Haemophilus influenzae

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HISTORICAL BACKGROUND

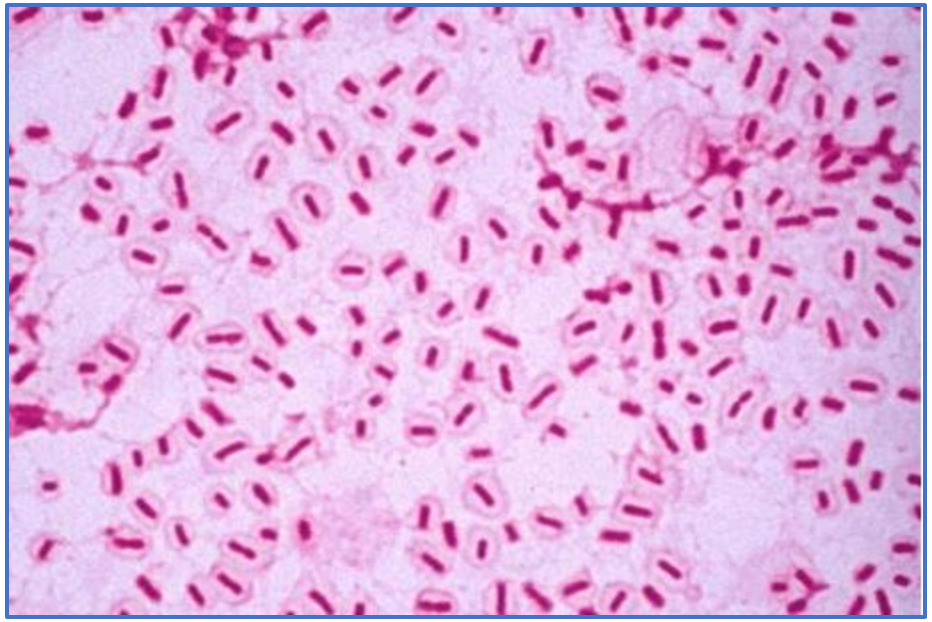
- **Discovery and Naming**
  - 1892: Richard Pfeiffer isolated the organism during influenza pandemic
  - Mistakenly thought to be the cause of influenza (hence the name "influenzae")
  - 1933: Influenza virus discovered - cleared the confusion
  - Despite the misnomer, the name persisted
- **Key Historical Fact:**
  - First free-living organism to have entire genome sequenced (1995)
  - Genome size: 1,830,138 base pairs

General characters

- Haemophilus influenzae are exclusive human bacteria found on the mucous membrane of the upper respiratory tract in humans and can live on dry hard surfaces for up to 12 days.
- Most strains of H. influenzae are **opportunistic** pathogens; they usually live in their host without causing disease, but cause problems only when other factors (such as a viral infection, reduced immune function or chronically inflamed tissues, e.g. from allergies) create an opportunity

MICROBIOLOGY - MORPHOLOGY

- **Microscopic Characteristics:**
  - **Gram stain:** Gram-negative coccobacillus
  - **Size:** Small (1 µm × 0.3 µm)
  - **Shape:** Pleomorphic (variable shapes)
  - **Motility:** Non-motile
  - **Spores:** Non-spore forming
  - **Oxygen requirement:** Facultative anaerobe, capnophilic



- **The Name "Haemophilus" = "Blood-loving"**
  - Requires blood-derived growth factors

GROWTH REQUIREMENTS

- Fastidious, requiring factors V (nicotinamide adenine dinucleotide [NAD]) and X (hematin) found in erythrocytes and are, therefore, grown on media enriched with lysed blood or blood products.
- **Essential Growth Factors:**
  1. **Factor X (Hemin)** - Heat-stable → Required for cytochrome synthesis
  2. **Factor V (NAD (Nicotinamide adenine dinucleotide))** - Heat-labile → Coenzyme for oxidation-reduction reactions

