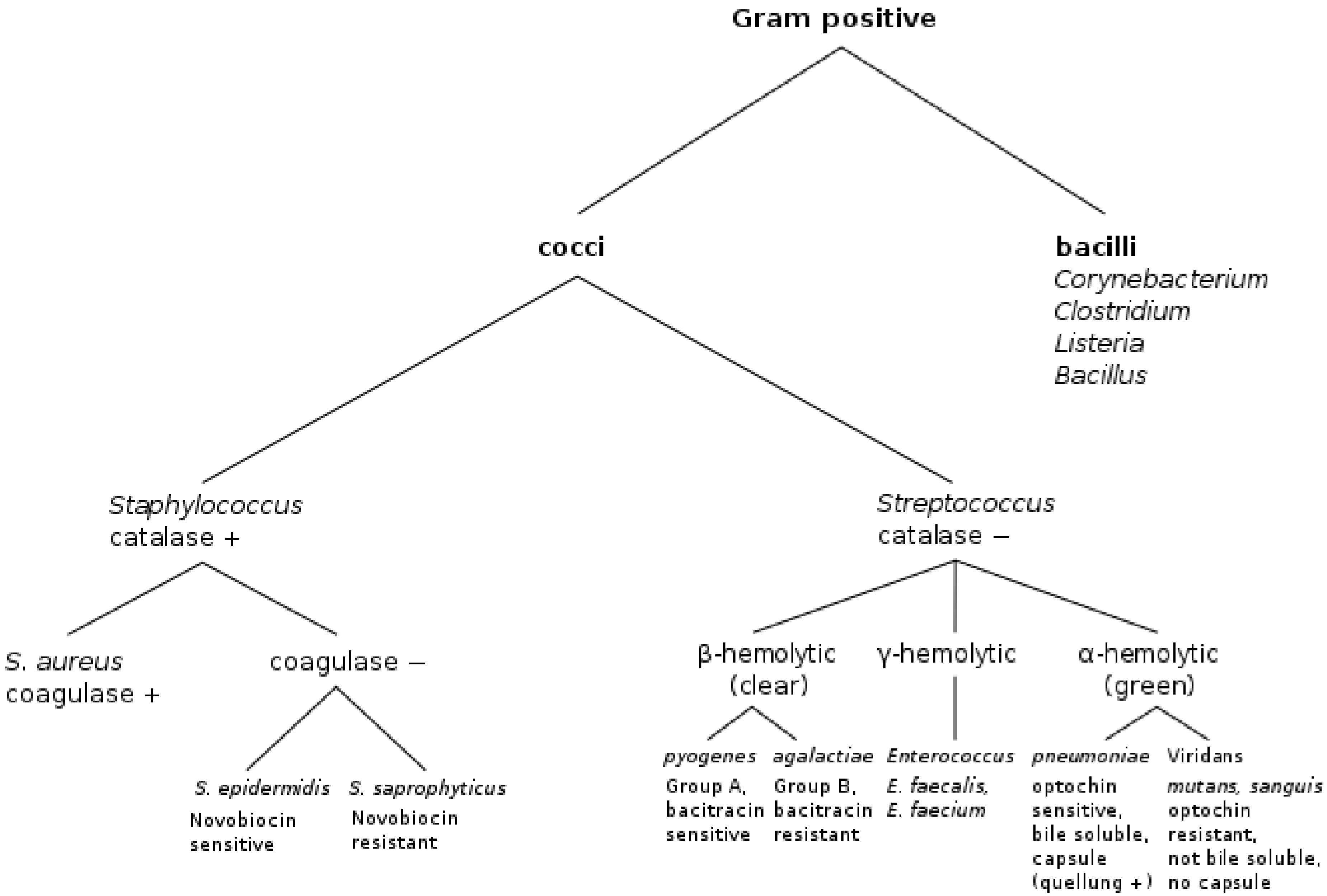
Introduction to Microbiology

Anas Abu-Humaidan
M.D. Ph.D.

Streptococci


Gram positive
cocci
bacilli
Corynebacterium
Clostridium
Listeria
Bacillus
Staphylococcus
catalase +
Streptococcus
catalase –
S. aureus
coagulase +
coagulase –
S. epidermidis
Novobiocin sensitive
S. saprophyticus
Novobiocin resistant
β-hemolytic (clear)
γ-hemolytic
α-hemolytic (green)
pyogenes
Group A, bacitracin sensitive
agalactiae
Group B, bacitracin resistant
Enterococcus
E. faecalis, E. faecium
pneumoniae
optochin sensitive, bile soluble, capsule (quellung +)
Viridans
mutans, sanguis
optochin resistant, not bile soluble, no capsule

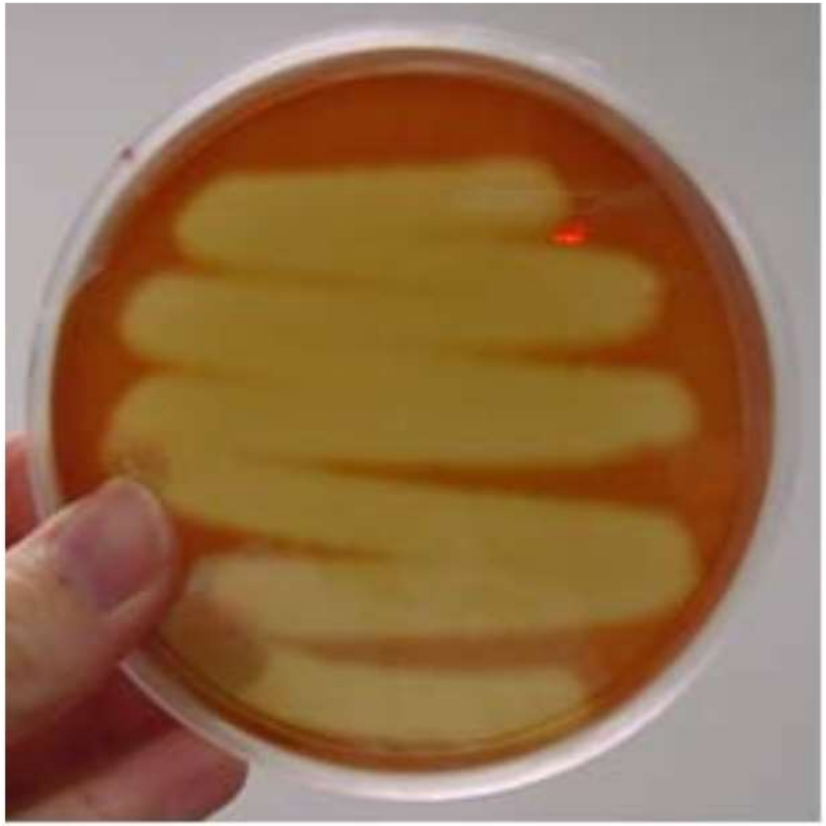
Streptococci / Classification

- The classification of more than 100 species within the genus *Streptococcus* is complicated because three different overlapping schemes are used:

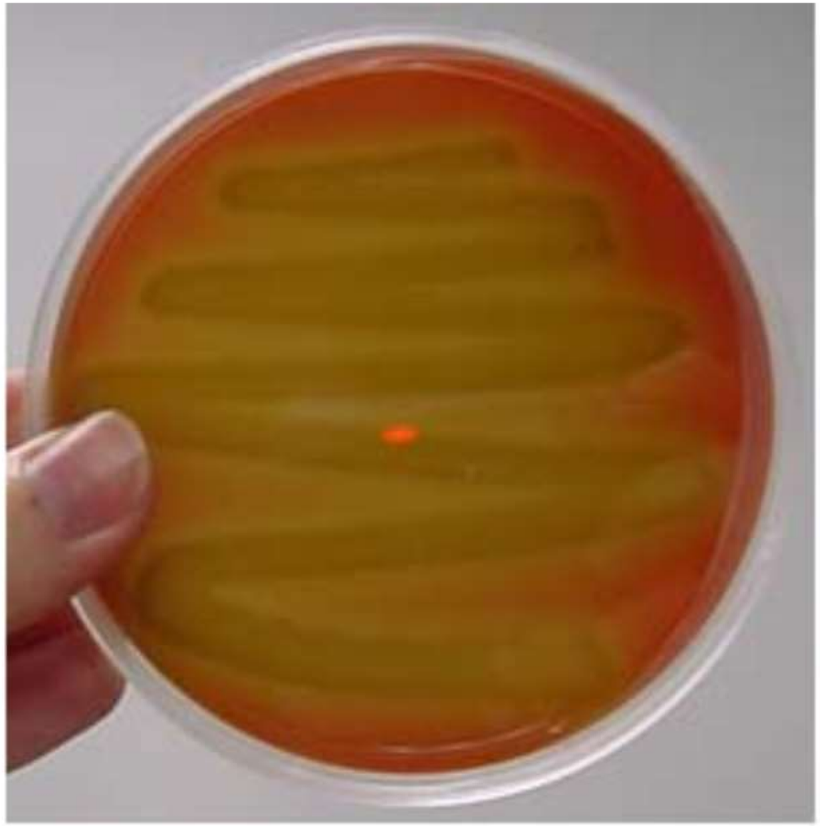
- (1) serologic properties: **Lancefield groupings** (originally A to W);
- (2) **hemolytic patterns:** complete (beta [β]) hemolysis, incomplete (alpha [α]) hemolysis, and no (gamma [γ]) hemolysis;
- (3) **biochemical (physiologic) properties.**

- The most important pathogenic streptococcal species for humans include *Streptococcus pyogenes* (group A streptococcus/ GAS) , Streptococcus agalactiae (GBS), ~~group D streptococcus~~ (enterococci), *Streptococcus pneumoniae*, and *Streptococcus viridans*.

