

به نام خدا

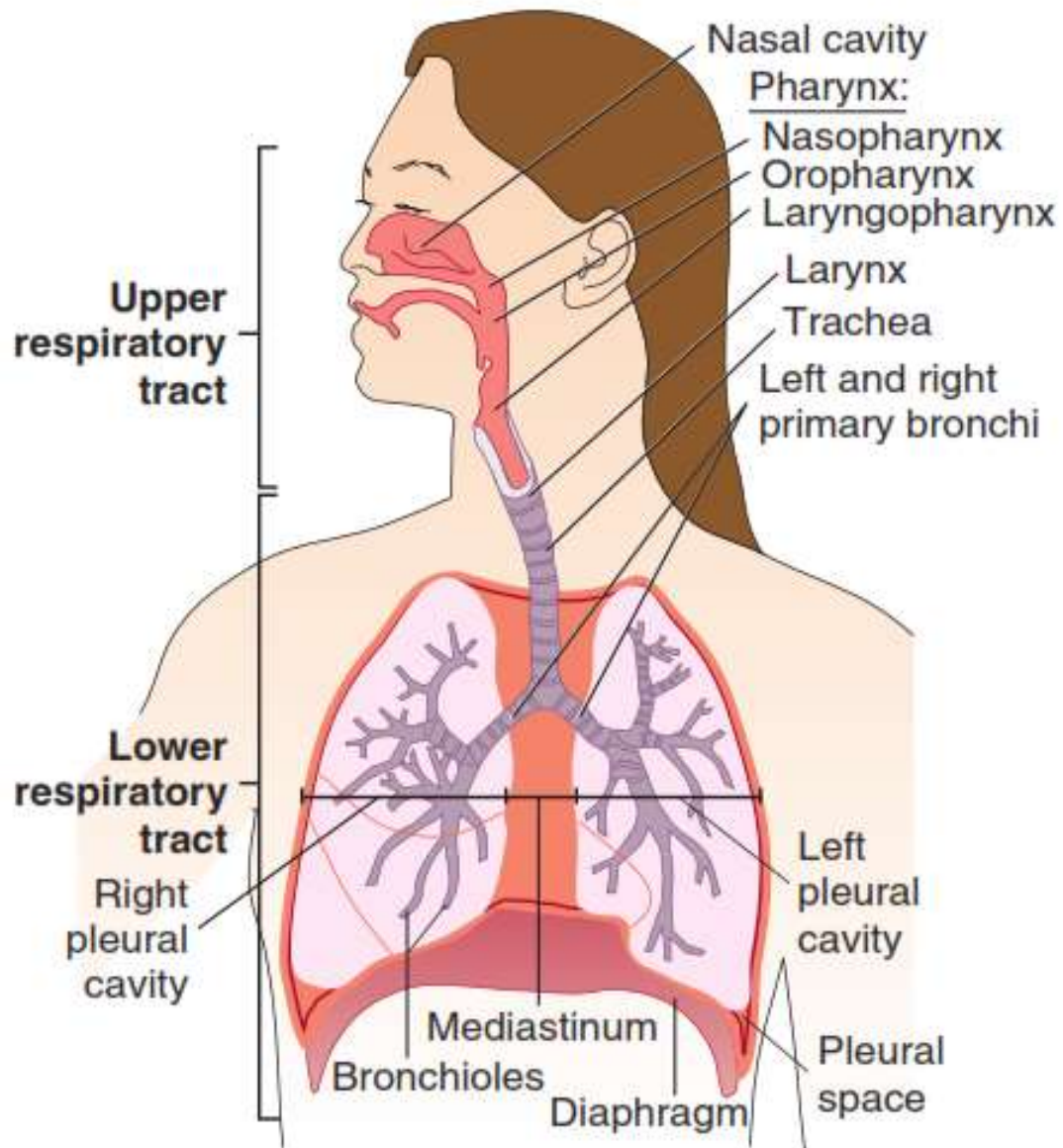


Bacteriology Lab 2

By: **Dr. A. Mohammadi**

Department of Biology,
Faculty of science,
University of Alzahra

میکروارگانیزم های دستگاه تنفس فوقانی



• BOX 68-1 Organisms Present in the Nasopharynx and Oropharynx of Healthy Humans

Possible Pathogens

Acinetobacter spp.
Viridans streptococci, including *Streptococcus anginosus* group
Beta-hemolytic streptococci
Streptococcus pneumoniae
Staphylococcus aureus
Neisseria meningitidis
Mycoplasma spp.
Haemophilus influenzae
Haemophilus parainfluenzae
Moraxella catarrhalis
Candida albicans
Herpes simplex virus
Enterobacteriaceae
Mycobacterium spp.
Pseudomonas spp.
Burkholderia cepacia
Filamentous fungi
Klebsiella ozaenae
Eikenella corrodens
Bacteroides spp.
Peptostreptococcus spp.
Actinomyces spp.
Capnocytophaga spp.
Actinobacillus spp., *Actinobacillus actinomycetemcomitans*
Aggregatibacter aphrophilus
Entamoeba gingivalis
Trichomonas tenax