GROWTH REQUIREMENTS

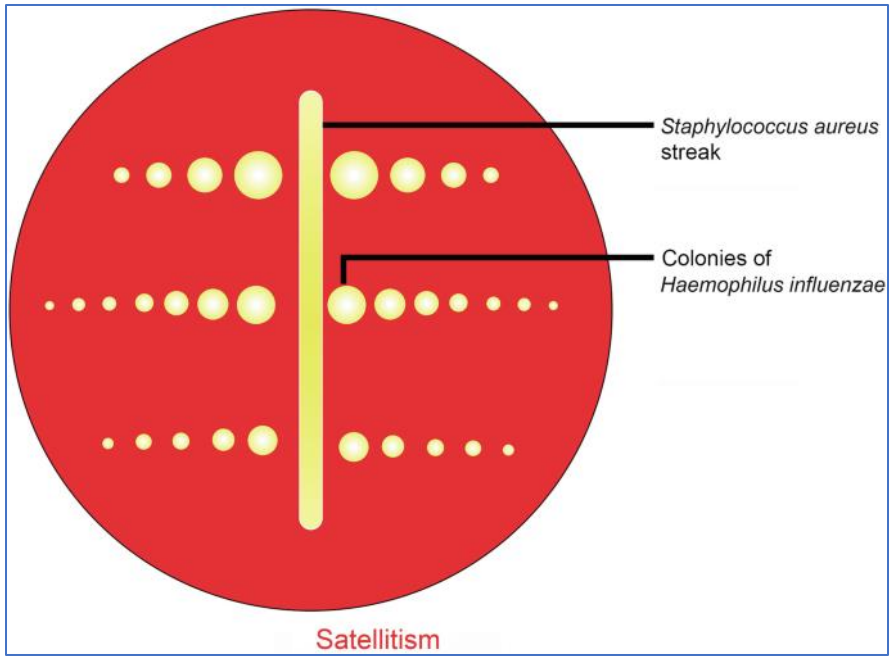
- **Culture Media:**
  - **Chocolate agar** (heated blood agar) ✓
  - Blood agar alone ✗ (NAD trapped inside RBCs)
  - **Satellitism phenomenon:** Growth on Blood agar around ***S. aureus*** colonies
    - *S. aureus* lyses RBCs, releasing Factor V
  - Nutrient agar alone ✗
    - Can grow in the presence of X & V factors

