Streptococcus spp.
(in respiratory system)

Istar Dolapci, MD, PhD

Ankara University School of Medicine

Department of Medical Microbiology

Objectives of today's class

- Describe the general characteristics of *Streptococcus* spp.
- Explain the classification system for *Streptococcus* spp.
- Identify the respiratory infections associated with *Streptococcus* spp.
- Identify a clinical isolate based on the results from standard laboratory diagnostic procedures

CONTENTS (Description Headings)

Streptococcus spp.

- General characteristics
- Classification

Streptococcus pyogenes

- Morphology
- Classification
- Cultural characteristics
- Biochemical reactions
- Resistance & antigenic structure
- Pathogenicity & virulence
- Toxins and other virulence factors
- Epidemiology
- Disease caused
- Laboratory diagnosis
- Treatment & prophylaxis

Case I

A 5 year-old boy with fever and sore throat

History:

Attends to a daycare center

Physical examination:

Fever 38.4^{o}C,

Red anterior pharynx & tonsillar region

Anterior lymph nodes enlarged

No rash

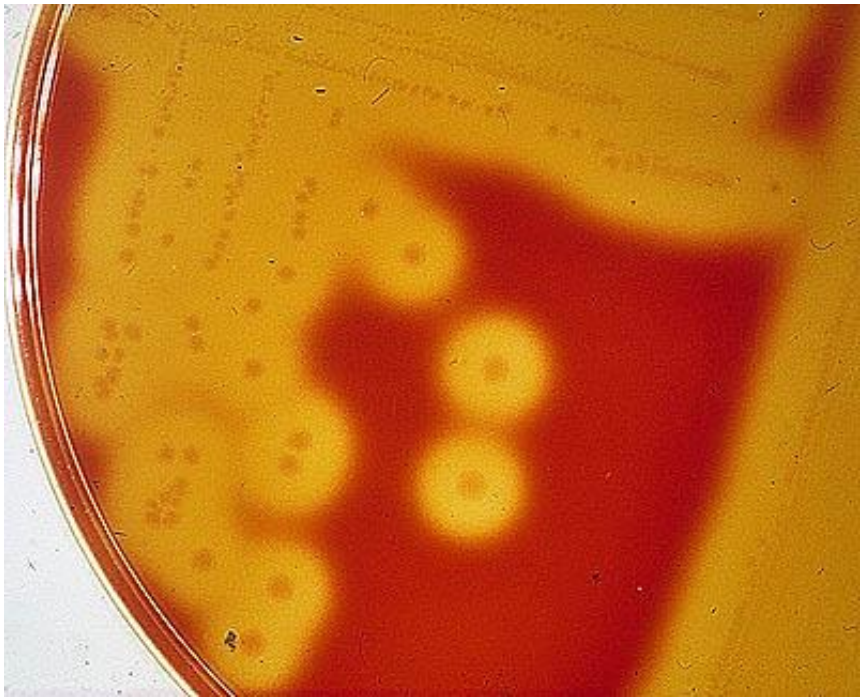
Follow up:

Throat culture

Penicillin therapy

Laboratory Findings of Case I

Gram positive cocci
Long chains



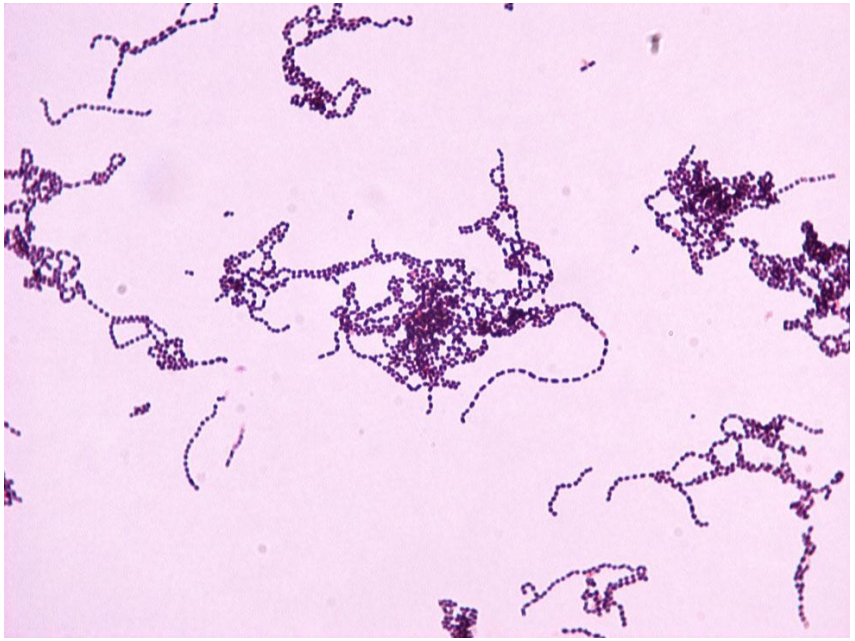
Small white colonies with large β-hemolysis zone on blood agar
Catalase negative
Susceptible to bacitracin

Questions should be cleared

- What is the most likely organism causing the infection?
- How can this organism be detected?
- What microbial factors are important in the pathogenesis of the disease?
- Why detection is important for the management of the disease?
- Is there any antimicrobial resistance problem for that microorganism?

INTRODUCTION

- Gram positive cocci
- Arranged in chains
- Facultative anaerobic
- Nutritionally fastidious
- Catalase negative
- Some encapsulated
- Non motile
- Part of normal flora of humans and animals



www.medscape.com/answers/225243-174800/what-is-the-pathophysiology-of-bacterial-pharyngitis

Classification of *Streptococcus* spp.

- **Hemolytic patterns**
- **Serologic properties**
- **Biochemical (physiologic) properties**

The streptococci can be divided into two groups:

1. The β-hemolytic streptococci, which are classified by Lancefield grouping, and
2. The α-hemolytic and γ-hemolytic streptococci, which are classified by biochemical testing

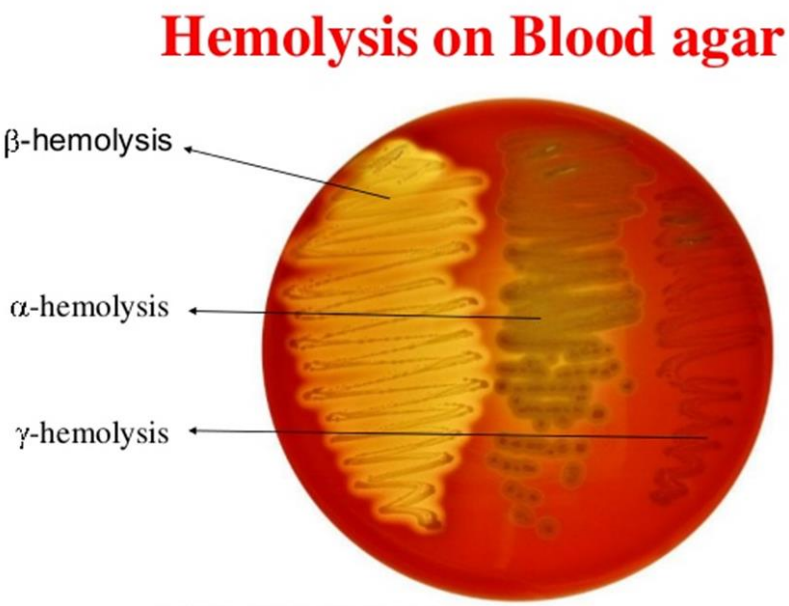
Classification of *Streptococcus* spp.