Rare Pathogens

Nonhemolytic streptococci
Micrococci
Corynebacterium spp.
Coagulase-negative staphylococci
Neisseria spp., other than *Neisseria gonorrhoeae* and *Neisseria meningitidis*
Lactobacillus spp.
Veillonella spp.
Spirochetes
Rothia dentocariosa
Leptotrichia buccalis
Selenomonas
Wolinella
Rothia mucilagenosus (formerly *Stomatococcus mucilagenosus*)
Campylobacter spp.

• BOX 68-2 Respiratory Tract Pathogens

Definite Respiratory Tract Pathogens

Corynebacterium diphtheriae (toxin-producing)
Mycobacterium tuberculosis
Mycoplasma pneumoniae
Chlamydia trachomatis
Chlamydia pneumoniae
Bordetella pertussis
Legionella spp.
Pneumocystis jiroveci
Nocardia spp.
Histoplasma capsulatum
Coccidioides immitis
Cryptococcus neoformans (may also be recovered from patients without disease)
Blastomyces dermatitidis
Viruses (respiratory syncytial virus, coronaviruses, human metapneumovirus, adenoviruses, enteroviruses, hantavirus, herpes simplex virus, influenza and parainfluenza virus, rhinoviruses, severe acute respiratory syndrome)

Rare Respiratory Tract Pathogens

Francisella tularensis
Bacillus anthracis
Yersinia pestis
Burkholderia pseudomallei
Coxiella burnetii
Chlamydia psittaci
Brucella spp.
Salmonella spp.
Pasteurella multocida
Klebsiella rhinoscleromatis
Varicella-zoster virus (VZV)
Parasites

TABLE A-15. Common Causes of Infectious Diseases of the Respiratory Tract*

Disease	Common Cause(s)
Common cold (rhinitis)	Rhinoviruses
Acute rhinosinusitis	Rhinoviruses, adenoviruses, parainfluenza viruses, influenza viruses, respiratory syncytial virus, coronaviruses
Acute bacterial rhinosinusitis	<i>Streptococcus pneumoniae</i> <i>Haemophilus influenzae</i>
Pharyngitis	Adenovirus Herpes simplex virus Epstein-Barr virus Coxsackie viruses <i>Streptococcus pyogenes</i> (group A <i>Streptococcus</i> is important because of the complications that can result (e.g., rheumatic fever, infections of contiguous tissues))
Viral croup	Parainfluenza virus Influenza virus Respiratory syncytial virus
Bacterial tracheitis	<i>Staphylococcus aureus</i>
Epiglottitis (bacterial croup)	<i>Haemophilus influenzae</i> type b
Bronchitis	Influenza viruses, parainfluenza viruses, adenovirus, respiratory syncytial virus, herpes simplex virus, rhinovirus, coxsackieviruses, and echovirus <i>Mycoplasma pneumoniae</i> <i>Chlamydia pneumoniae</i> <i>Streptococcus pyogenes</i>
Bronchiolitis	Respiratory syncytial virus
Pneumonia	Neonatal (0–1 month): <i>Escherichia coli</i> and <i>Streptococcus agalactiae</i> Infants (1–6 months): <i>Chlamydia trachomatis</i> and respiratory syncytial virus Children (6 months–5 years): Respiratory syncytial virus and parainfluenza virus Children (5–15 years): <i>Mycoplasma pneumoniae</i> and influenza A virus Young adults (16–30 years): <i>Mycoplasma pneumoniae</i> Older adults (> 30 years): <i>Streptococcus pneumoniae</i>

*Note that this is not an exhaustive list and does not include all possible etiologies for a particular disease.

