were given, and how you reached this conclusion. **State how this contributed to your final diagnosis** as to whether or not the person has streptococcal pharyngitis. The possible results for blood agar and Taxo A® disc were discussed in the beginning pages of this lab.

3. If he has streptococcal pharyngitis, state the genus and species of this bacterium.

B. Case Study #1 from Lab 14: Unknown #2

Each member of the group must:

- 1. Print a copy of each of the two rubrics from the links above.*
- 2. Print and fill out a copy of the Team Member Evaluation Form from the link above.*
- 3. Staple them together and hand them in to me the day your Lab 14 Case Study Lab Report is due.*

A 21 year old male complains of a sore throat and painful swallowing. A physical exam of the throat shows tonsillopharyngeal edema and erythema, a patchy exudate, petechiae on the soft palate, and a red, swollen uvula. He has a temperature of 101.6 °F. He doesn't have a cough or a noticeably runny nose.

1. Patient's signs and symptoms

Read the case study. Explain how the patient's signs and symptoms contributed to your diagnosis of the type of infectious disease seen here. You are urged to use the computers in lab to search reliable medically

oriented Internet sources to support this. Reliable sources you might consider are *Medscape* (http://emedicine.medscape.com/infectious_diseases) and The Centers for Disease Control and Prevention (CDC) at <http://www.cdc.gov/>. Cite any sources you use at the end of this Patient's Symptoms section in APA style (<http://www.apastyle.org/>).

The **patient's signs and symptoms should suggest a general type of infectious disease** that is present, such as a urinary tract infection, a wound infection, gastroenteritis, pharyngitis, pneumonia, septicemia, etc. **You need to determine the general type of infection in order to determine what microbiological tests to perform to identify the bacterium causing the infection.** Search at least one medically-oriented reference article from a reliable site such as *Medscape* and **use this article to support your diagnosis of the type of infectious disease seen here.** Don't forget to **cite any sources you used in APA style directly under this Patient's Signs and Symptoms sections** of this Lab Report.

2. Microbiological lab tests you performed in Lab 14

a. Blood agar with Taxo A® (bacitracin) disc: Unknown #2

Give the results of the Blood agar with Taxo A® (bacitracin) disc you performed on the unknown you were given, and how you reached this conclusion. **State how this contributed to your final diagnosis** as to whether or not the person has streptococcal pharyngitis. The possible results for blood agar and Taxo A® disc were discussed in the beginning pages of this lab.

3. If he has streptococcal pharyngitis, state the genus and species of this bacterium.

C. Case Study #2

You must print a copy of the rubric for both case study #1 and case study #2 and staple them to your Lab 14 Lab Report before you submit the report to your instructor!

A hospitalized 57 year old female with an indwelling urinary catheter and multiple antibiotic therapies presents suprapubic discomfort, lower back pain, and a temperature of 101.1°F. A complete blood count (CBC) shows leukocytosis with a left shift. A urine dipstick shows a positive leukocyte esterase test, a negative nitrite test, 30mg of protein per deciliter, and red blood cells in the urine.

1. Patient's history and predisposing factors

Read the case study. Explain how any relevant parts of the patient's history contributed to your diagnosis of the type of infectious disease seen here. You are urged to use the computers in lab to search reliable medically oriented Internet sources to support this. Reliable sources you might consider are *Medscape* (http://emedicine.medscape.com/infectious_diseases) and The Centers for Disease Control and Prevention (CDC) at <http://www.cdc.gov/>. Cite any sources you use at the end of this Patient's History section in APA style (<http://www.apastyle.org/>).

The **patient's history should suggest a general type of infectious disease** that is present, such as a urinary tract infection, a wound infection, gastroenteritis, pharyngitis, pneumonia, septicemia, etc. **Do not look up the bacterium you eventually identify** as the cause of this infectious disease. You don't know the causative bacterium at this point. **You need to determine the general type of infection in order to determine what microbiological tests to perform to identify the bacterium causing the infection.** Search at least one medically-oriented reference article from a reliable site such as *Medscape* and **use this article to support your diagnosis of the type of infectious disease seen here.** Don't forget to **cite any sources you used in APA style directly under this Patient's History and Patient's Symptoms sections** of this Lab Report.

2. Patient's signs and symptoms

Read the case study. Explain how the patient's signs and symptoms contributed to your diagnosis of the type of infectious disease seen here. You are urged to use the computers in lab to search reliable medically oriented Internet sources to support this. Reliable sources you might consider are *Medscape* (http://emedicine.medscape.com/infectious_diseases) and The Centers for Disease Control and Prevention (CDC) at <http://www.cdc.gov/>. Cite any sources you use at the end of this Patient's Symptoms section in APA style (<http://www.apastyle.org/>).