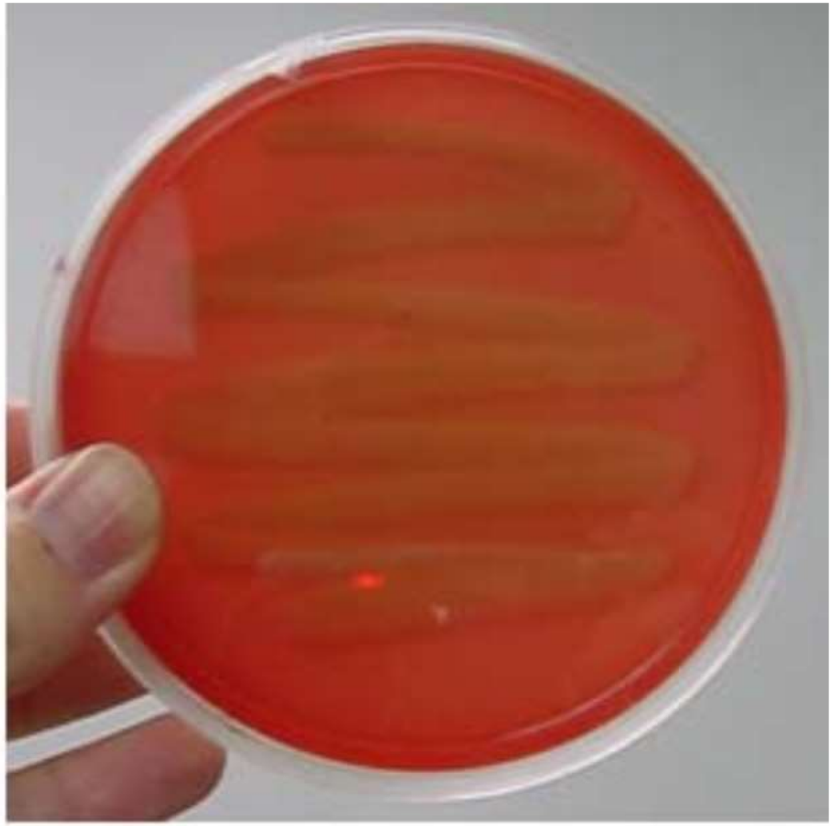
Streptococci / Classification / hemolytic patterns



Beta Hemolysis

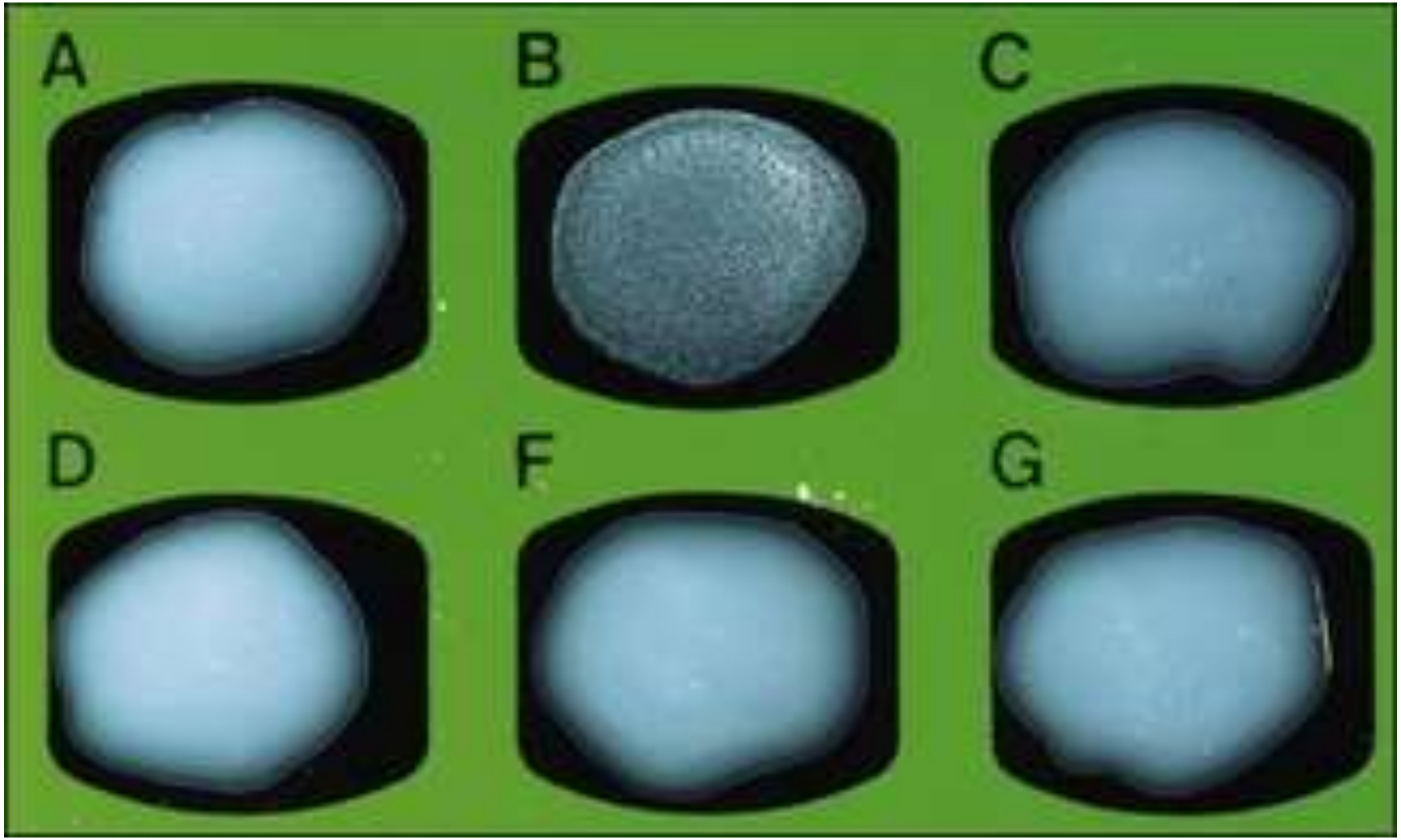


Alpha Hemolysis

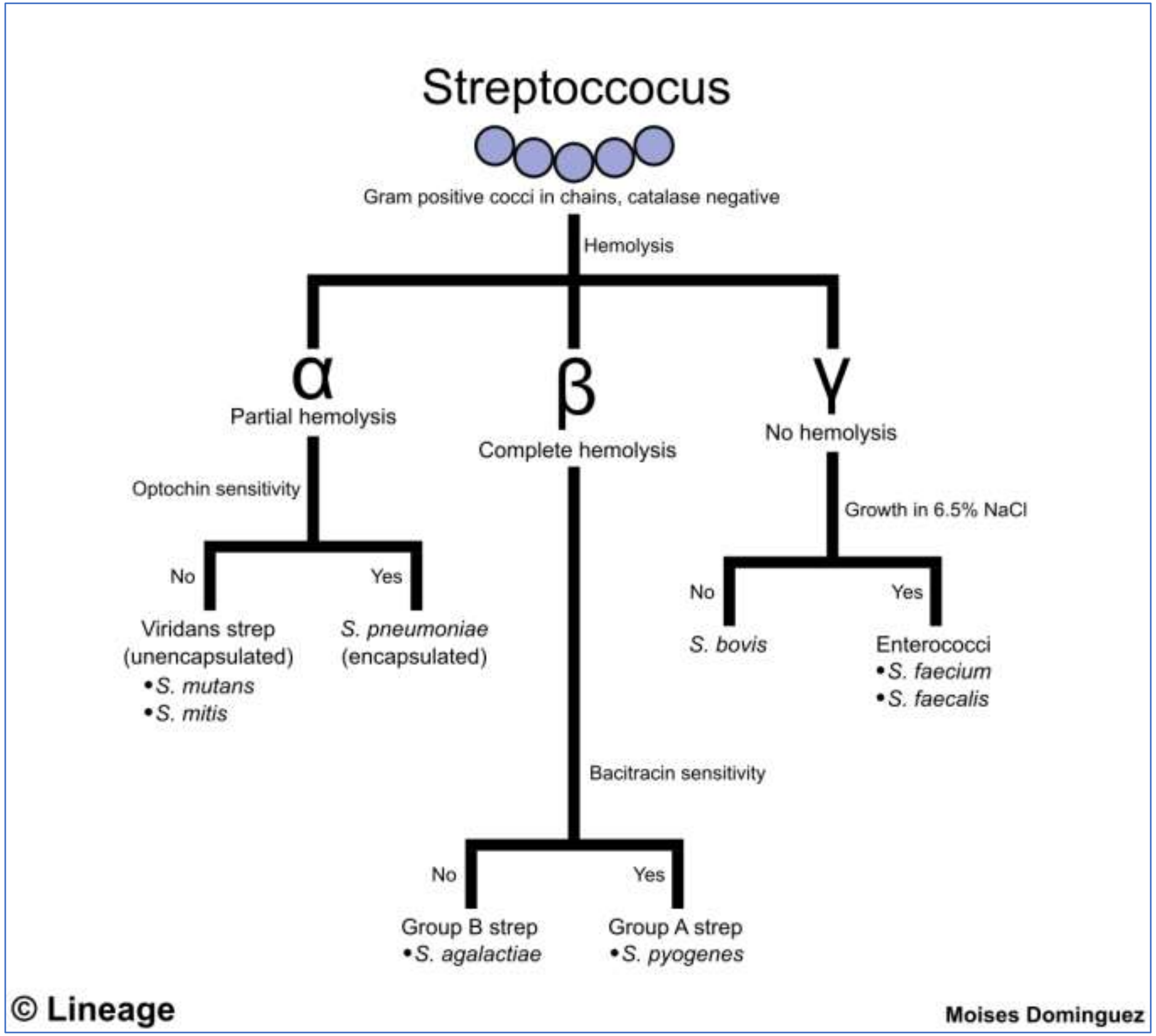


Gamma Hemolysis

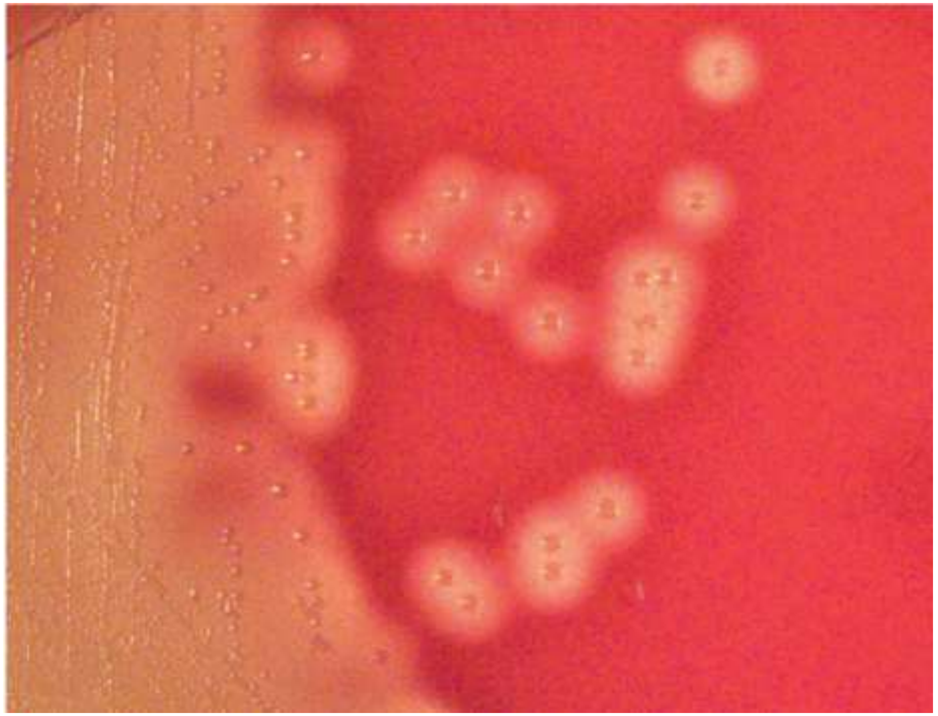
Streptococci / Classification / Lancefield groupings