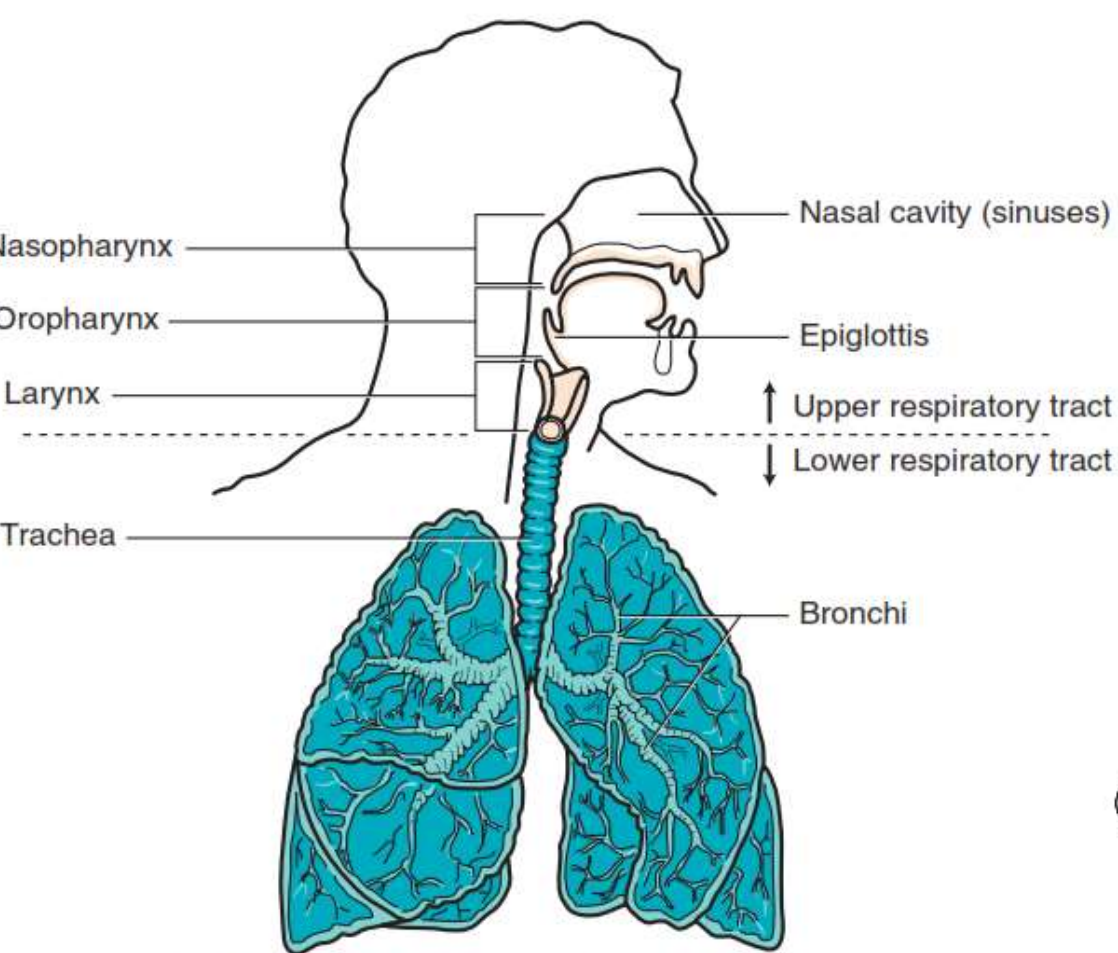
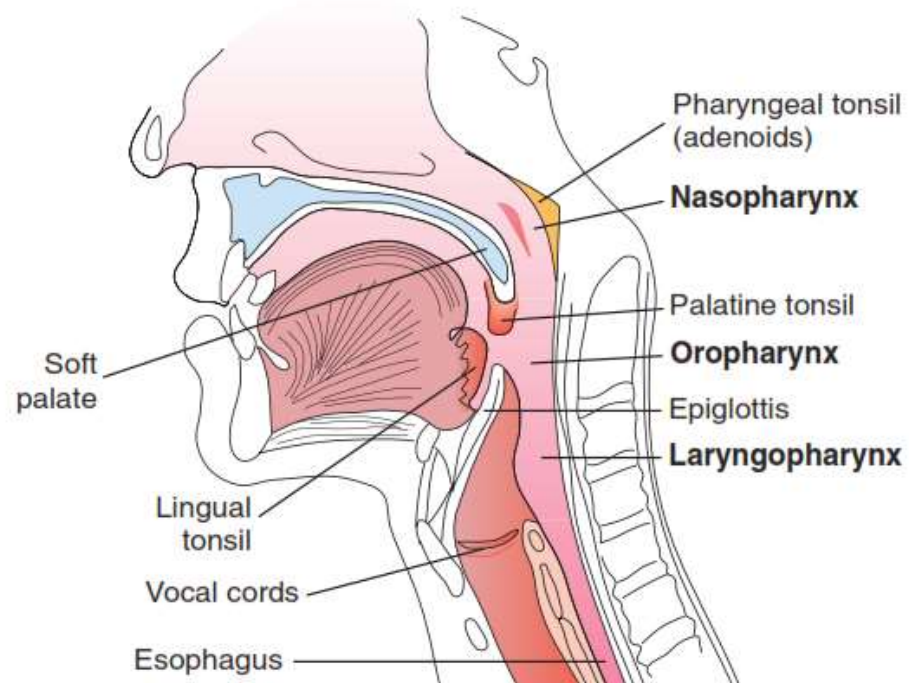


FIGURE 32-1 Anatomy of the respiratory tract.