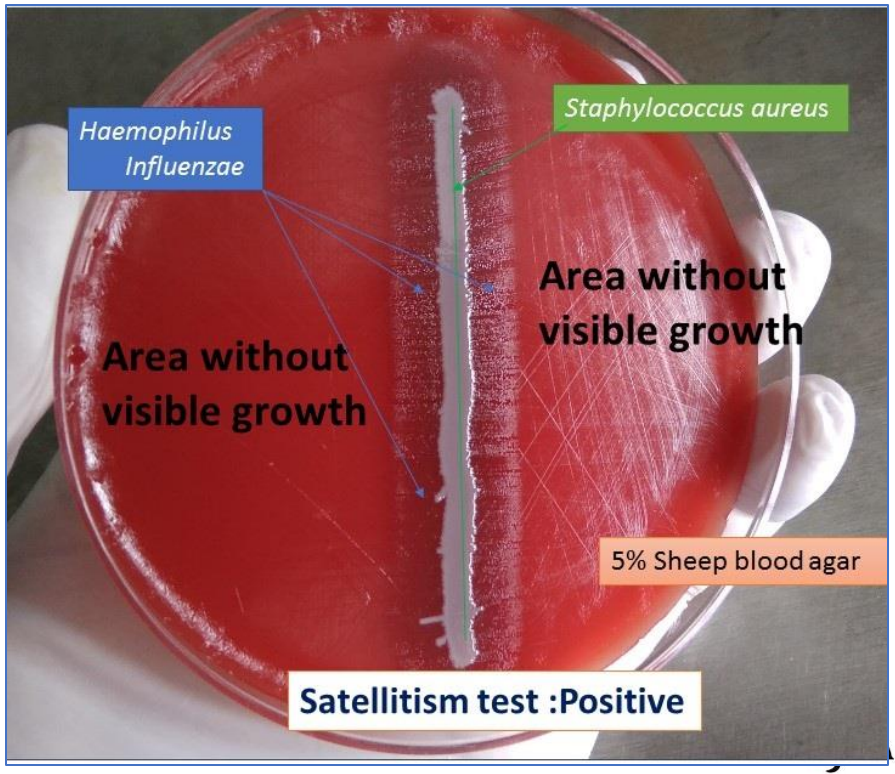
chocolate agar

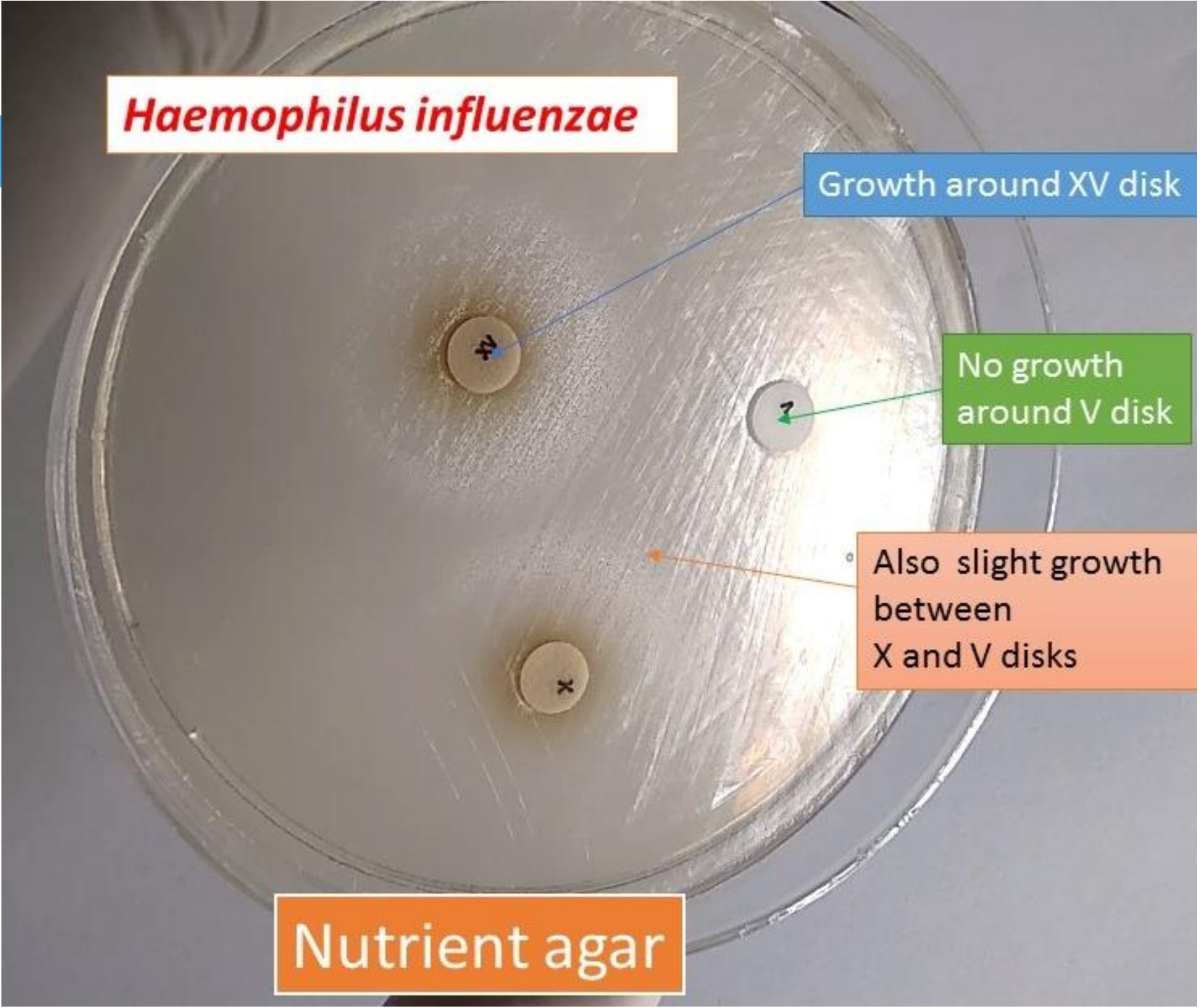
Principle of Satellitism Test

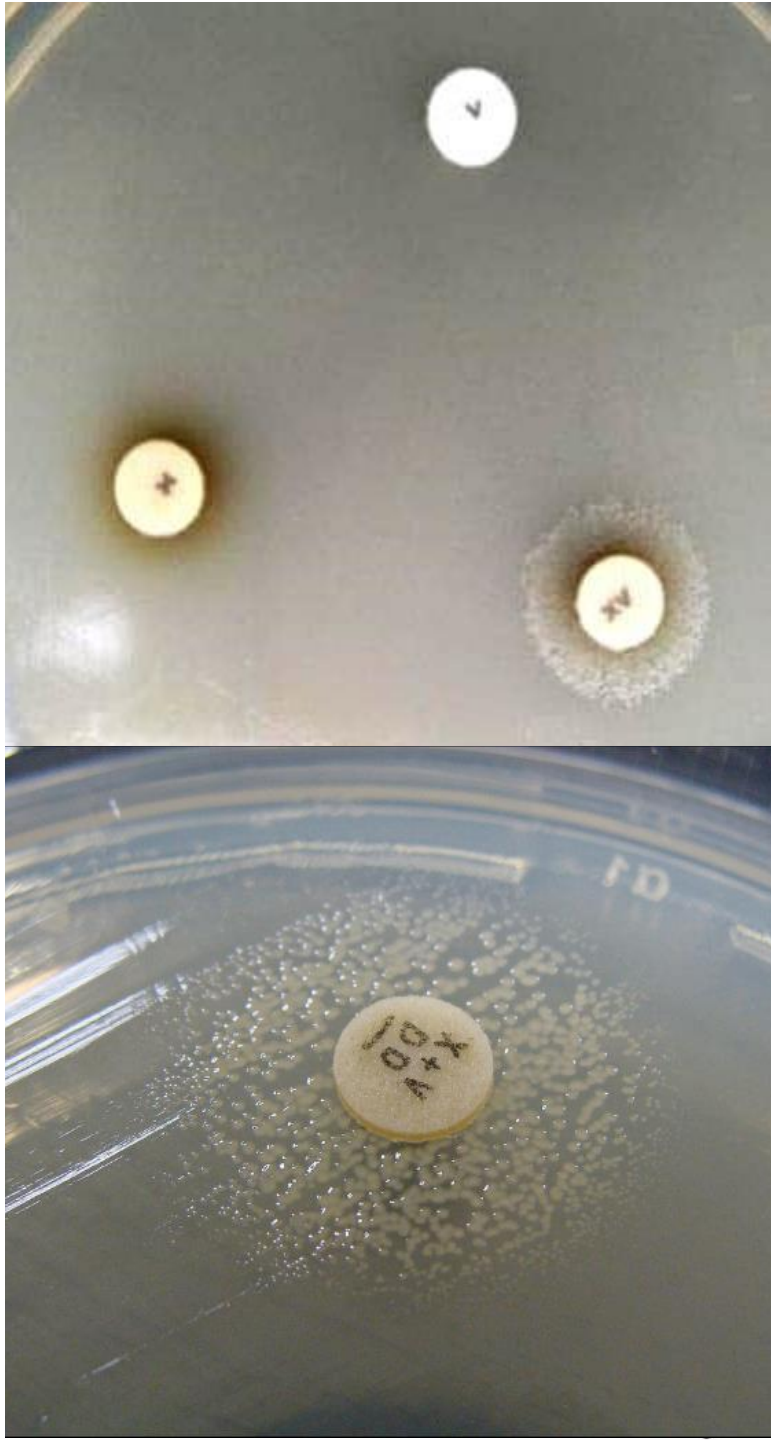
Blood agar medium provides only an X-factor, but for obtaining a V-factor, the erythrocytes present in the blood agar must be hemolyzed. *H. influenzae* can neither hemolyze the blood nor grow without the V-factor, so H. influenzae alone can't grow in a blood agar medium.

Staphylococcus aureus is Beta-hemolytic, and its presence in the blood agar medium makes V-factor (NAD) available in the medium. Hence, H. influenzae can grow in the vicinity of S. aureus colonies in the blood agar medium. This phenomenon is called 'Satellitism'.