Hemolytic patterns

- Initial classification based on hemolysis on sheep blood agar plates
  - α (partial / incomplete hemolysis),
  - β (complete hemolysis), and
  - γ (no hemolysis)

Hemolysis Patterns of *Streptococcus* spp. on 5% sheep blood agar

ASM MicrobeLibrary.org © Buxton



Dr. Nabil El-Aila
Diagnostic Microbiology

Classification of *Streptococcus* spp.

Serologic properties

- 1930's: Lancefield defines cell wall antigen groups
  - Lancefield groupings originally A to W
  - Especially for β-hemolytic species

Based on Lancefield groupings;

- Groupable streptococci
  - A, B and D; frequent
  - C, G and F; less frequent
- Non groupable streptococci
  - *S. pneumoniae*; causes pneumonia
  - Viridans streptococci
    - e.g. *S. mutans*; causes dental caries

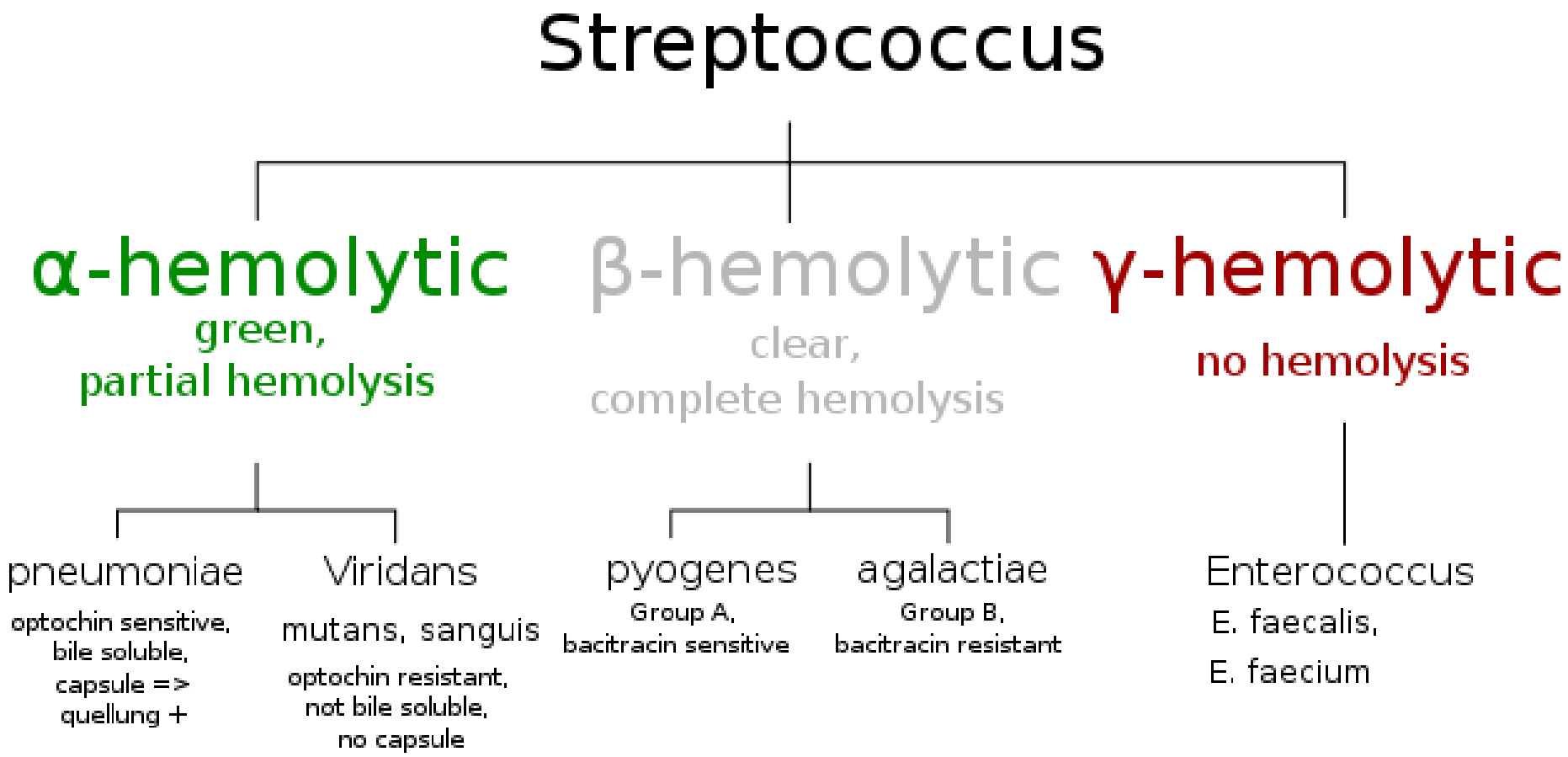
Classification of *Streptococcus* spp.

Biochemical (physiologic) properties

- Sugar fermentation reactions
- Investigation of the presence of enzymes
- Determination of resistance or sensitivity to various chemical agents

Classification of *Streptococcus* spp.

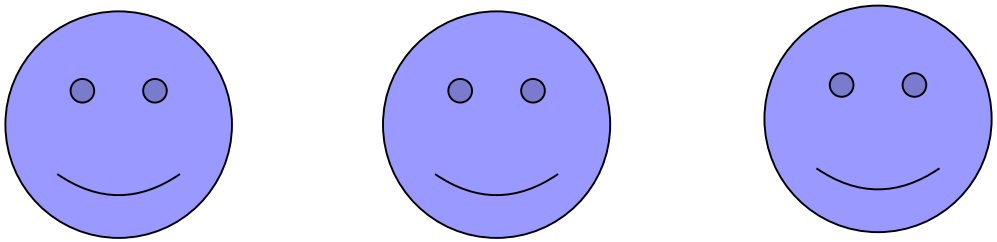
Biochemical Classification	Serologic Classification	Hemolysis Patterns
S. pyogenes	A	β
S. agalactiae	B	β; rare γ
S. dysgalactiae, *S. equi,* *S. zooepidemicus*	C	β
S. bovis	D	α; γ; rare β
S. anginosus group	A, C, F, G, non groupable	β; α or rare γ
S. canis	G	β
Viridans group	Non groupable	α or γ
S. pneumoniae	Non groupable	α



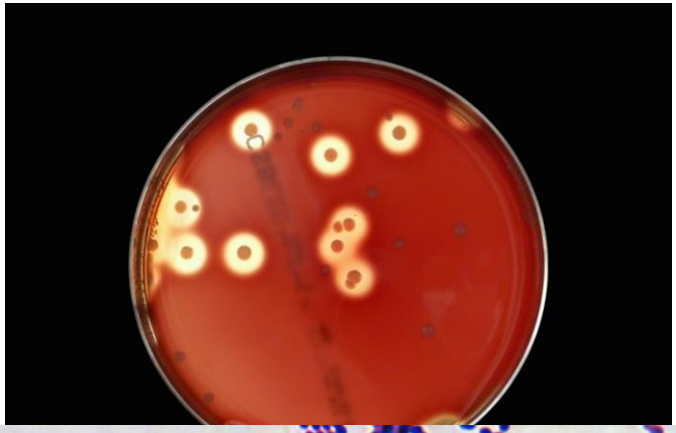
ATTENTION: Group C and G also beta hemolytic !