• Figure 69-1 The pharynx, including its three divisions and nearby structures.

BOX 32-1 Normal Nasopharyngeal and Oropharyngeal Organisms

Bacteria

Usually Present

Streptococcus mitis, *Streptococcus salivarius*, and other viridans streptococci

Streptococcus pneumoniae

Non-group A β -hemolytic streptococci

Streptococcus pyogenes

Nonhemolytic streptococci

Veillonella spp.

Bacteroides spp.

Fusobacterium spp.

Prevotella spp.

Porphyromonas spp.

Coagulase-negative staphylococci

Neisseria spp.

Diphtheroids

Micrococcus spp.

Eikenella spp.

Capnocytophaga spp.

Occasionally Present

Haemophilus influenzae

Haemophilus parainfluenzae

Peptostreptococcus

Actinomycetes

Staphylococcus aureus

Mycoplasma

Fungus

Candida spp.

Viruses

Herpes simplex

BOX 32-2 Selected Nonviral Pathogens in the Respiratory Tract

Primary Pathogens

Streptococcus pneumoniae

Group A β -hemolytic streptococci

Neisseria meningitidis

Neisseria gonorrhoeae

Bordetella pertussis

Mycobacterium kansasii

Mycobacterium tuberculosis

Legionella pneumophila

Toxin-producing *Corynebacterium diphtheriae*

Mycoplasma pneumoniae

Chlamydia trachomatis

Chlamydophila pneumoniae

Pneumocystis jirovecii

Possible Pathogens

Acinetobacter spp.

Enterics and other gram-negative bacilli

Fungi

Nocardia spp.

Staphylococcus aureus

Haemophilus influenzae

β -Hemolytic streptococci, non-group A

Moraxella catarrhalis

Anaerobes

Mycobacterium spp.

Actinomycetes

Dr A.Mohammadi

TABLE 32-1 Upper Respiratory Tract Infections

Clinical Syndrome	Causative Agents	Specimen Collection	Other
Pharyngitis	Children: <i>Streptococcus pyogenes</i> Adults: Viruses	For <i>S. pyogenes</i> , swab tonsils and posterior pharynx, and place in transport media; do not allow to dry; viral cultures not necessary	Culture for <i>Neisseria gonorrhoeae</i> or <i>Corynebacterium diphtheriae</i> if clinically indicated
Sinusitis	<u>Most common</u> Rhinovirus Parainfluenza virus Influenza virus <u>Less common</u> <i>Streptococcus pneumoniae</i> <i>Haemophilus influenzae</i>	Direct sinus sampling	Direct sampling indicated for patients who fail empiric therapy, who are severely ill, immunocompromised, or if an unusual pathogen is suspected
Otitis media	<u>Most common</u> <i>S. pneumoniae</i> <i>H. influenzae</i> <u>Less common</u> <i>S. pyogenes</i> <i>Moraxella catarrhalis</i> <i>Staphylococcus aureus</i>	Direct culture by tympanocentesis	Direct culture indicated for patients who are severely ill, immunocompromised, or if unusual pathogen is suspected
Epiglottitis	<u>Most common</u> Streptococci Staphylococci <i>H. influenzae</i> type B <u>Less common</u> <i>H. parainfluenzae</i>	Direct swab of epiglottis, blood cultures	Direct swab should be performed only if airway is secure
Pertussis	<u>Most common</u> <i>Bordetella pertussis</i> <i>Bordetella parapertussis</i> <u>Less common</u> <i>Bordetella bronchiseptica</i> <i>Bordetella holmesii</i>	Nasopharyngeal swab: 1. PCR testing 2. Plate directly onto Bordet-Gengou or Regan-Lowe medium for culture	If very late in the course of the disease, serology testing may be more beneficial if other testing is negative

TABLE 69-1 Examples of Bacteria That Can Cause Acute Pharyngitis and Tonsillitis

Organism	Disease	Relative Frequency
<i>Streptococcus pyogenes</i>	Pharyngitis/tonsillitis/rheumatic fever/scarlet fever	15%-35%
Group C and G beta-hemolytic streptococci	Pharyngitis/tonsillitis	<3%-11%
<i>Arcanobacterium (Corynebacterium) haemolyticum</i>	Pharyngitis/tonsillitis/rash	<1%-10%
<i>Neisseria gonorrhoeae</i>	Pharyngitis/disseminated disease	Rare*
<i>Corynebacterium ulcerans</i>	Pharyngitis	Rare
<i>Mycoplasma pneumoniae</i>	Pneumonia/bronchitis/pharyngitis	Rare
<i>Yersinia enterocolitica</i>	Pharyngitis/enterocolitis	Rare
Human immunodeficiency virus-1	Pharyngitis/acute retroviral disease	Rare

*Less than 1%.