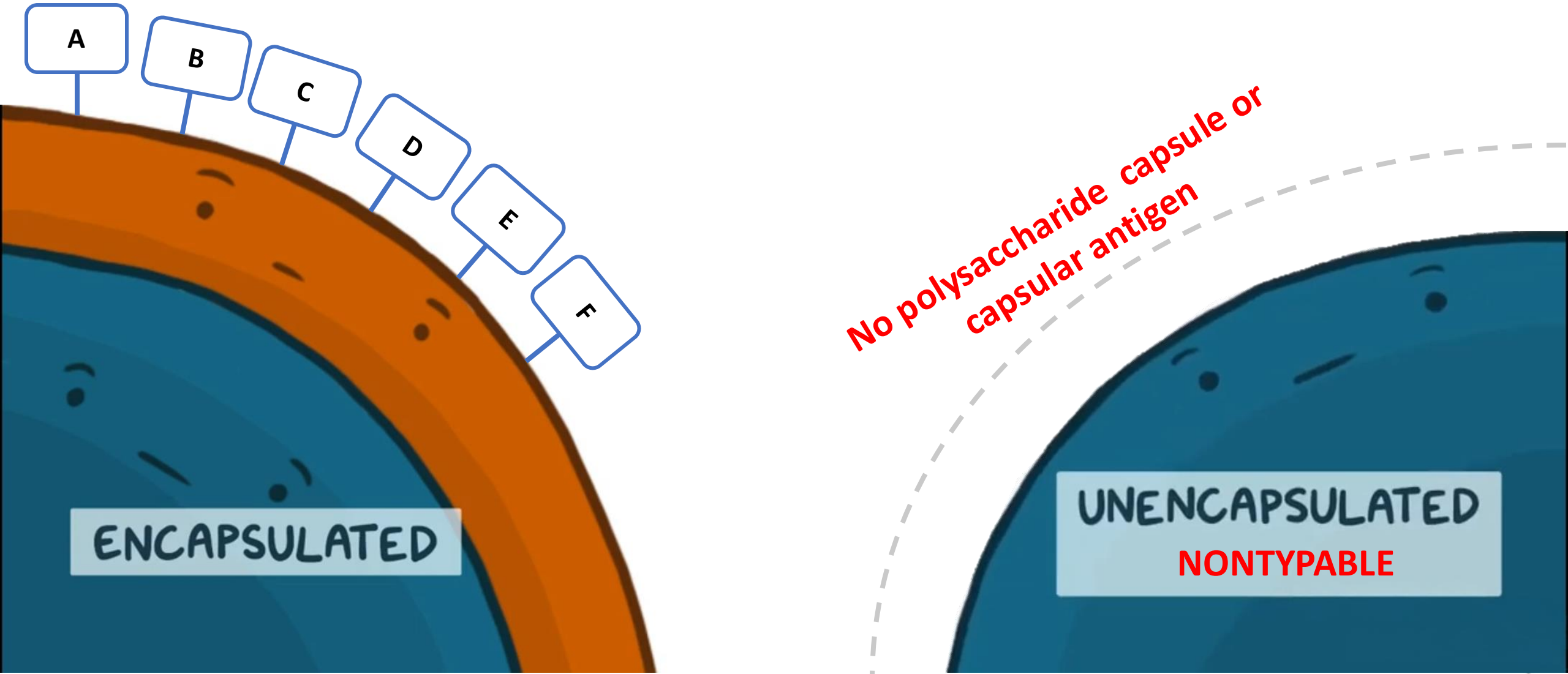





Haemophilus influenzae
Growth around XV disk
No growth around V disk
Also slight growth between X and V disks
Nutrient agar



Strains that cause diseases in humans are Hib and nontypable



CLASSIFICATION

- **Based on Capsular Polysaccharide:**
  - **Encapsulated Strains** (Typeable):
    - 6 serotypes: a, b, c, d, e, f
    - Distinguished by different capsular polysaccharides
    - **Type b (Hib):** Most virulent historically
  - **Non-encapsulated Strains:**
    - **Nontypeable H. influenzae (NTHi)**
    - No capsule
    - Do not agglutinate with antisera
    - Currently more common cause of disease

EPIDEMIOLOGY - PRE-VACCINE ERA

- **Before Hib Vaccine (1980s):**
- **United States:**
  - ~20,000 cases/year of invasive Hib disease
  - Incidence: 40-100 per 100,000 children <5 years
  - **Most common cause** of bacterial meningitis in children 6 months - 2 years
- **Global Burden:**
  - 3 million cases of serious illness annually
  - 386,000 deaths/year (mostly children <5 years)
  - Mainly meningitis and pneumonia