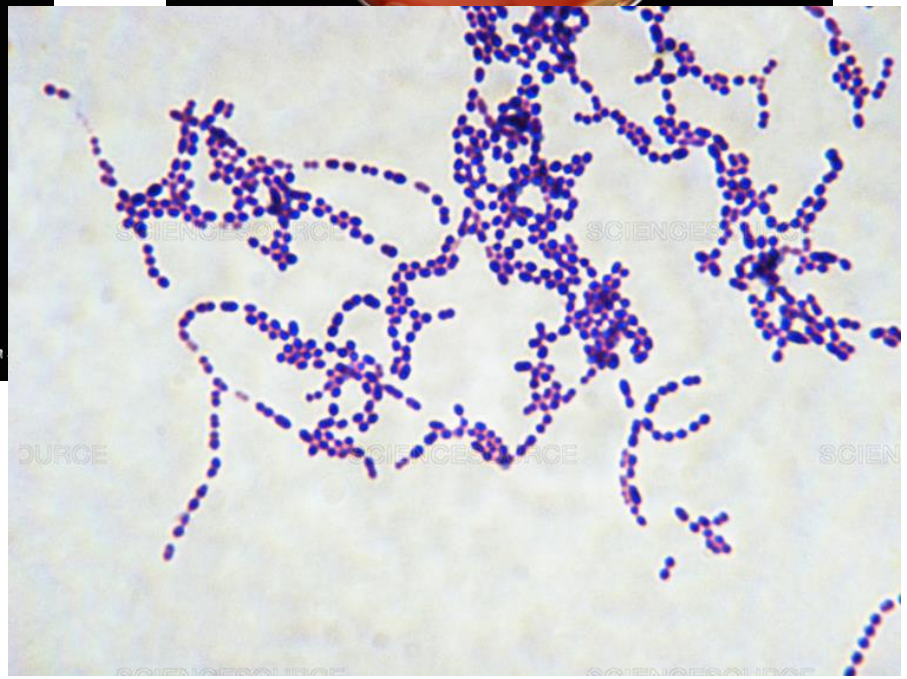
Classification of Common β-Hemolytic Streptococci

Group	Representative species	Disease
A	*S. pyogenes*	**Pharyngitis**, skin and soft-tissue infections, bacteremia, rheumatic fever, acute glomerulonephritis
A, C, F, G	*S. anginosus* group	Abscess
B	*S. agalactiae*	Neonatal disease, endometritis, wound infections, urinary tract infections, bacteremia, **pneumonia** (in newborns), skin and soft-tissue infections
C	*S. dysgalactiae*	**Pharyngitis**, acute glomerulonephritis
F,G	*S. anginosus* group	Abscesses
C	*S. dysgalactiae*	**Pharyngitis**, acute glomerulonephritis



Streptococcus pyogenes (GAS)



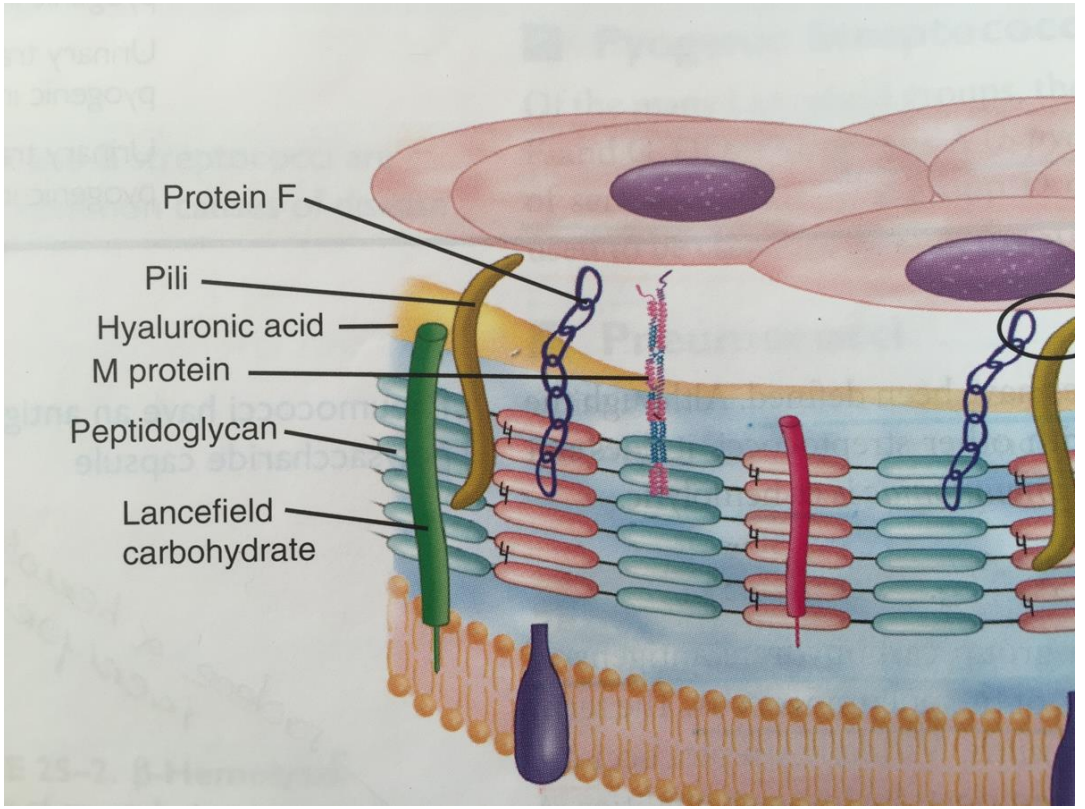


Streptococcus Pyogenes-Science Source /sciencesource.com

Streptococcus pyogenes

Antigenic structure

- **Group specific carbohydrates**
  - Lancefield (group A) antigen
- **Type specific protein**
  - M protein; 150 serotype
    - M protein- major virulence protein
    - Binding to host cell
    - Antiphagocytic and complement inactivation
    - Cause to acute rheumatic fever (ARF)