• BOX 69-1 Viral Agents That Can Cause Rhinitis

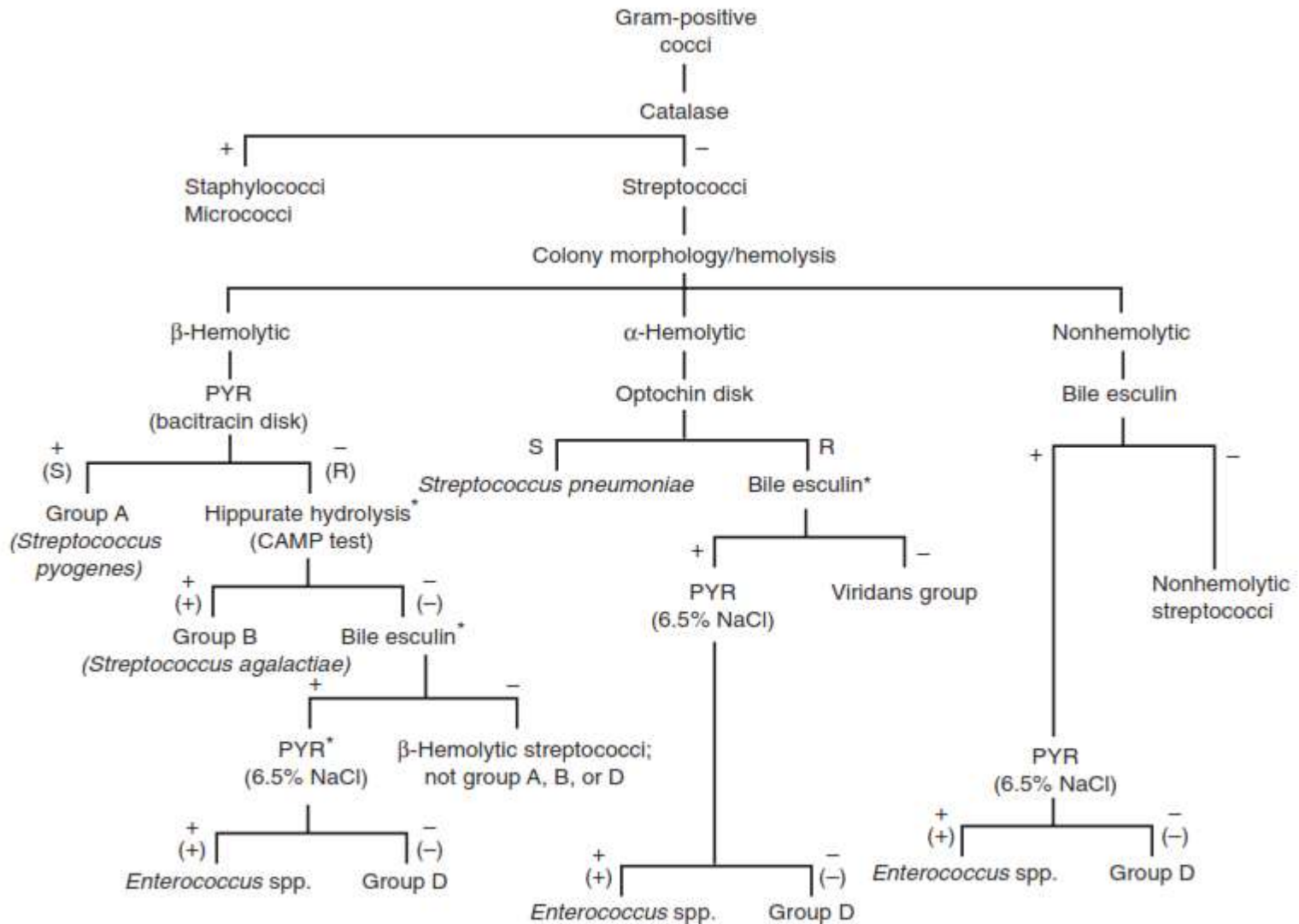
- Rhinoviruses
- Coronaviruses
- Adenoviruses
- Parainfluenza and influenza viruses
- Respiratory syncytial virus
- Enterovirus

**TABLE
69-2**

**Medium and Atmosphere for Incubation of
Cultures to Recover Group A Streptococci
from Pharyngeal Specimens**

Media	Atmosphere of Incubation
Sheep blood agar	Anaerobic
Sheep blood agar with coverslip over the primary area of inoculation	Aerobic
Sheep blood agar with trimethoprim- sulfamethoxazole	5%-10% CO ₂ or anaerobic

تشخیص آزمایشگاهی



*Perform additional tests if isolate is from nonrespiratory source.