Streptococci / Classification / biochemical (physiologic) properties

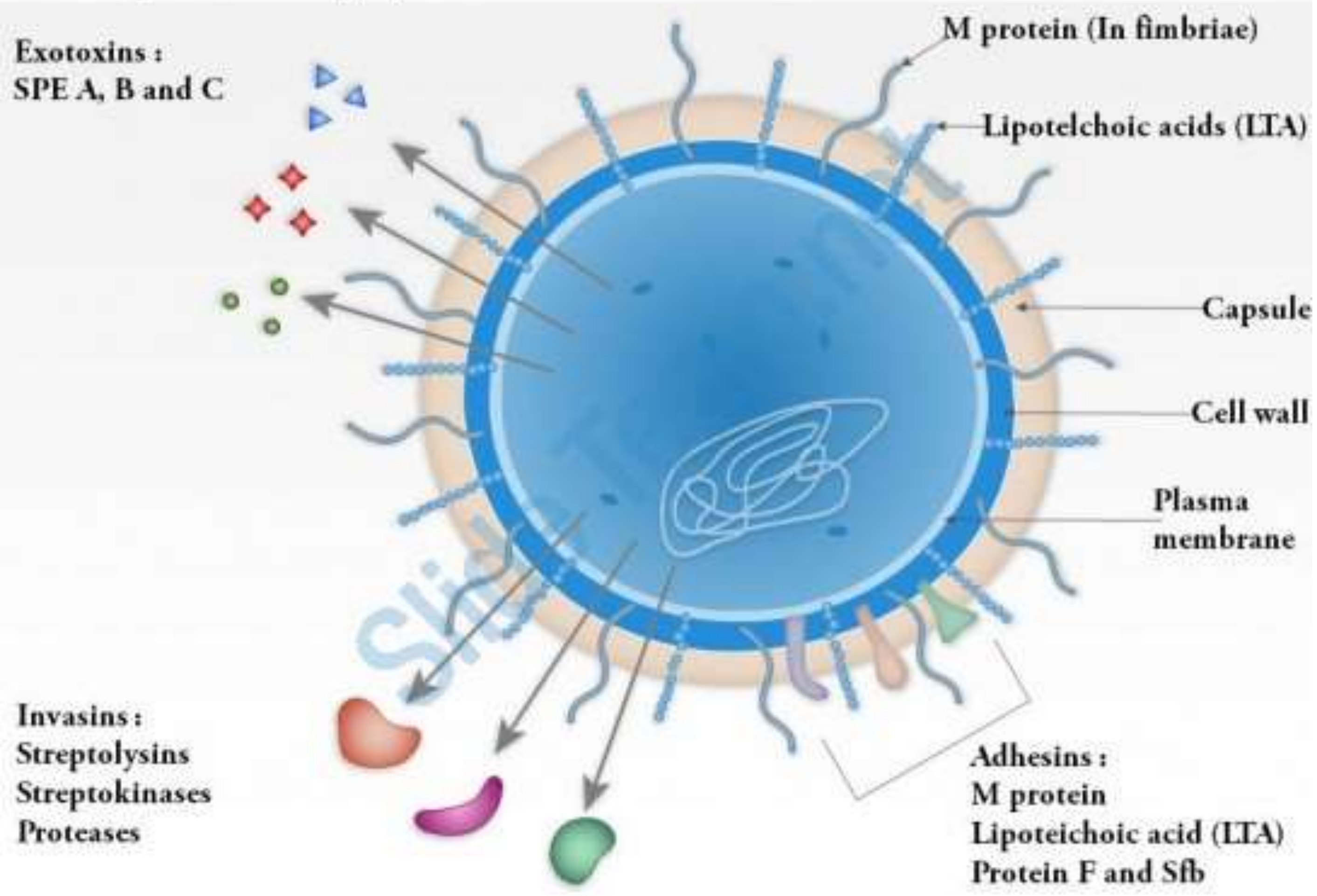


Streptococcus pyogenes



- *S. pyogenes* are **spherical cocci**, 1 to 2 µm in diameter, **arranged in chains**.
- After 24 hours of incubation, 1- to 2-mm white colonies with **large zones of β-hemolysis** are observed
- Harbors the **Lancefield group A antigen**, and is often called group A streptococcus (**GAS**)

Streptococcus pyogenes bacterium



- *S. pyogenes* has multiple mechanisms for **avoiding opsonization and phagocytosis.** The **hyaluronic acid capsule** is a poor immunogen and interferes with phagocytosis. The **M proteins** also interfere with phagocytosis by blocking the binding of the complement component C3b . The **M proteins** also facilitate **adherence to host cells** *S. pyogenes* can **invade into epithelial cells,** a process that is mediated by **M protein** and **F protein.**
- *S. pyogenes* has **C5a peptidase** on the surface. This serine protease inactivates C5a.

Streptococcus pyogenes / Toxins and enzymes

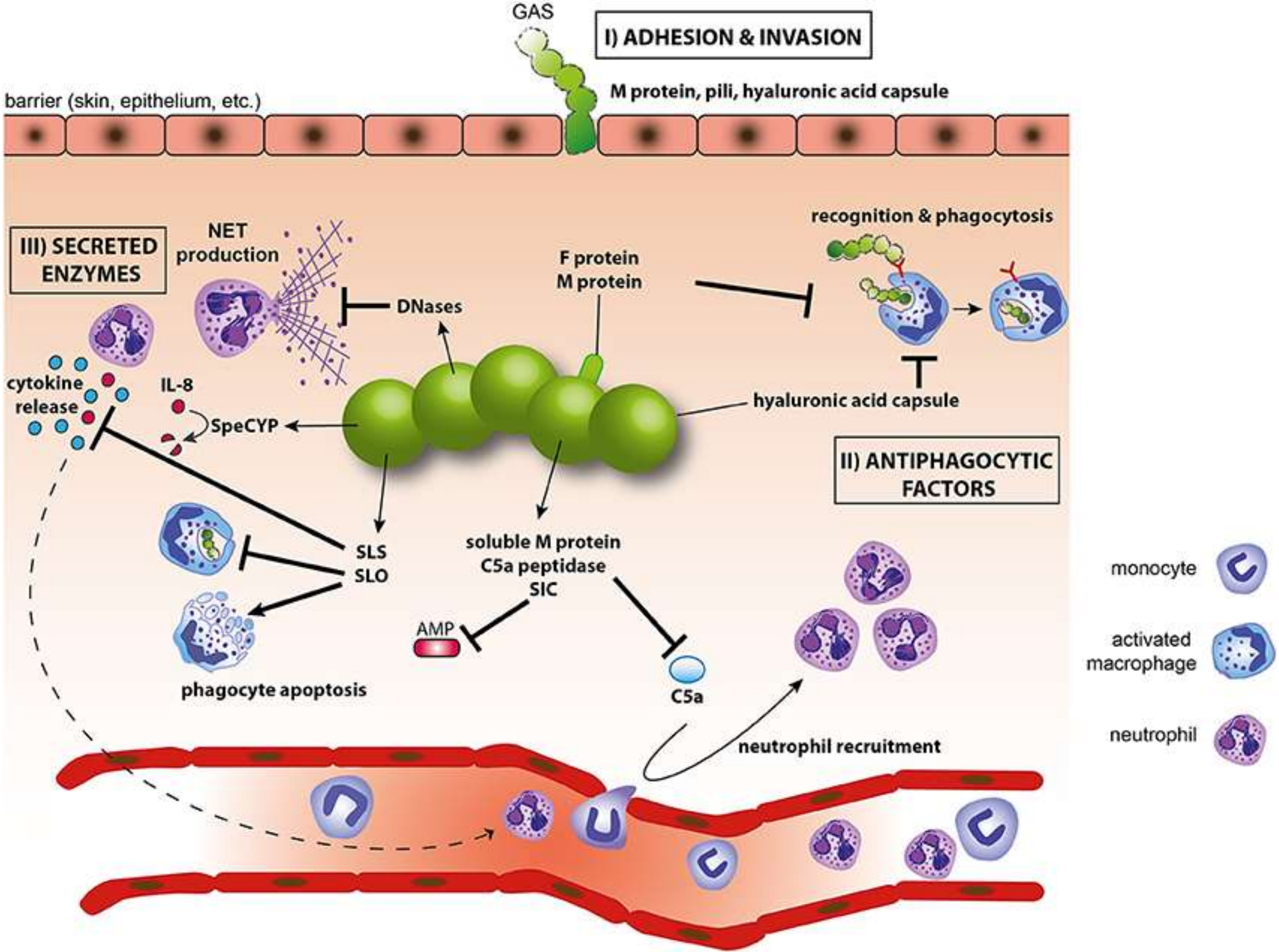
- The **streptococcal pyrogenic exotoxins (Spe),** originally called *erythrogenic toxins,* are produced by lysogenic strain. The toxins act as superantigens.