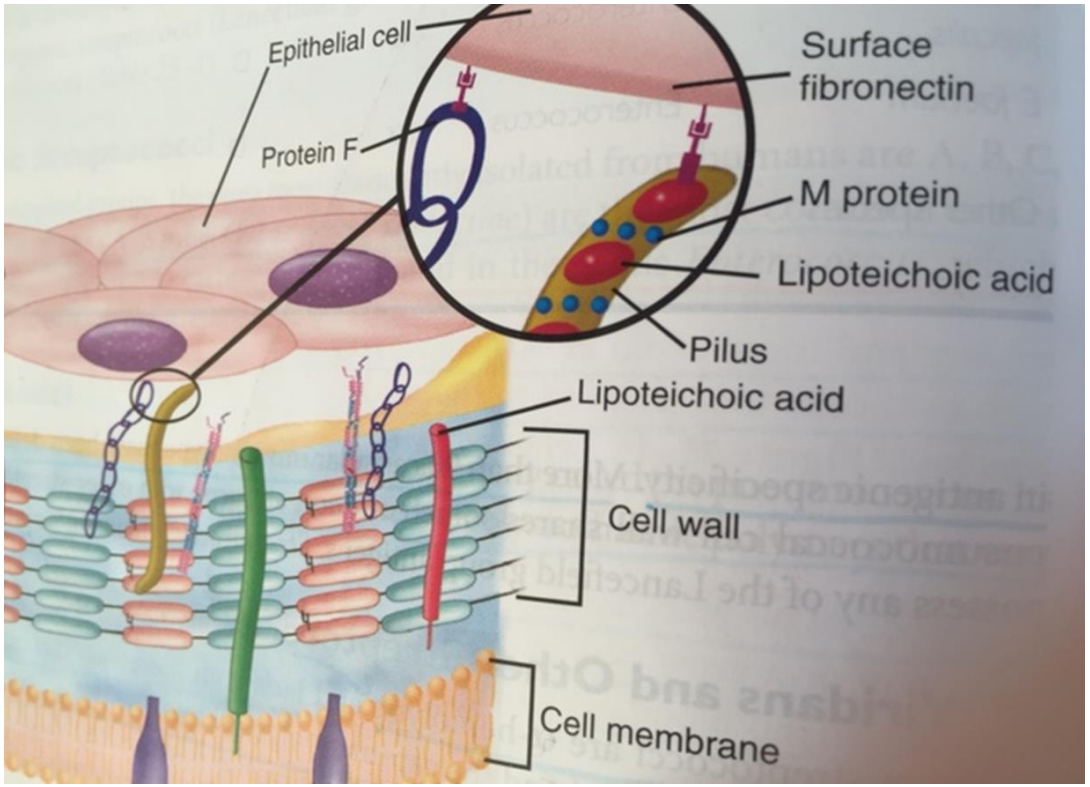


Jawetz, Melnick & Adelberg's Medical Microbiology; 27th Ed; McGraw Hill Lange; 2016

Streptococcus pyogenes

Antigenic structure

- **Other surface components**
  - M like proteins
  - Lipoteichoic acid-attachment
  - Fimbria-F protein - attachment
- **Capsule (some strains)**
  - Hyaluronic acid-protects from phagocytosis

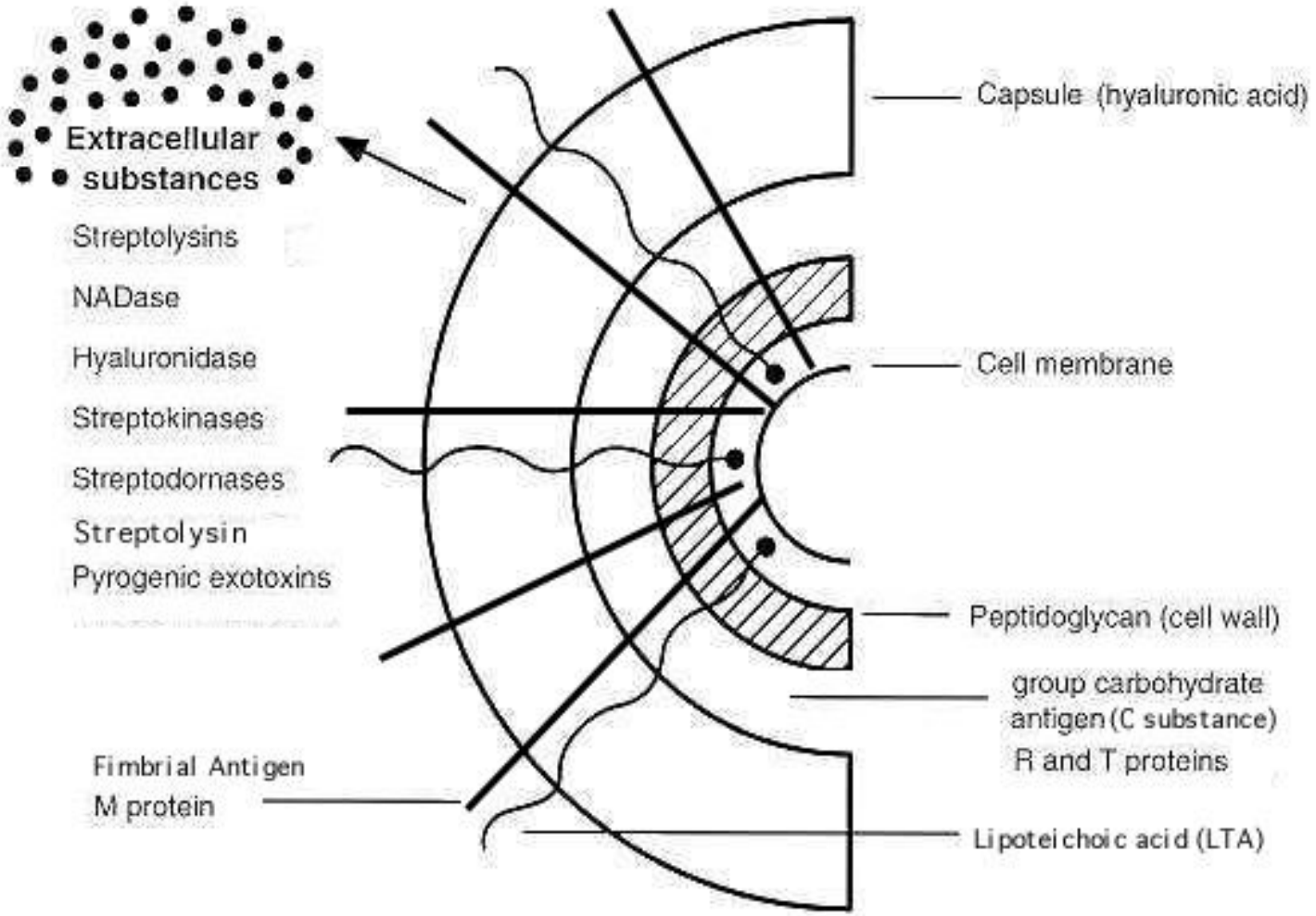


Jawetz, Melnick & Adelberg's Medical Microbiology; 27th Ed; McGraw Hill Lange; 2016