FIGURE 15-15 Schematic diagram for the presumptive identification of gram-positive cocci. R, Resistant; S, susceptible.

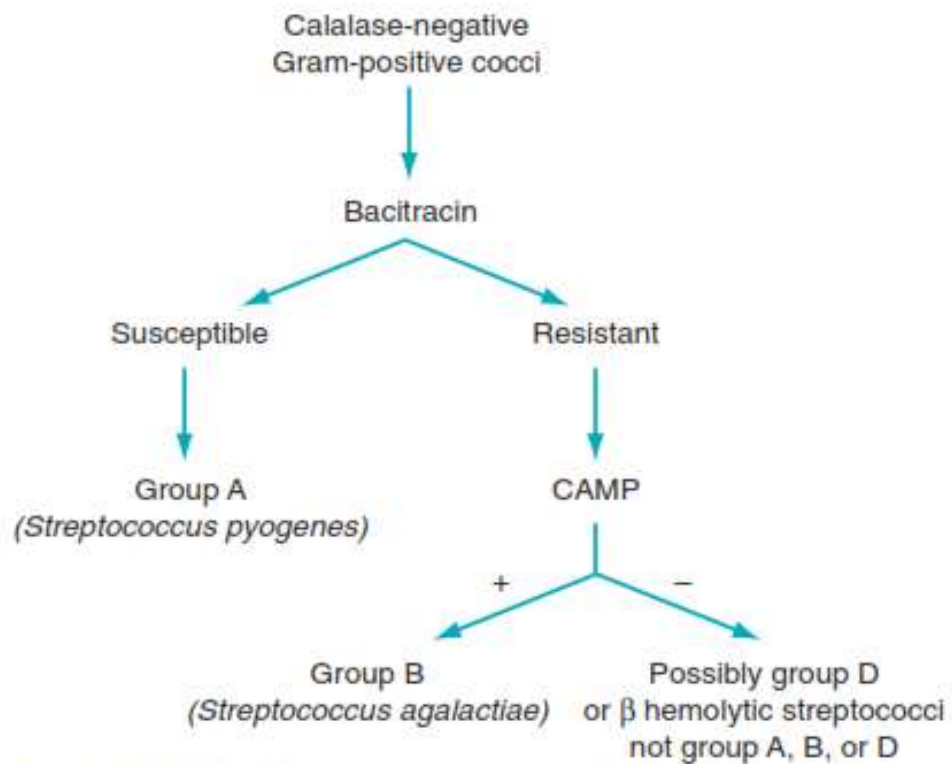


FIGURE 15-6 Schematic diagram for differentiation of group A streptococci (GAS) from group B streptococci (GBS).

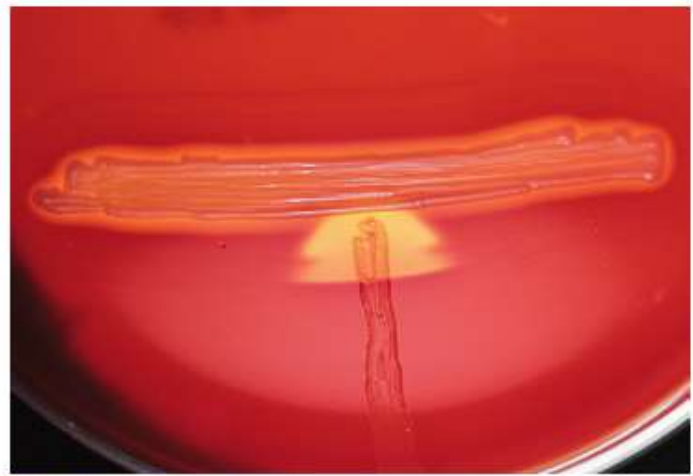


FIGURE 15-18 CAMP test for presumptive identification of group B streptococci (GBS). *Streptococcus agalactiae* shows the classic arrow shape near the streptococcal streak.



FIGURE 15-19 Modification of the CAMP test showing the enhanced hemolysis produced by *Streptococcus agalactiae* when a drop of extracted β -lysin is placed on the colony.