- **Streptolysin S** is an oxygen-stable, nonimmunogenic, cell-bound hemolysin that can lyse erythrocytes, leukocytes, and platelets, and is responsible for the characteristic β-hemolysis seen on blood agar media.

- **Streptolysin O** is an oxygen-labile hemolysin capable of lysing erythrocytes, leukocytes, platelets, and cultured cells. Antibodies are readily formed against streptolysin O **(antistreptolysin O [ASO] antibodies),** and are useful for documenting recent group A streptococcal infection (**ASO test**). patients with **cutaneous infections do not develop ASO** antibodies.

- **Streptokinase** lyse blood clots and fibrin deposits and facilitate the rapid spread of *S. pyogenes* in infected tissues.

- **DNases A to D** can depolymerize free deoxyribonucleic acid (DNA) present in pus.


GAS
I) ADHESION & INVASION
M protein, pili, hyaluronic acid capsule
barrier (skin, epithelium, etc.)
recognition & phagocytosis
NET production
III) SECRETED ENZYMES
DNases
F protein
M protein
cytokine release
IL-8
SpeCYP
hyaluronic acid capsule
II) ANTIPHAGOCYTIC FACTORS
SLS
SLO
soluble M protein
C5a peptidase
SIC
AMP
C5a
phagocyte apoptosis
neutrophil recruitment
monocyte
activated macrophage
neutrophil

Streptococcus pyogenes / Epidemiology

- The Centers for Disease Control and Prevention (CDC) has estimated that at least 10 million cases of noninvasive disease occur annually, with **pharyngitis and pyoderma** the most common infections. Group A streptococci **can colonize the oropharynx of healthy** children and young adults in the absence of clinical disease.

- *S. pyogenes* disease is caused by recently acquired **strains that can establish an infection** of the pharynx or skin before specific antibodies are produced or competitive organisms are able to proliferate.

- The pathogen is spread from **person to person through respiratory droplets**. Crowding, such as in classrooms and **day-care facilities**, increases the opportunity for the organism to spread, particularly during the winter months

Streptococcus pyogenes / Clinical correlations

***Streptococcus pyogenes* (Group A)**

Suppurative Infections

Pharyngitis: reddened pharynx with exudates generally present; cervical lymphadenopathy can be prominent

Scarlet fever: diffuse erythematous rash beginning on the chest and spreading to the extremities; complication of streptococcal pharyngitis

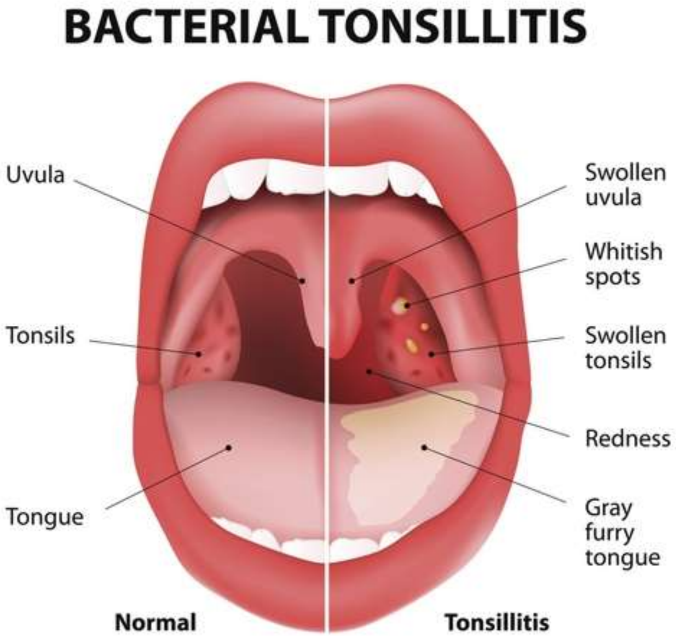
Pyoderma: localized skin infection with vesicles progressing to pustules; no evidence of systemic disease

Erysipelas: localized skin infection with pain, inflammation, lymph node enlargement, and systemic symptoms

Cellulitis: infection of the skin that involves the subcutaneous tissues

Necrotizing fasciitis: deep infection of skin that involves destruction of muscle and fat layers

Streptococcal toxic shock syndrome: multiorgan systemic infection resembling staphylococcal toxic shock syndrome; however, most patients are bacteremic and with evidence of fasciitis

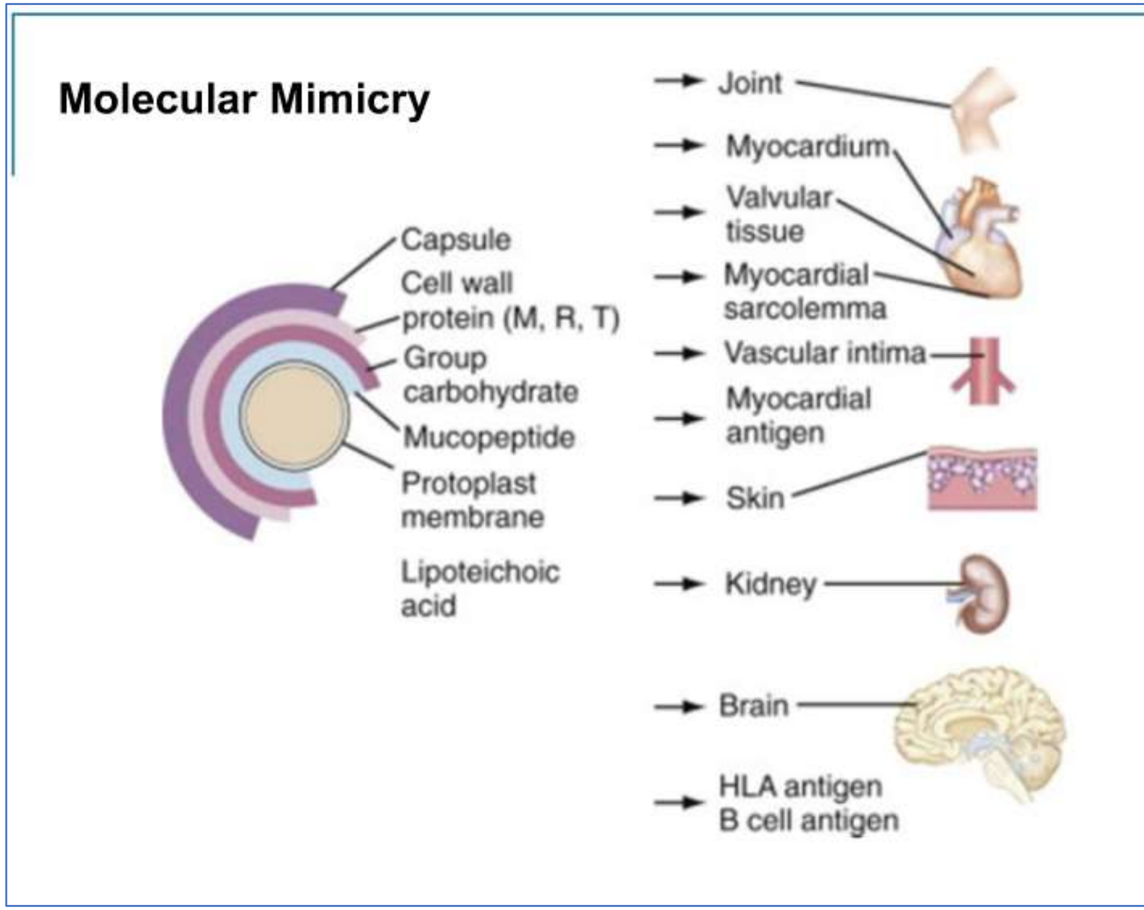

BACTERIAL TONSILLITIS
Uvula
Tonsils
Tongue
Swollen uvula
Whitish spots
Swollen tonsils
Redness
Gray furry tongue
Normal
Tonsillitis


A
B

Streptococcus pyogenes / Clinical correlations

- **Post-streptococcal glomerulonephritis (PSGN)** is an immunologically-mediated sequela of pharyngitis or skin infections caused by nephritogenic strains of Streptococcus pyogenes.

- **Rheumatic fever (RF)** is an inflammatory disease that can involve the heart, joints, skin, and brain. The disease typically develops two to four weeks after a streptococcal throat infection.



Nonsuppurative Infections

Rheumatic fever: characterized by inflammatory changes of the heart (pancarditis), joints (arthralgias to arthritis), blood vessels, and subcutaneous tissues

Acute glomerulonephritis: acute inflammation of the renal glomeruli with edema, hypertension, hematuria, and proteinuria