Streptococcus pyogenes

Group A Streptococcus (GAS);
human-restricted pathogen

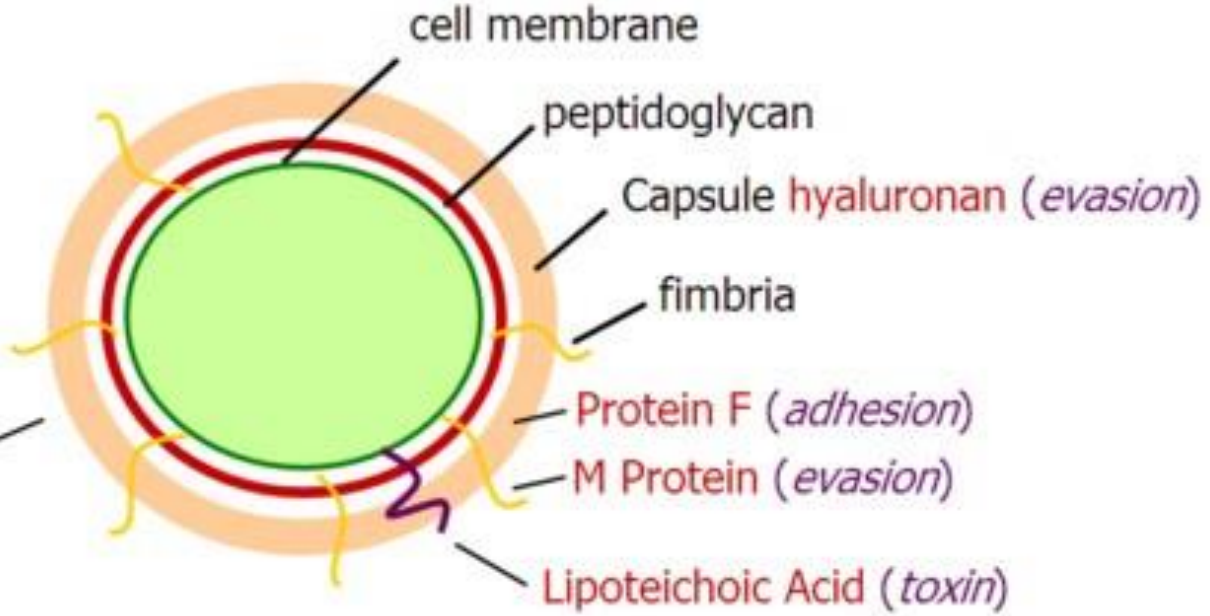
- Cell-associated virulence factors
  - **Hyaluronic acid capsule**
  - **M protein**
    - over 150 types
  - **Lipoteichoic acid** (LTA)
- Extracellular products
  - **Streptolysin S** (SLS)
  - **Streptolysin O** (SLO)
  - **DNases**
  - **Streptokinase**
  - **Spe** (streptococcal pyogenic exotoxin)
  - **Superantigens**



Cell surface structure of *Streptococcus pyogenes* and secreted products involved in virulence

http://textbookofbacteriology.net/streptococcus_2.html

GAS Virulence Factors



invasion:
Streptokinase
Hyaluronidase

evasion:
Streptolysin O and S
Streptodornase
C5a Peptidase
Streptococcal Chemokine Protease

toxin:
Streptococcal Pyrogenic Exotoxins

GAS Virulence Factors

- **Cell-associated virulence factors**
  - **Hyaluronic acid capsule**
    - Antiphagocytic
    - Not immunogenic
  - **M protein** (over 150 types); other genes with similar proteins
    - Antiphagocytic
    - Bind IgG, IgA
    - Iron transport
    - Resistance to antimicrobial peptides
  - **Lipoteichoic acid** (LTA)

GAS Virulence Factors

- **Enzymes-** Help invasion and evasion
  - **Streptolysin O** (Immunogenic- O_2 sensitive)
    - Induce antistreptolysin O (ASO) antibodies
  - **Streptolysin S** (Non immunogenic-O_2 stable)
    - Responsible hemolysis seen on blood agar

GAS Virulence Factors

- **Enzymes-** Help invasion and evasion
  - **Streptokinase** (A and B)
    - Promotes lysis of fibrin clots by activating a plasminogen
    - BIOLOGICAL ROLE : It breaks down the fibrin barrier around the lesion & facilitate spreading of infection
    - Intravenous streptokinase is given for the treatment of early MI & other thromboembolic disorders

GAS Virulence Factors

- **Enzymes-** Help invasion and evasion
  - **DNase (streptodornase)** (A-D)
    - Causes depolymerization of DNA
    - Pyogenic exudates contain large amount of DNA derived from nuclei of necrotic cells
    - Helps to liquefy the thick pus & hence responsible for the thin serous character of streptococcal exudates
    - Demonstration of Anti-DNAase B antibody is used in the retrospective diagnosis of *S.pyogenes* infection
      - THERAPEUTIC APPLICATION: To liquefy localized collection of thick exudates as in empyema, a preparation of streptokinase and streptodornase are used

GAS Virulence Factors

- **Enzymes-** Help invasion and evasion
  - **Hyaluronidase**
    - Breaks down hyaluronic acid of tissues
    - Thus favours spread of infection along intercellular spaces

GAS Virulence Factors

- **Toxins**
  - **Streptococcal pyrogenic exotoxin (Spe), (Erythrogenic / Dick / Scarlatinal toxin)**
    - Produced by lysogenic strains of streptococci
    - Heat labile 4 toxins- SpeA, SpeB, SpeC and Spe F
      - Types A & C are coded by bacteriophage genes
      - Type B gene is chromosomal
    - Also some strains of C and G group streptococci produce
    - Induced fever
      - That is the T cell mitogen that induce a massive release of inflammatory cytokines, causing fever, shock and tissue damage
    - Superantigen
    - **Diseases: Toxic shock syndrome, Scarlet fever, Necrotizing fasciitis, Puerperal fever**

Epidemiology

Transmission: Droplets or direct contact
Can colonize the oropharynx of healthy children and young adults in the absence of clinical disease (carrier state)

Season: Winter

Risk Groups: Crowding, classrooms or day care

Age: Common 5-15-year of age

Clinical Diseases

- **Suppurative Streptococcal Diseases**
  - **Pharyngitis**
  - Erysipelas
  - Cellulitis
  - Pyoderma
  - Impetigo
- **Toxin Mediated Streptococcal Diseases**
  - Necrotizing fasciitis (streptococcal gangrene)
  - Streptococcal toxic shock syndrome
  - Scarlet fever
  - Puerperal fever
- **Systemic Infections**
  - **Pneumonia**
  - Septicemia
- **Immunologic (poststreptococcal) Diseases**
  - Rheumatic fever
  - Glomerulonephritis