Dr A.Mohammadi

PATHOGENIC STREPTOCOCCAL SPECIES

TABLE A-4. Laboratory Tests Used to Differentiate the Pathogenic Streptococcal Species

Organism	Lancefield Grouping	Hemolysis* on Blood Agar Plates	Biochemical Tests
<i>S pyogenes</i>	A	Beta	Sensitive to bacitracin (A disc)
<i>S agalactiae</i>	B	Beta usually; gamma sometimes	Positive CAMP-test; hippurate hydrolysis
<i>S pneumoniae</i>	Nongroupable; Viridans group	Alpha	Soluble in bile; sensitive to Optochin (P disc)

CAMP, Christie, Atkinson, Munch, Peterson.

*There are three different types of hemolysis. **Alpha hemolysis** is not really hemolysis of the erythrocytes in the blood agar plates but rather a conversion of hemoglobin to a form of hemoglobin that appears green in the agar around the bacterial colony. **Beta hemolysis** is true hemolysis with actual lysis of the erythrocytes in the blood agar around the bacterial colony. **Gamma hemolysis** is not really a hemolytic event either. The organism is gamma hemolytic when no lysis of the erythrocytes or color change occurs in the media around the colony. In other words, these organisms are nonhemolytic.



FIGURE 14-2 Group A β -hemolytic streptococci (*Streptococcus pyogenes*) after growth overnight on a 10-cm plate with 5% sheep blood agar. The small (0.5–1 mm diameter) white colonies are surrounded by diffuse zones of β -hemolysis 7–10 mm in diameter. (Courtesy of H Reyes.)

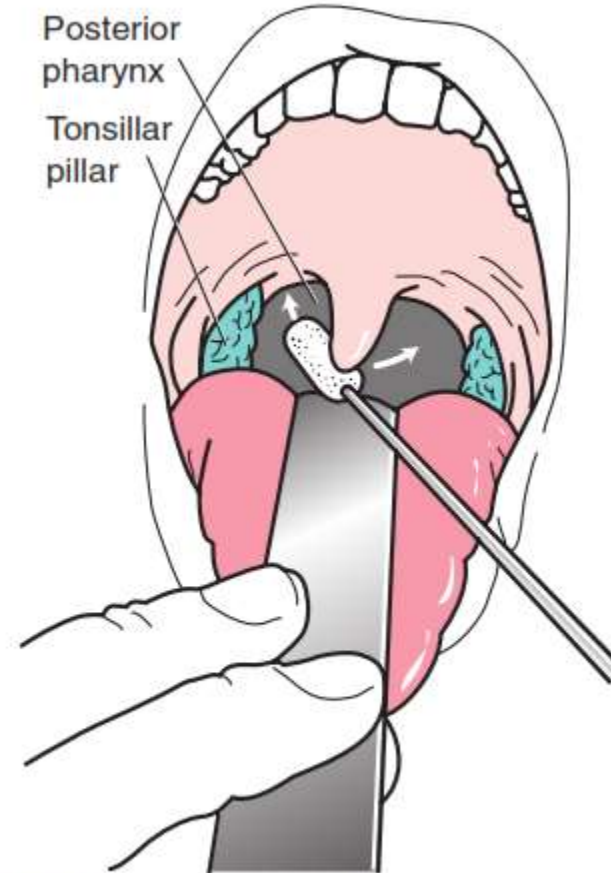
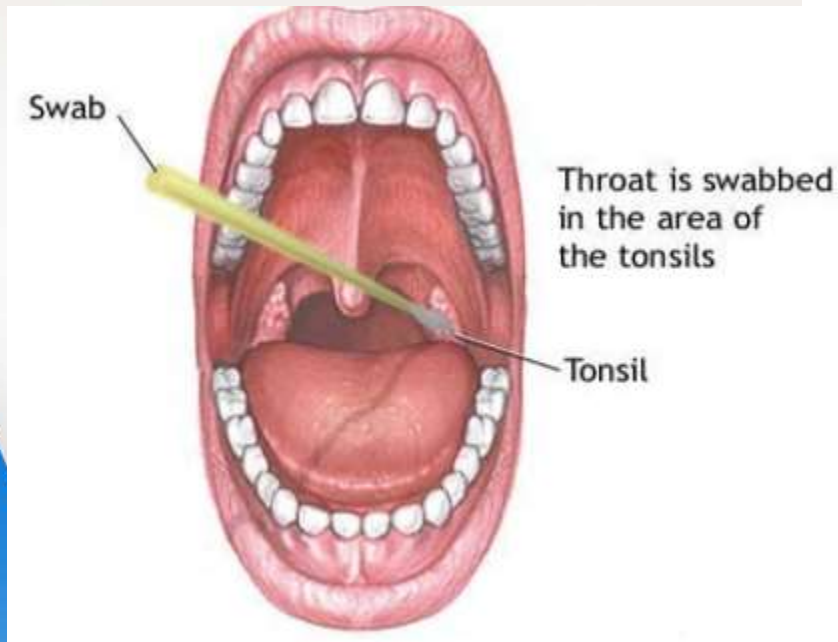


FIGURE 32-2 Specimen collection from the throat.