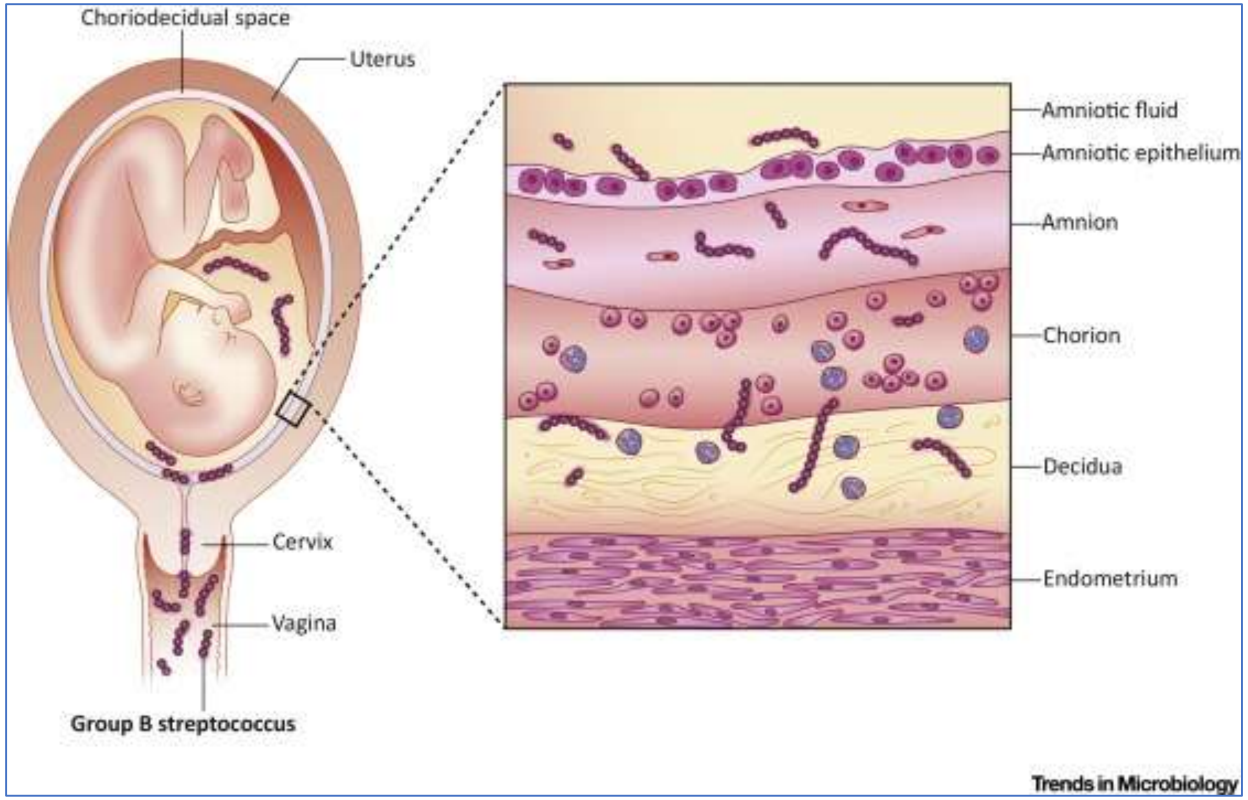
Streptococcus agalactiae

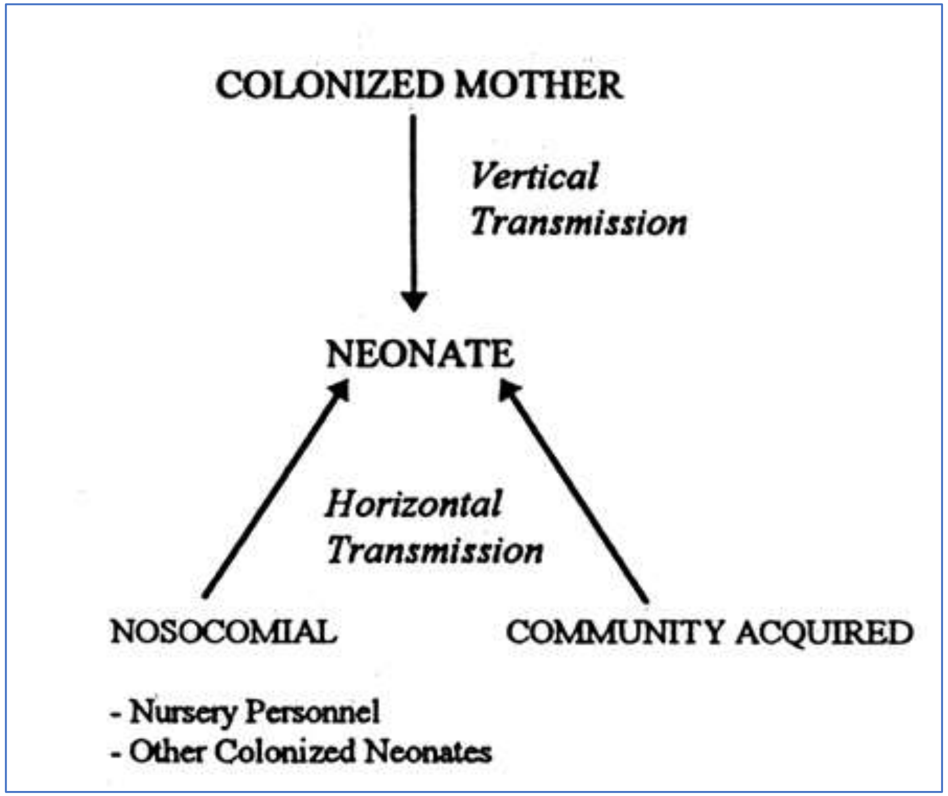
- Distinction of GBS from other streptococci can be based on **GBS antigen detection** or on biochemical reactions including **resistance to bacitracin**

- Group B streptococci colonize the lower gastrointestinal tract and the genitourinary tract. Transient **vaginal carriage** has been observed in 10% to 30% of **pregnant women**.

- men and nonpregnant women with group B streptococcal infections are generally **older and have debilitating underlying conditions**. The most common presentations are **bacteremia, pneumonia, bone and joint infections,** and **skin and soft-tissue infections**

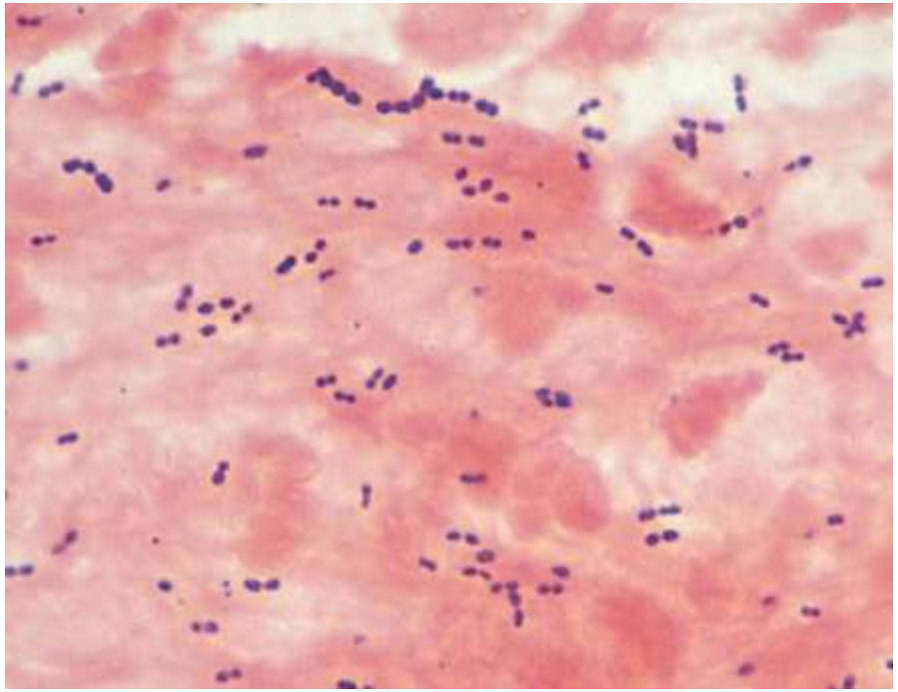
Streptococcus agalactiae





- Neonatal infection is divided into **early-onset** infection, occurring at less than 7 days of age; and **late-onset** infection, occurring at or beyond 7 days of age. Both are characterized by **bacteremia** or **meningitis.**

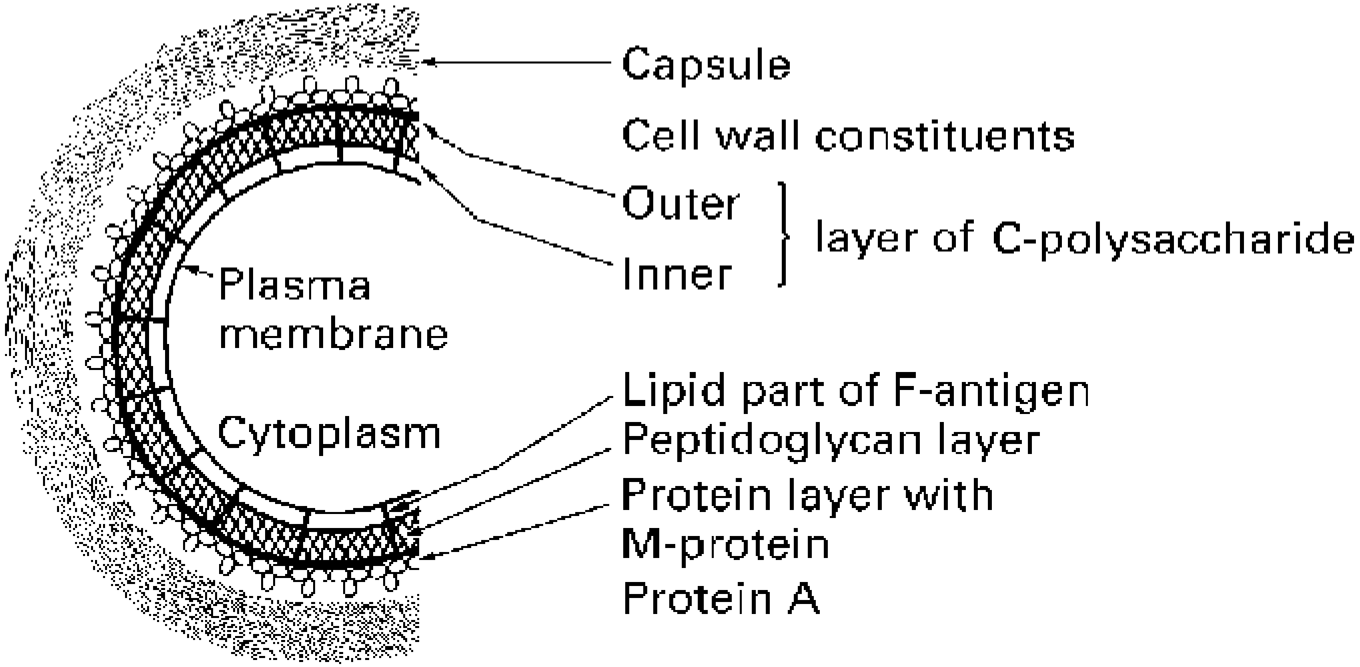
Streptococcus pneumoniae



- The pneumococcus is an **encapsulated** gram-positive coccus. The cells are 0.5 to 1.2 µm in diameter, oval, and arranged in pairs (commonly referred to as **diplococci**)

- The α-hemolytic appearance results from production of **pneumolysin,** an enzyme that degrades hemoglobin, producing a green product