Pharyngitis

- Adheres to the pharyngeal epithelium by **lipoteichoic acid–covered surface pili** and by **hyaluronic acid** in encapsulated strains

- Develops in 2 to 4 days after exposure to the pathogen

Pharyngitis

In infants and small children;

- It occurs as a subacute nasopharyngitis
  - A thin serous discharge
  - Little fever
  - Extension to the middle ear and the mastoid
  - Enlarged cervical lymph nodes
- The illness may persist for weeks

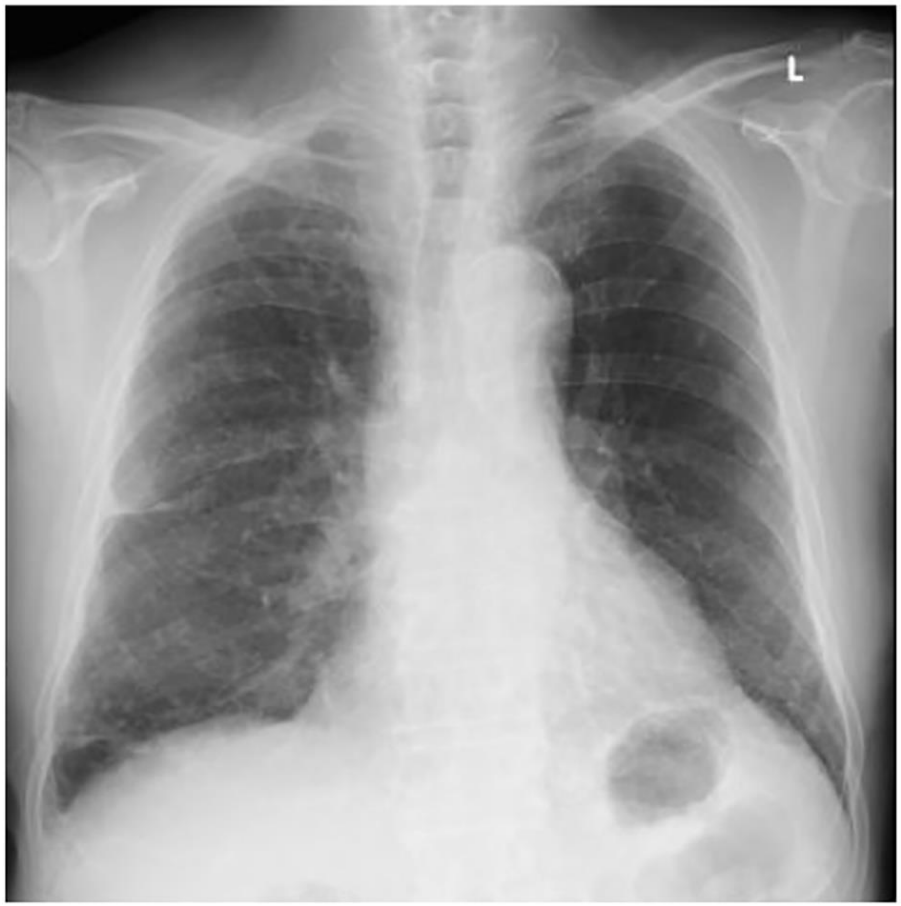
Pharyngitis

In older children and adults;

- It is more acute
  - Intense nasopharyngitis
  - Tonsillitis
  - Intense redness and edema of the mucous membranes
  - Purulent exudate
  - Enlarged, tender cervical lymph nodes
  - A high fever (usually)

Pharyngitis / Pneumonia

- Usually does not involve the lungs
- When pneumonia does occur, is rapidly progressive and severe
  - It is most commonly a sequela to viral infections



Journal of Clinical Medicine Research 11 (8): 831-5, 2016

Pharyngitis / Scarlet fever

Circumoral pallor

Pastia lines

- **Scarlet fever** is a complication of pharyngitis, if pathogen produces pyrogenic exotoxin

Strawberry appearance

Erythematous rash

www.zmescience.com/medicine/genetic/drug-resistant-scarlet-89242423/

Laboratory Diagnosis

- Microscopy
- Antigen detection
- Nucleic acid based tests
- Culture
- Identification
- Antibody detection

Laboratory Diagnosis

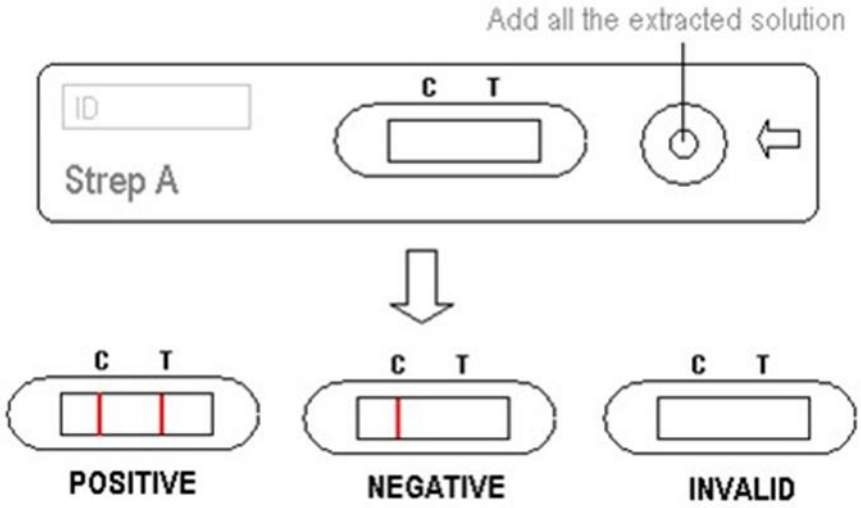
Microscopy;