مواد و روش ها

ردیف	مواد لازم:		
۱	سواب	۷	انکوباتور ۳۷ درجه
۲	لوپ	۸	کیت رنگ آمیزی گرم
۳	محیط BA , MHA , بیل اسکولین	۹	NB 6.5% NaCl
۴	معرف تست کاتالاز	۱۰	
۵	دیسک باسیتراسین	۱۱	

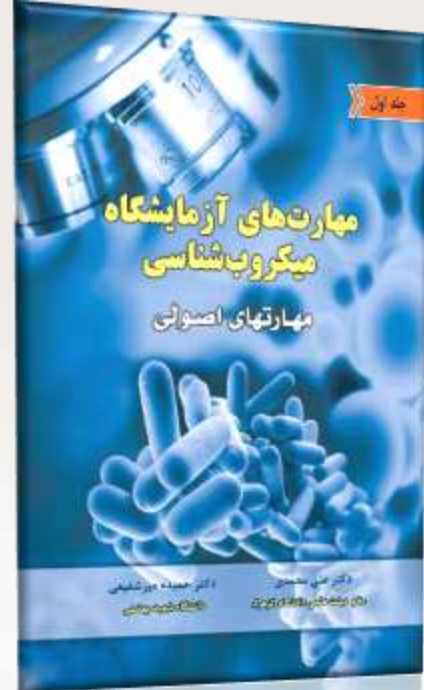
نمونه گیری.

- (۱) در صورت التهاب اپی گلوت اقدام به جمع آوری کشت گلو نشود.
- (۲) پایین نگه داشتن زبان با آپلیکاتور مخصوص و وادار نمودن بیمار به گفتن "تتتت".
- (۳) کشیدن سواب استریل به پشت زبان کوچک و بین منافذ لوزه ای
- (۴) سعی شود سواب با فک و زبان یا زبان کوچک تماس نیابد .
- (۵) کشیدن سواب به آرامی ولی محکم به عقب و جلو در طول قسمت خلفی حلق ، سطح لوزه ای و هر سطح ملتهب و زخمی قابل مشاهده.

کار عملی

ادامه.

- (۱) نمونه ی گلو صرفا برای جداسازی استرپتوکوکس پایوجنز به کار می رود. لذا تست ها بر محوریت استرپتوکاسه و گروه های لانسفیلد
- (۲) سواب را به بلاد آگار انتقال داده و با کمک لوپ کشت چهار منطقه ای خطی بدهید. سپس پلیت را در مجاورت ۵-۱۰% CO₂ در ۳۵ درجه قرار دهید. تا فعالیت همولیزین در شرایط بیهوازی دیده شود.
- (۳) برخی آزمایشگاه ها محیط اختصاصی SBA به همراه SXT (سولفومتاکسازومل- تری متوپریم) برای جلوگیری از رشد فلور طبیعی نازوفارنکس اضافه شده است. با توجه به فراوانی فلور در این منطقه رنگ آمیزی گرم در این مرحله به کار نمی رود.
- (۴) نمونه های دارای بتا همولیز، را رنگ آمیزی گرم نمائید و سپس با انجام تست کاتالاز آن ها را تائید نمائید.
- (۵) برای تائید نهایی می توانید کلنی ها کاتالاز منفی را با تست آنتی بیوگرام برای حساسیت به باسیتراسین ومقاومت به SXT تست نمائید.



منابع:

- **مهارت های آزمایشگاه میکروب شناسی** ، جلد ۱- ۳
- دکتر علی محمدی-عضو هیئت علمی دانشگاه الزهرا (س).
- دکتر حمیده میرشفیعی - دانشگاه شهید بهشتی
- Mahon, C. R., Lehman, D. C., & Manuselis, G. (2018). *Textbook of Diagnostic Microbiology-E-Book*. Elsevier Health Sciences.
- Tille, P. (2016). *Bailey & Scott's Diagnostic Microbiology-E-Book*. Elsevier Health Sciences.
- Chamberlain, N. (2008). *Medical microbiology: The big picture*. McGraw Hill Professional.
- Carroll, K. C., Butel, J., & Morse, S. (2016). *Jawetz Melnick & Adelbergs Medical Microbiology 27 E*. McGraw Hill Professional.