Streptococcus pneumoniae / Structure



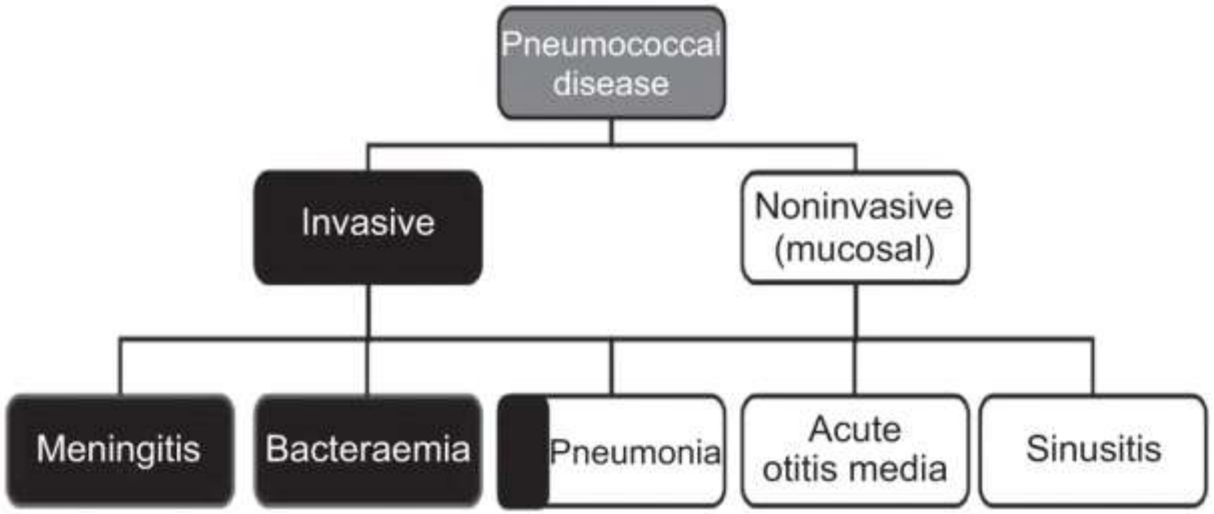
- Virulent strains of *S. pneumoniae* are covered with a complex **polysaccharide capsule,** used for the serologic classification of strains.
- The most commonly isolated serotypes are used in a **polyvalent vaccine.**

Streptococcus pneumoniae / Epidemiology

- *S. pneumoniae* is a common inhabitant of **the throat and nasopharynx** in healthy people, with colonization more common in children than in adults and more common in adults living in a household with children.

- Pneumococcal disease occurs when organisms colonizing the nasopharynx and oropharynx **spread** to the lungs (pneumonia), paranasal sinuses (sinusitis), ears (otitis media), or meninges (meningitis).

- The **introduction of vaccines** for pediatric and adult populations has reduced the incidence of disease caused by *S. pneumoniae* .



Streptococcus pneumoniae / Pathogenesis

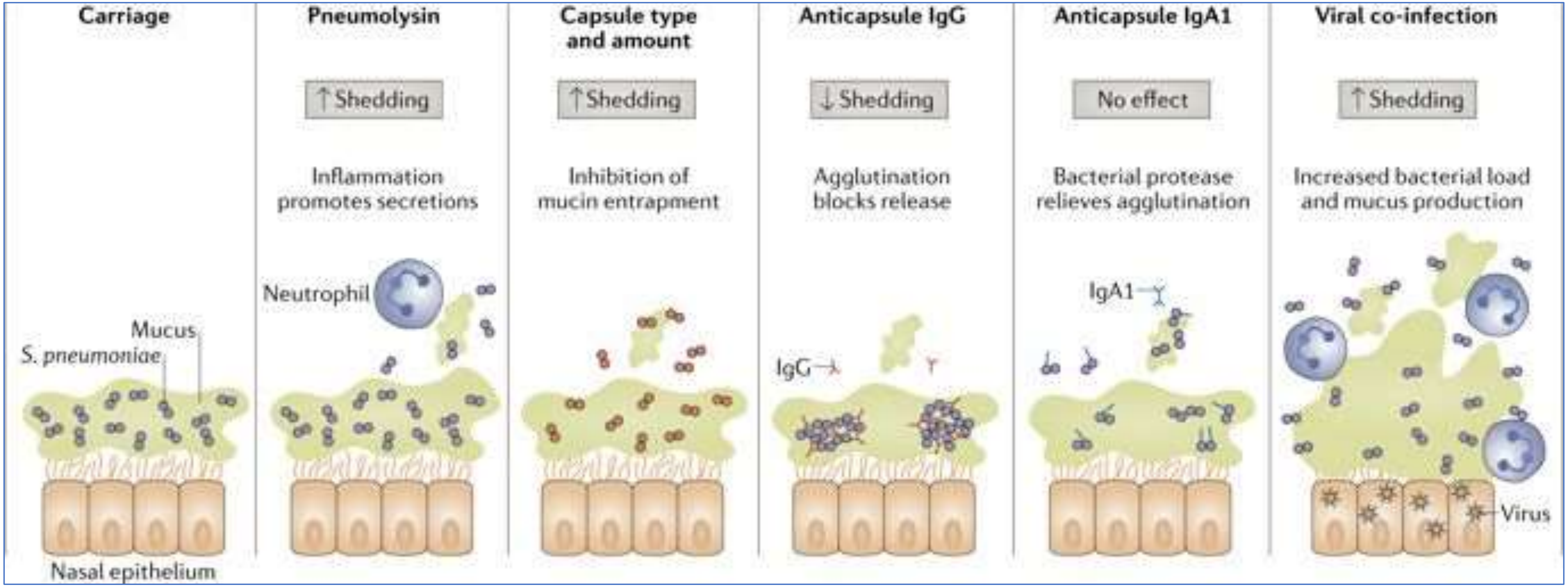
- The disease manifestations are caused **primarily by the host response** to infection rather than the production of organism-specific toxic factors

- After colonization in upper respiratory tract (e.g. oropharynx) bacteria can migrate to lower respiratory tract**, Secretory IgA in mucus** traps bacteria by binding the bacteria to mucin with the Fc region of the antibody. The bacterial **IgA protease** prevents this interaction. **Pneumolysin** can destroy the ciliated epithelial cells and phagocytic cells.

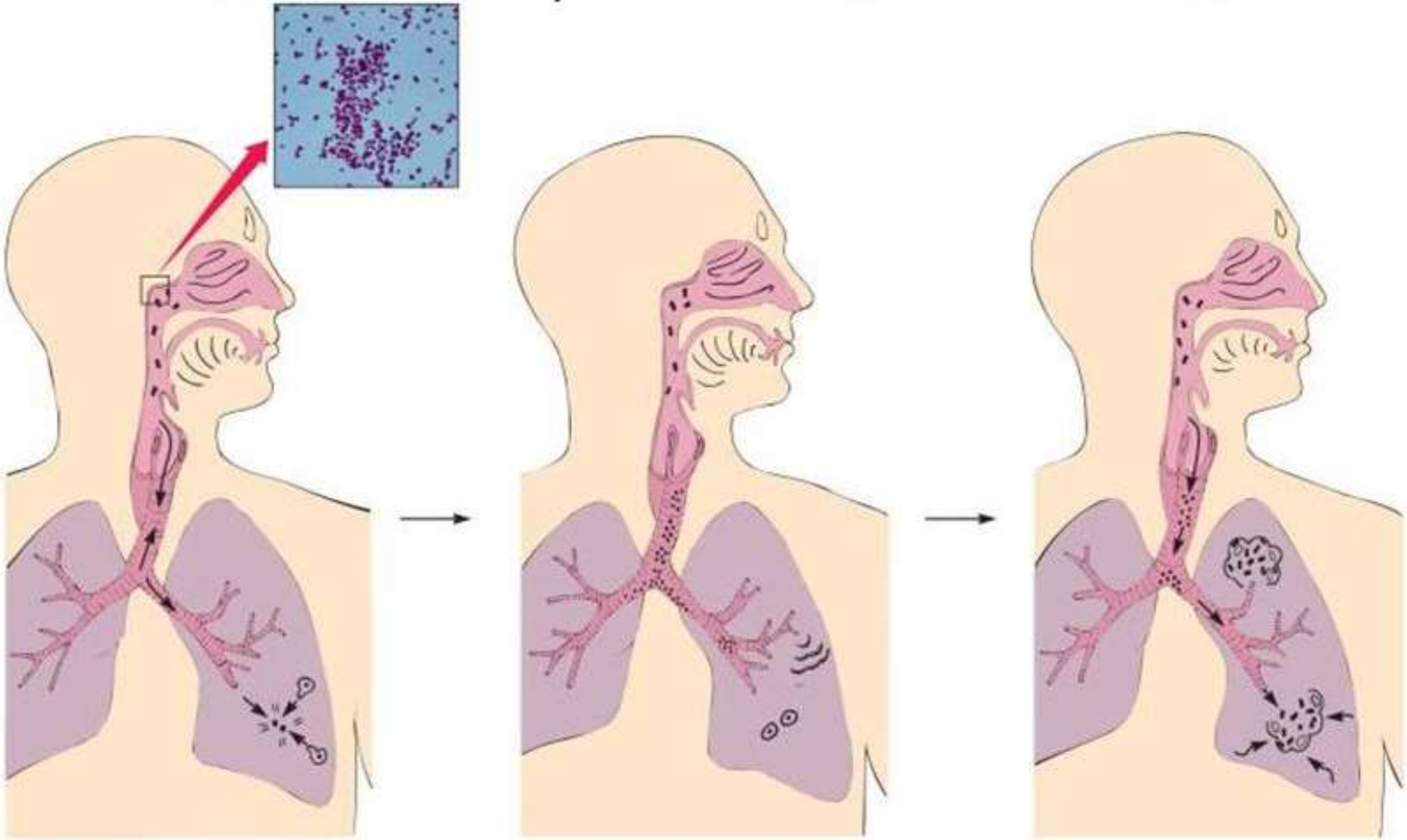
- A characteristic of pneumococcal infections is the mobilization of inflammatory cells to the focus of infection. A**midase,** enhances release of the cell wall components (**Teichoic acid** and the **peptidoglycan fragments**) which can activate complement and initiate an immune response.

- **Phosphorylcholine** present in the bacterial cell wall can bind to receptors for platelet-activating factor , and helps in host cell invasion, and is important for **amidase** function.

- The virulence of *S. pneumoniae* is a direct result of the **capsule**. **Encapsulated** (smooth) strains can cause disease in humans and experimental animals, whereas **nonencapsulated** (rough) strains are avirulent.