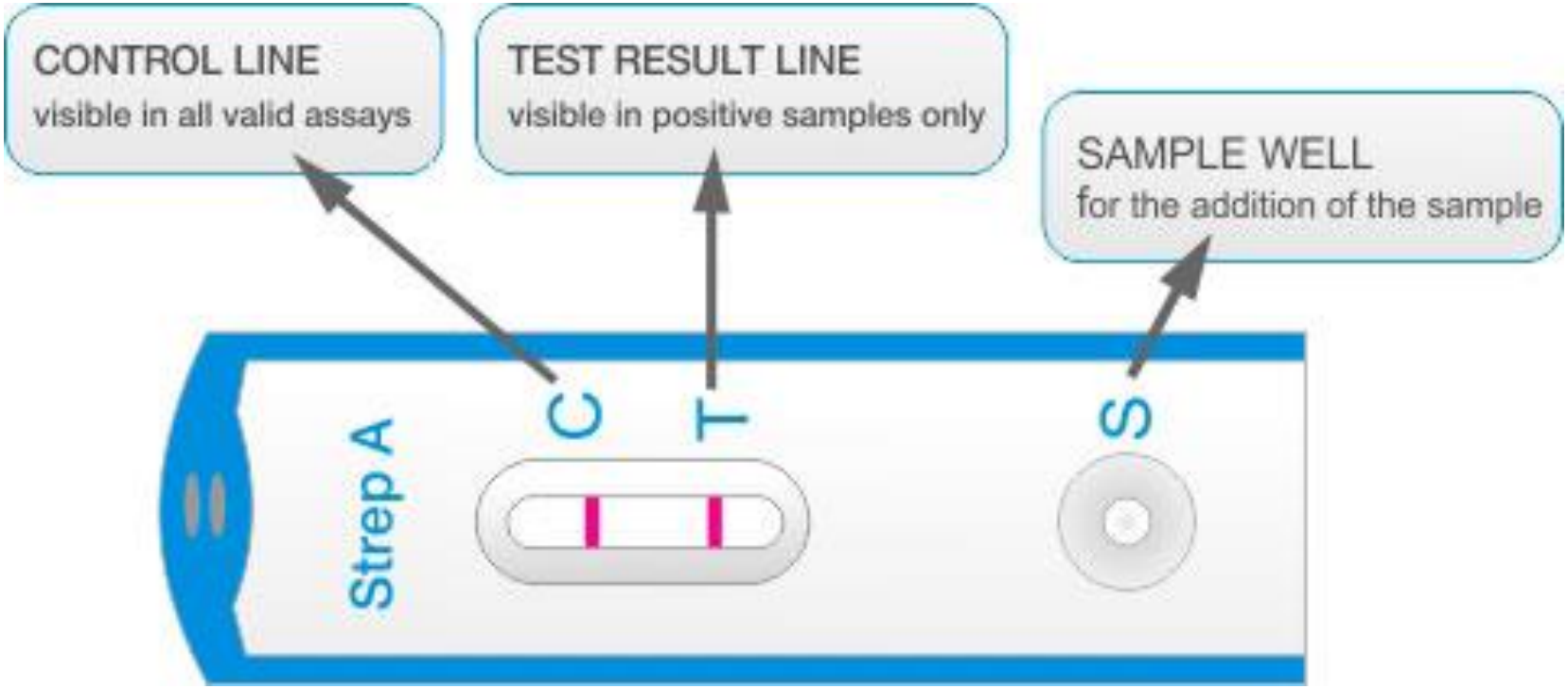
- Only for the diagnosis of soft tissue infection on skin samples
  - Streptococci are not observed in Gram stains of uninfected skin
- Smears are of no value in infections of throat
  - Many species of streptococci are part of the normal oropharyngeal flora

Laboratory Diagnosis

Antigen detection;

- Swab test
  - Only for group A
- Rapid
- Inexpensive
- Specific
- Low sensitivity



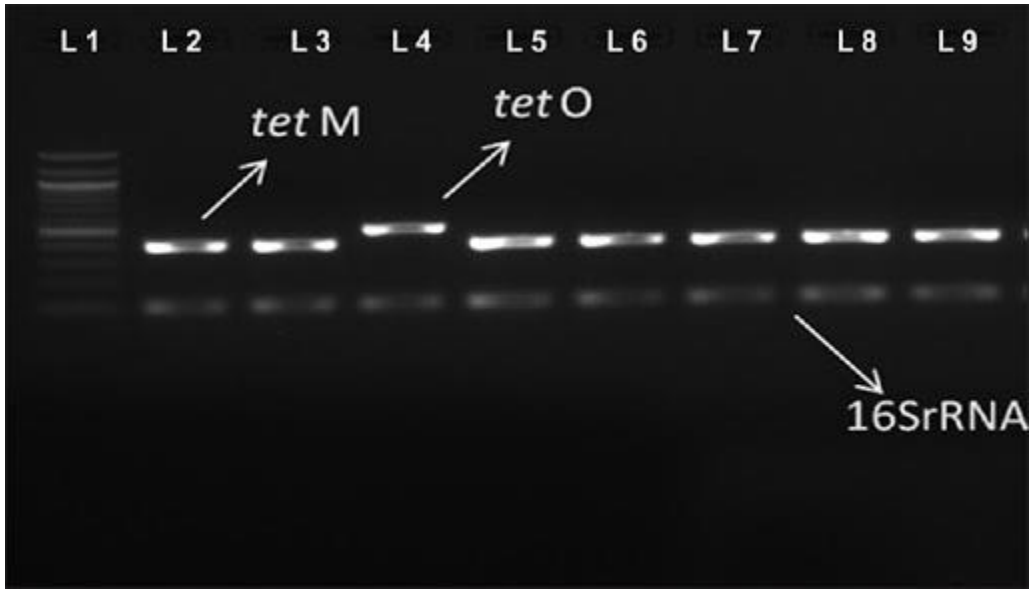


http://bioplus.gr/en/products2012/rapid-tests/strep-a/

Laboratory Diagnosis

Nucleic Acid Based Assays;

- PCR or probes; limited
  - Probe assays are less sensitive than culture
  - Amplification assays are as sensitive as culture
    - They are the test of choice where available



Int J Med Microbiol, 36 (2):186-91, 2018

Laboratory Diagnosis

Culture;