The **patient's signs and symptoms should suggest a general type of infectious disease** that is present, such as a urinary tract infection, a wound infection, gastroenteritis, pharyngitis, pneumonia, septicemia, etc. **Do not look up the bacterium you eventually identify** as the cause of this infectious disease. You don't know the causative bacterium at this point. **You need to determine the general type of infection in order to determine what microbiological tests to perform to identify the bacterium causing the infection.** Search at least one medically-oriented reference article from a reliable site such as *Medscape* and **use this article to support your diagnosis of the type of infectious disease seen here.** Don't forget to **cite any sources you used in APA style directly under this Patient's History and Patient's Symptoms sections** of this Lab Report.

3. Results of laboratory test given in the case study

List each lab test given and explain how the results of that test helps to contribute to your diagnosis. The CBC and urinalysis tests are described in [Appendix C](#) and [Appendix D](#) of this lab manual.

4. Microbiological lab tests you performed in Lab 14

a. Gram stain and catalase test results

Give the Gram reaction (Gram-positive or Gram negative and how you reached this conclusion) and the shape and arrangement of the unknown you were given. **State how this contributed to your decision as to which microbiological tests and/or media to use next.** The Gram stain is discussed in [Lab 6](#); the catalase test in [Lab 8](#)

b. SF broth

Give the results of the SF broth tube you inoculated with the unknown you were given, and how you reached those conclusions. **State how this contributed to your final diagnosis** of the bacterium causing this infection. The possible results for SF broth were discussed earlier in this lab.

c. Bile esculin agar

Give the results of the Bile esculin agar slant you inoculated with the unknown you were given, and how you reached those conclusions. **State how this contributed to your final diagnosis** of the bacterium causing this infection. The possible results for Bile esculin agar were discussed earlier in this lab.

Genus of bacterium: _____

Infection: _____

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PERFORMANCE OBJECTIVES FOR LAB 14

After completing this lab, the student will be able to perform the following objectives:

A. THE GENUS *STREPTOCOCCUS*

1. State the Gram reaction and morphology of the streptococci.
2. State two ways the streptococci are classified.
3. Describe alpha hemolysis, beta hemolysis, and gamma reaction on Blood agar plates.
4. State what is meant by the Lancefield system.
5. State the Lancefield group of streptococcus that is the most common cause of acute streptococcal infections in humans and name five other Lancefield groups that frequently cause human infections.

1. THE BETA STREPTOCOCCI

DISCUSSION

1. State what the term "group A beta" means when referring to streptococci.
2. State the genus and species of the group A beta streptococci.
3. State the most common infection caused by *Streptococcus pyogenes* and name six other infections it may cause.
4. Name two autoimmune diseases associated with the group A beta streptococci.
5. State the genus and species of the group B streptococci.
6. State the normal habitat of the group B streptococci, name three infections they may cause in newborns, and describe how the infants become colonized.
7. Name three infections the group B streptococci may cause in adults.

ISOLATION AND IDENTIFICATION OF GROUP A BETA STREPTOCOCCI

1. Describe the appearance of group A beta streptococci on Blood agar.
2. State why Blood agar is usually stabbed during streaking when isolating beta streptococci.
3. Describe the reaction of group A beta streptococci to a Taxo A® disc containing bacitracin.

RESULTS FOR GROUP A BETA STREPTOCOCCI

1. Identify an organism as a group A beta streptococcus (or *Streptococcus pyogenes*) and state the reasons why when it is seen growing on a Blood agar plate with a Taxo A® disc containing bacitracin.
2. Recognize beta hemolysis on Blood agar.

2. THE PNEUMOCOCCUS

DISCUSSION

1. State the genus and species of the pneumococcus.
2. State the Gram reaction and morphology of *Streptococcus pneumoniae*.
3. State the natural habitat of *Streptococcus pneumoniae* and name four infections it may cause in humans.

ISOLATION AND IDENTIFICATION OF PNEUMOCOCCI

1. Describe the appearance of *Streptococcus pneumoniae* on Blood agar with a Taxo P® disc containing the drug optochin.

RESULTS OF PNEUMOCOCCI

1. Identify an organism as *Streptococcus pneumoniae* and state the reasons why when it is seen growing on a Blood agar plate with a Taxo P® disc containing optochin.
2. Recognize alpha hemolysis on Blood agar.

3. THE VIRIDANS STREPTOCOCCI

1. State the normal habitat of the viridans streptococci and name three infections they may cause in humans.
2. State the hemolytic reactions of the viridans streptococci on Blood agar.

B. THE GENUS *ENTEROCOCCUS*

DISCUSSION

1. Name the most common enterococcus that infects humans and state its normal habitat.
2. State the Lancefield group of the enterococci.
3. Name four infections commonly caused by *Enterococcus faecalis*.

ISOLATION AND IDENTIFICATION OF ENTEROCOCCI

1. Describe the reactions of enterococci in SF broth and on Bile Esculin agar.
2. State the Gram reaction and morphology of the enterococci.

RESULTS FOR THE ENTEROCOCCI

1. Identify an organism as an *Enterococcus* and state the reasons why when it is seen growing in SF broth and on Bile Esculin agar.

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SELF-QUIZ

[Self-quiz](#)

[Answers](#)

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