Carriage
Pneumolysin
Capsule type and amount
Anticapsule IgG
Anticapsule IgA1
Viral co-infection
↑Shedding
↑Shedding
↓Shedding
No effect
↑Shedding
Inflammation promotes secretions
Inhibition of mucin entrapment
Agglutination blocks release
Bacterial protease relieves agglutination
Increased bacterial load and mucus production
Neutrophil
Mucus
S. pneumoniae
IgG
IgA1
Virus
Nasal epithelium

Strep Pneumonia

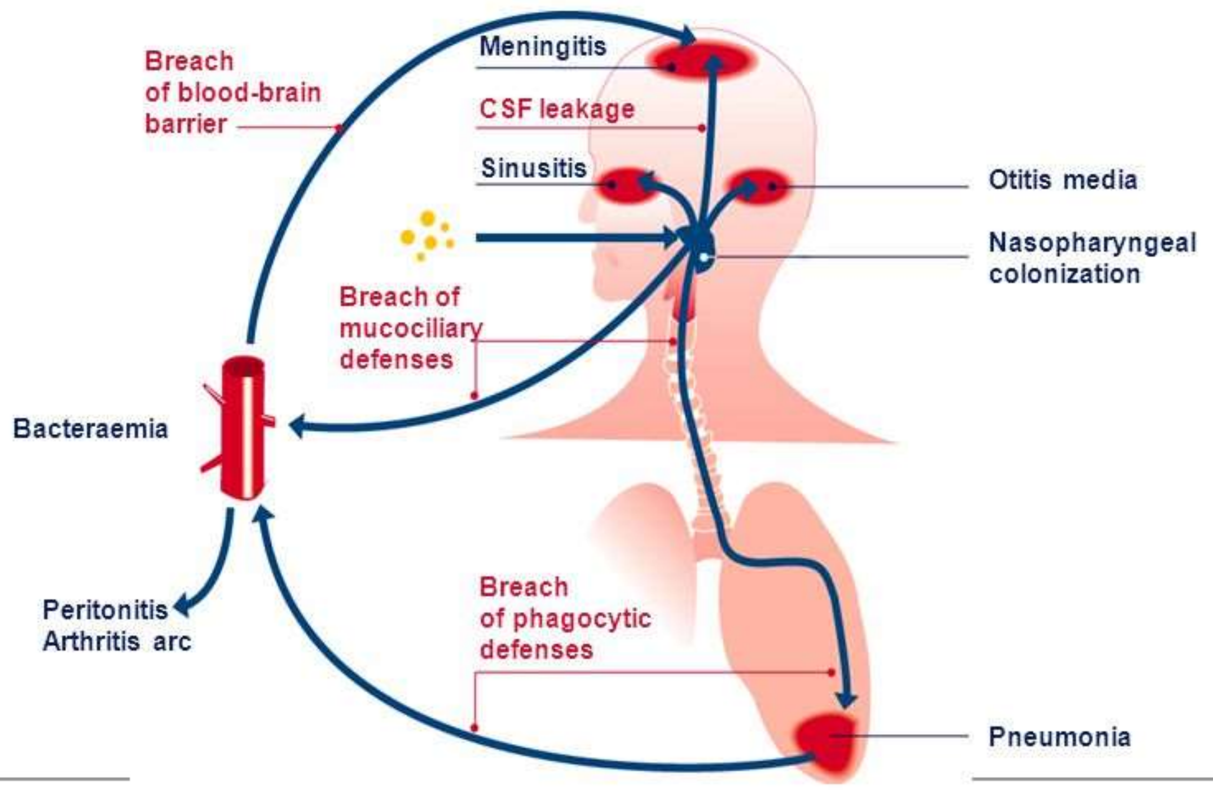


Normal condition
1 Periodic colonization with streptococci
2 Some penetration into lower respiratory tract
3 Streptococci trapped by mucus and removed by ciliary action
4 Phagocytosed by macrophages

Predisposing factors
5 Ciliated epithelium damaged by viruses, toxins, smoking, chemicals
6 Fluid accumulation
7 Decreased activity of macrophages

Development of pneumonia
8 Growth of streptococci on damaged ciliated epithelium
9 Growth in fluids and in alveoli, both of which stimulate increased fluid accumulation

S. Pneumoniae: Pathogenesis



Adapted from [...] Salyers & Whitt. In: *Bacterial Pathogenesis: A Molecular Approach*, 1994

SANOFI PASTEUR

Viridans Streptococci

- The viridans group of streptococci is a heterogeneous collection of α-hemolytic (producing a **green coloration** on blood agar plates (hence the name "**viridans**", from Latin "vĭrĭdis", green)) and nonhemolytic streptococci.
- The viridans streptococci colonize the **oropharynx**, gastrointestinal tract, and genitourinary tract.

Group	Representative Species	Diseases
Anginosus	*S. anginosus*, *S. constellatus*, *S. intermedius*	Abscesses in brain, oropharynx, or peritoneal cavity
Mitis	*S. mitis*, *S. pneumoniae*, *S. oralis*	Subacute endocarditis; sepsis in neutropenic patients; pneumonia; meningitis
Mutans	*S. mutans*, *S. sobrinus*	Dental caries; bacteremia
Salivarius	*S. salivarius*	Bacteremia; endocarditis
Bovis	*S. gallolyticus* subsp. *gallolyticus*, subsp. *pasteurianus*	Bacteremia associated with gastrointestinal cancer (subsp. *gallolyticus*); meningitis (subsp. *pasteurianus*)
Ungrouped	*S. suis*	Meningitis; bacteremia; streptococcal toxic shock syndrome

Enterococcus

- The enterococci are gram-positive cocci, typically arranged in **pairs and short chains** .

- As their name implies, **enterococci are enteric bacteria** that are commonly recovered in feces. *E. faecalis* is found in the **large intestine** in high concentrations (e.g., 10^5 to 10^7 organisms per gram of feces) and in the **genitourinary tract**. Similar distribution for *E. faecium.*

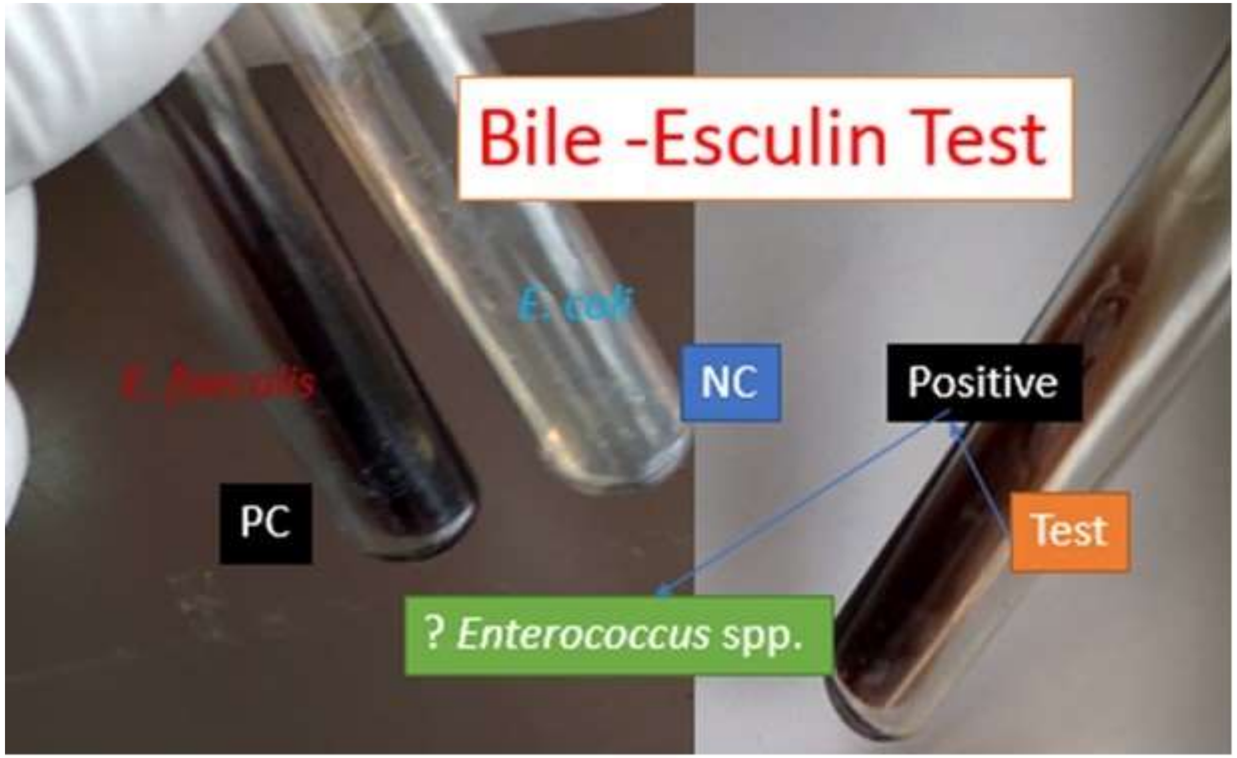
- The cocci grow both aerobically and anaerobically in a **broad temperature range** (10° C to 45° C), in a **wide pH range** (4.6 to 9.9), and in the presence of high concentrations of sodium chloride **(NaCl)** and **bile salts .**

- **Variable hemolysis pattern.**

- Virulence is mediated by two general properties: (1) **ability to adhere to tissues and form biofilms** and (2) **antibiotic resistance**

- enterococci are one of the most common causes of infections acquired in the hospital **(nosocomial infection).** The **urinary tract is the most common site of enterococcal** infections, and infections are **frequently associated with urinary catheterization or instrumentation .**