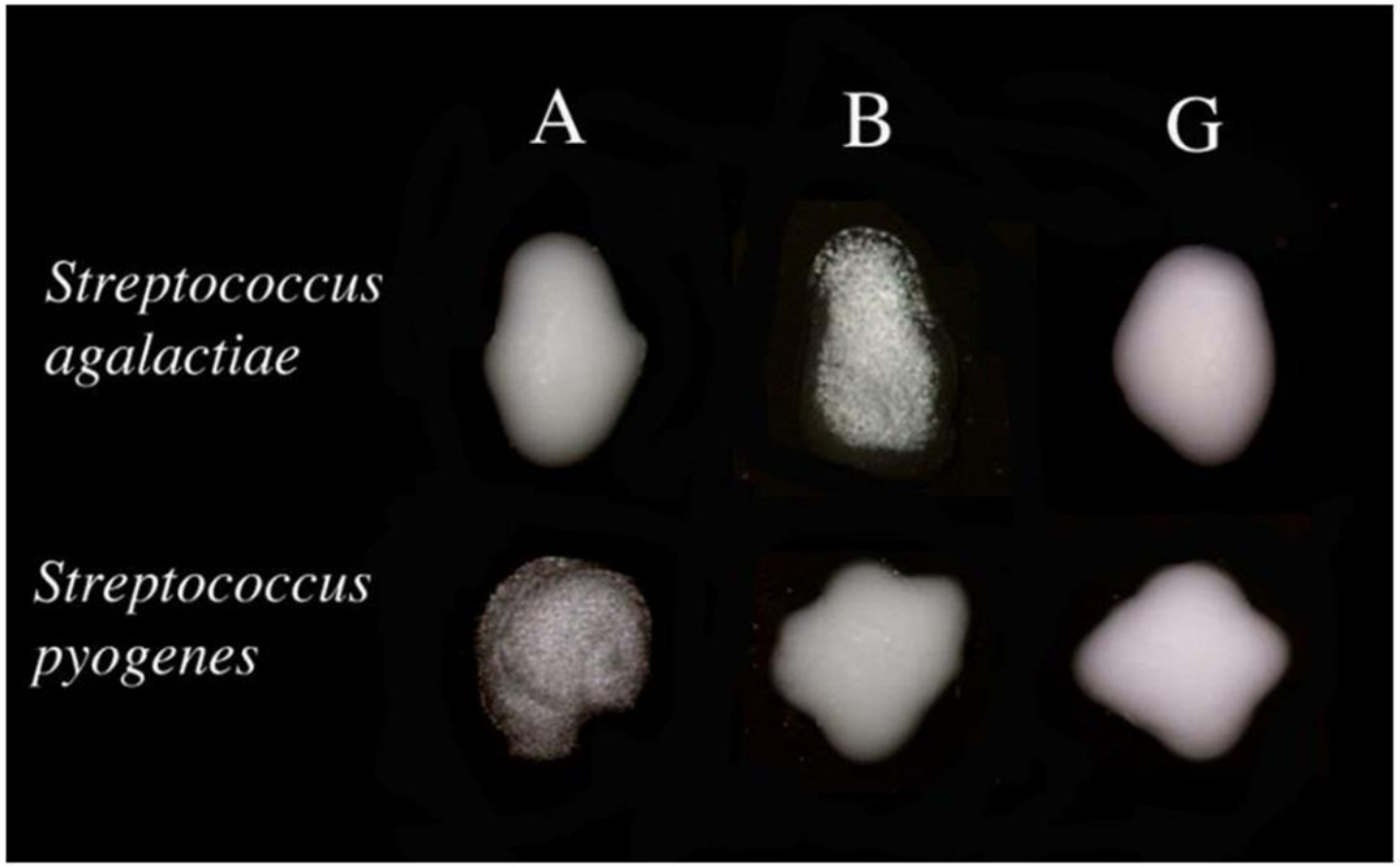
- The posterior oropharynx (e.g., tonsils) specimens
- Gold standard
- Beta hemolysis on sheep blood 5% agar
  - Prolonged incubation (2 to 3 days)

Laboratory Diagnosis

Identification;

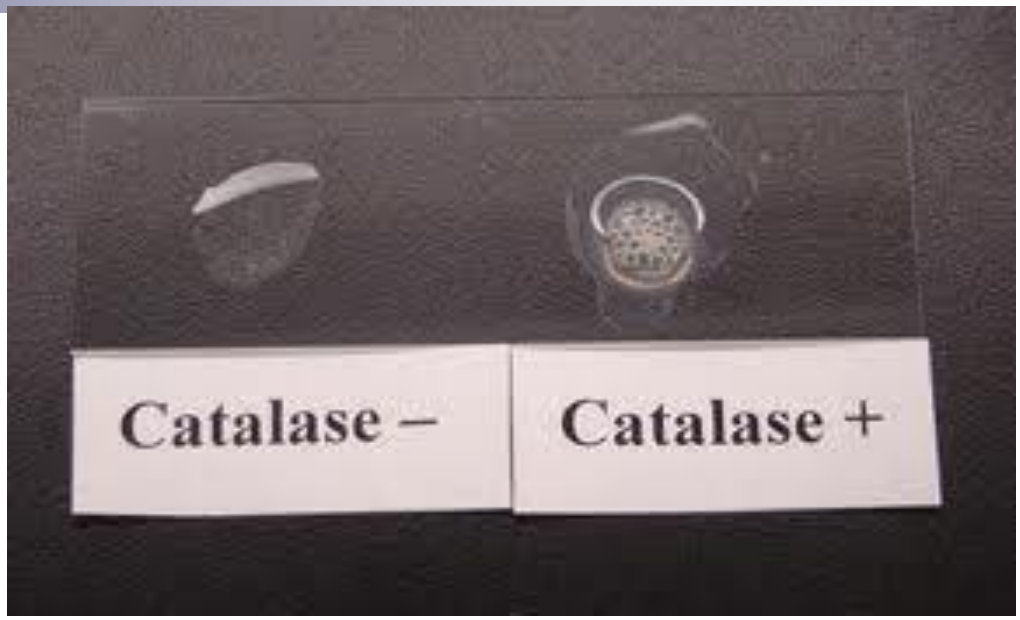
- Catalase negative
- Susceptibility to bacitracin
- L-pyrrolidonyl arylamidase (PYR) test
- Group specific carbohydrate detection (card test)

Slide Agglutination Tests



https://www.slideserve.com/auryon/mlab-2434-microbiology-keri-brophy-martinez

Laboratory Diagnosis



https://microbeonline.com/catalase-test-principle-uses-procedure-results/

Identification;

- Catalase test
- H_2O_2 + catalase ⟶ $H_2O + O_2$

Catalase (+)	*Staphylococcus spp.*
Catalase (-)	*Streptococcus spp.*

Laboratory Diagnosis

Identification;

- Susceptibility to bacitracin

Laboratory Diagnosis

Identification;

- L-pyrrolidonyl arylamidase (PYR) test

Substrate: **L-pyrolidonyl-beta-naphtylamide**
Enzyme: **L-pyrrolidonyl arylamidase**
Product: **Beta-naftilamid**
Reagent: **Cinnamaldehit**
Result: **A red compaund**

PYR (+)	*Staphylococcus* spp., *Enterococcus* spp., ***Streptococcus pyogenes***
PYR (-)	Other *streptococcus* spp.

https://microbiologynotes.com/pyr-l-pyrrolidonyl-β-naphthylamide-test-principle-uses-procedure-result-interpretaion-examples-and-limitation/

Laboratory Diagnosis

Antibody detection

- Antibodies against the M protein (type-specific antibodies)
  - important for maintaining immunity
  - are not useful for diagnosis
- ASO
  - 3 to 4 weeks after the initial exposure
  - shows a recent streptococcal pharyngeal infection, not immunity
- Anti-DNAse
  - occurs after either streptococcal pyoderma or pharyngitis
  - mainly used in diagnosis of streptococcal pyoderma for which ASO titers is of much less value

Treatment

- Sensitive to **penicillin** (No resistance to penicillins)
  - Oral penicillin V or amoxicillin
  - Oral cephalosporin or macrolide

- Resistance or poor clinical response has limited the usefulness of the **tetracyclines** and **sulfonamides**
- Resistance to **erythromycin** and the newer **macrolides** (e.g., azithromycin, clarithromycin) is increasing in frequency

References

- Medical Microbiology; Murray, Rosenthal, Pfaller; 7th Ed; Elsevier Saunders; 2013

- Jawetz, Melnick & Adelberg's Medical Microbiology; Brooks G, Carroll KC, Butel J, Morse S (Eds); 27th Ed; McGraw Hill Lange; 2016

- Sherris Medical Microbiology; 6th Ed; Ryan KJ, Ray CG; McGraw Hill Education; 2014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