Enterococcus



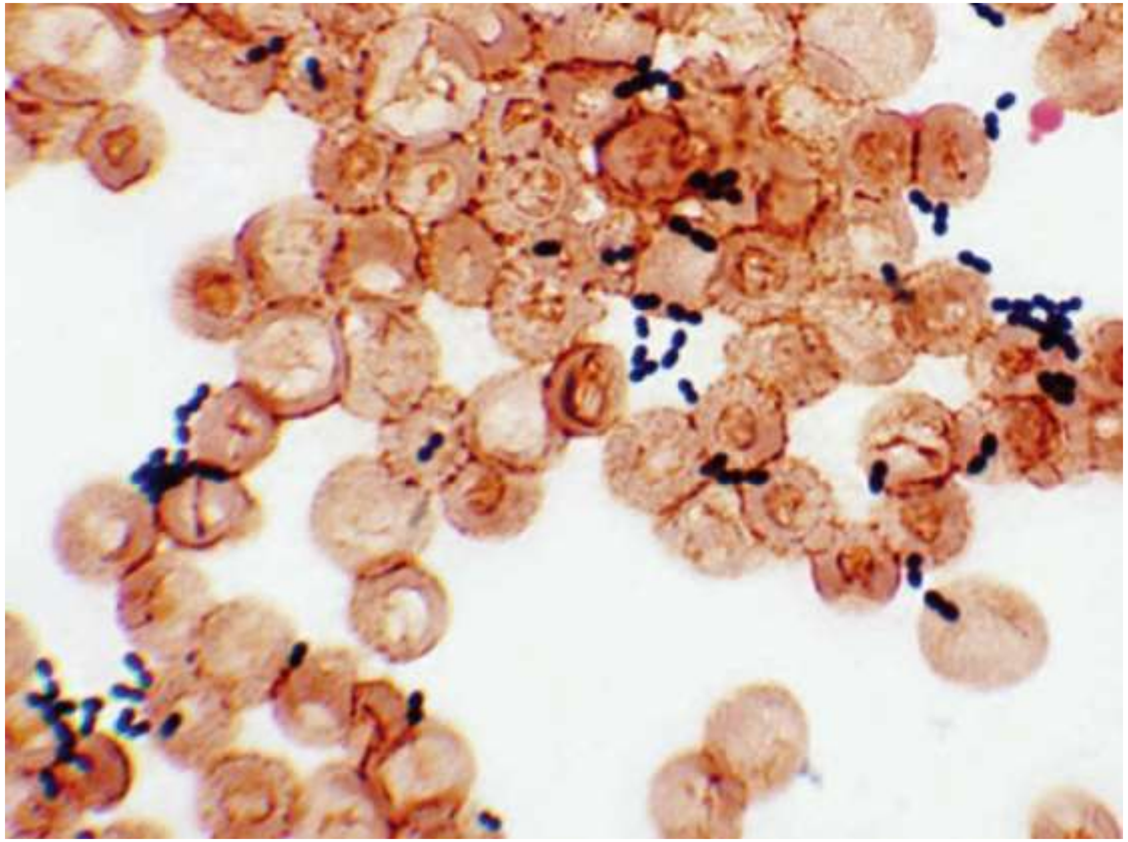
Positive control (**PN**): E. faecalis (ATCC 29212)

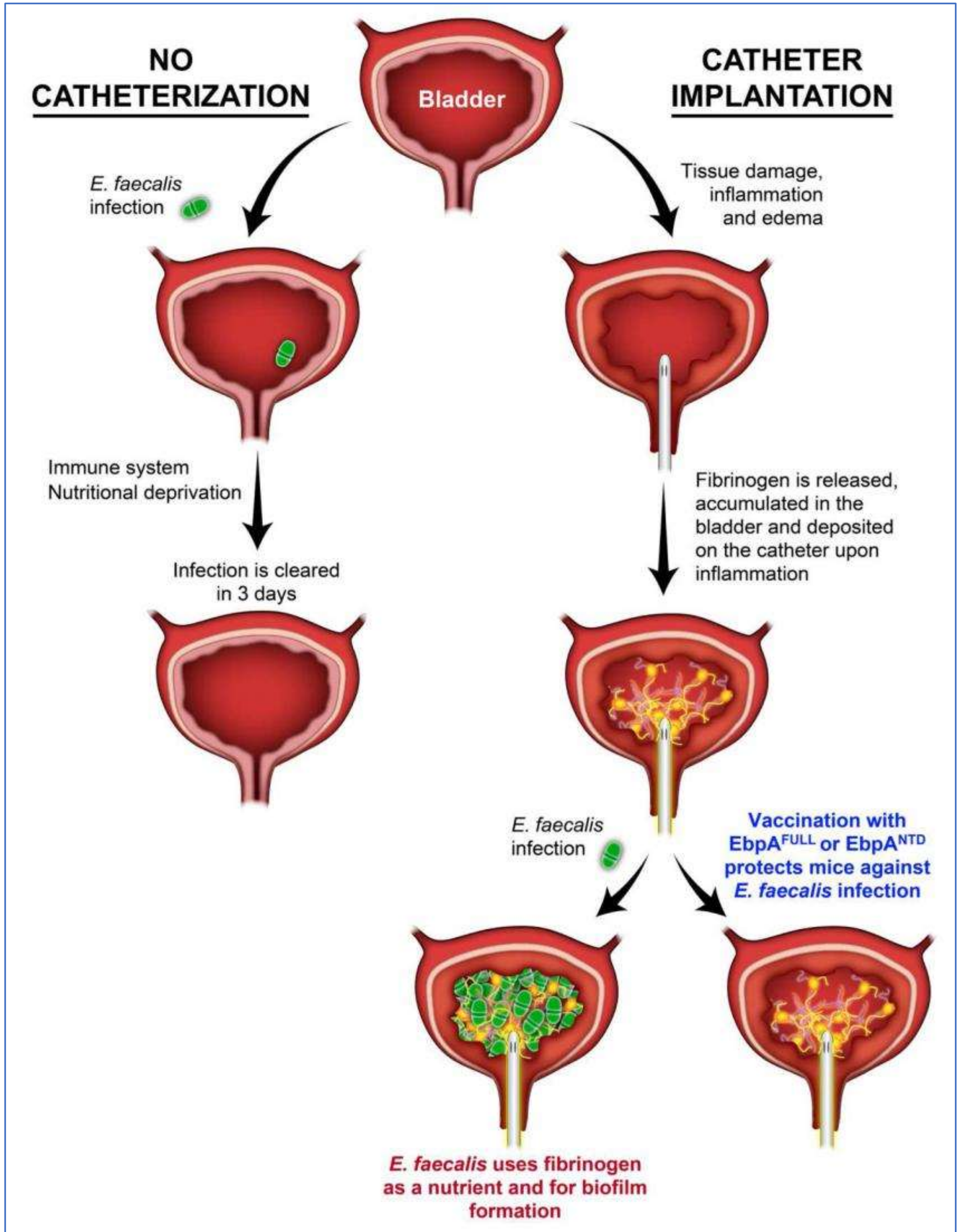
Negative control (**NC**): Escherichia coli (ATCC25923)

Test: Positive

- Principle of Bile Esculin test

Bacteria that are bile-esculin positive, first of all, able to grow in the presence of bile salts. Hydrolysis of the esculin in the medium results in the formation of glucose and esculetin. Esculetin reacts with ferric ions present ferric citrate in the medium to form a phenolic iron complex which produces dark brown or black color.




NO CATHETERIZATION
Bladder
CATHETER IMPLANTATION
E. faecalis infection
Tissue damage, inflammation and edema
Immune system
Nutritional deprivation
Infection is cleared in 3 days
Fibrinogen is released, accumulated in the bladder and deposited on the catheter upon inflammation
E. faecalis infection
Vaccination with EbpA^FULL or EbpA^NTD protects mice against E. faecalis infection
E. faecalis uses fibrinogen as a nutrient and for biofilm formation

- Age: 65
- Medical History: Type 2 diabetes, hypertension
- Hospitalized post operation with a urinary catheter
- Complaint: Painful urination and fever
- Clinical History:
- Mr. X reports a low-grade fever and a sense of general malaise. Urine is turbid and he has suprapubic tenderness .
- Gram stain shows gram positive cocci that is bile-esculin positive

What is the most probable pathogen?

- Age: 7
- Medical History: None significant
- Presenting Complaint:
- Emily is brought to the pediatrician by her parents with complaints of a sore throat, fever, and a red rash.

- Clinical History:
- Over the past 24 hours, Emily developed a sudden-onset sore throat and fever. Her parents noticed a fine red rash that started on her neck and spread to her chest and extremities.

- Physical Examination:

- Vital Signs: Temperature 101.8°F (38.8°C), heart rate 110 bpm, respiratory rate 20/min.
- Throat Examination: Presence of redness and swollen tonsils with yellowish-white exudate.
- Skin Examination: Diffuse red rash with a sandpaper-like texture.
- Diagnostic Tests:

- Throat Culture: Positive for Group A Streptococcus (Streptococcus pyogenes).
- Scarlet Fever Rash Confirmation: The rash, along with the clinical presentation and positive throat culture, confirms scarlet fever.
- Medical Management:

- Antibiotic Therapy: Initiated with oral penicillin to eradicate the streptococcal infection.
- Symptomatic Relief: Paracetamol for fever and throat lozenges for pain.

- Follow-up:
- 48 Hours Later: Emily's fever subsides, and the rash begins to fade.
- Completion of Antibiotics: The full course of antibiotics is administered to prevent complications like rheumatic fever.
- Question:
- <u>Describe the clinical features that aid in the diagnosis of scarlet fever in Emily.</u>
- <u>Why is it important to promptly diagnose and treat Group A Streptococcus infections, such as scarlet fever, and what potential complications should be considered in the management of this condition in pediatric patients?</u>

Amman, July 29 (Petra) -- A 5-year-old child died after eating contaminated food from a restaurant in Ein al-Basha, northwest of Amman, in a mass poisoning incident, the Ministry of Health announced Wednesday.

Minister of Health Sa'ad Jaber said that the incident left 700 people ill, all of whom were admitted to hospitals, indicating that most of the cases are in good health while a few of them have moderate symptoms.

A statement by the ministry revealed that laboratory tests have confirmed the presence of two types of bacteria in the Shawerma meals, including the enterococcus faecalis bacteria and campylobacter.

"After examining the restaurant, it was found that the cooling unit inside the shawarma cooking room was found non-functional, and that the preparation process takes place outside in an unhealthy environment and without adhering to the safety measures" the statement added.

Further reading:

- Jawetz, Melnick & Adelberg's Medical Microbiology, 26th edition-
  Section 3: Bacteriology-
  Chapter 14: The Streptococci, Enterococci, and Related Genera

- Murray - Medical Microbiology 8th Edition
  Section 4: Bacteriology
  Chapter 19: STREPTOCOCCUS AND ENTEROCOCCUS