EPIDEMIOLOGY - POST-VACCINE ERA

- **After Hib Vaccine Introduction (1990s):**
- **Dramatic Impact:**
  - >99% reduction in invasive Hib disease
  - By 2016: Only 30 cases in children ≤5 years in US
- **Current Epidemiology:**
  - **Emerging serotypes:** Hia, Hif more prevalent
  - **NTHi:** Now predominant cause of disease
  - High-risk groups:
    - Immunocompromised patients
    - Asplenic patients
    - Adults >65 years

TRANSMISSION & RESERVOIR

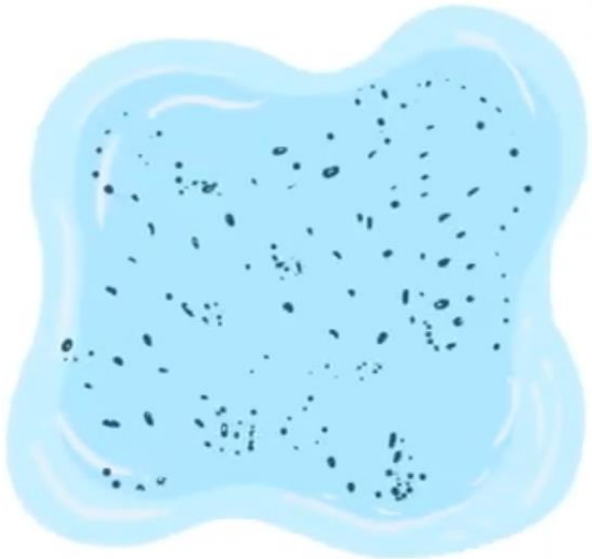
- **Reservoir:**
  - **HUMANS ONLY** - no animal reservoir
- **Transmission:**
  - **Respiratory droplets** (coughing, sneezing)
  - **Direct contact** with respiratory secretions
  - Close contact required (household, daycare)
- **Colonization Rates:**
  - 3-5% of children are colonized with Hib (encapsulated) while 20-80% of healthy children colonized with NTHi
  - Hib colonization now rare in vaccinated populations
- **Incubation Period:**
  - - Variable, estimated **2-4 days**

VIRULENCE FACTORS

1. **Capsular Polysaccharide (PRP)**
   - **Polyribosyl ribitol phosphate** (in type b)
   - Anti-phagocytic properties
   - Allows penetration through epithelial/endothelial barriers
   - Essential for invasiveness
2. **Pili & Adhesins**
   - Facilitate attachment to respiratory epithelium
3. **IgA1 Protease**
   - Cleaves secretory IgA
   - Enables mucosal colonization

VIRULENCE FACTORS (Continued)

4. **Lipopolysaccharide (LPS/Endotoxin)**
   - Triggers inflammatory cascade
   - Causes tissue damage in meningitis
5. **Outer Membrane Proteins (OMPs)**
   - Immune evasion
   - Iron acquisition
6. **Biofilm Formation (NTHi)**
   - Chronic infections (otitis media, COPD)
   - Increased antibiotic resistance



PATHOGENESIS - INVASIVE DISEASE

- **Sequential Steps:**

1. **Colonization** of nasopharynx
   ↓
2. **Mucosal invasion**
   - Capsule prevents phagocytosis
   - IgA protease aids penetration
   ↓
3. **Bloodstream invasion** (Bacteremia)
   - Capsule resists complement-mediated lysis
   ↓
4. **Hematogenous spread** to:
   - Meninges → Meningitis
   - Epiglottis → Epiglottitis
   - Joints → Septic arthritis
   - Lungs → Pneumonia

PATHOGENESIS - NON-INVASIVE DISEASE

- **Local Mucosal Infections (mainly NTHi):**
- **Mechanisms:**
  - Local inflammation without invasion
  - Impaired mucociliary clearance
- **Common Sites:**
  - Middle ear → Otitis media
  - Sinuses → Sinusitis
  - Bronchi → Bronchitis/COPD exacerbations
  - Conjunctiva → Conjunctivitis
- **Predisposing Factors:**
  - Viral upper respiratory infections, Smoking, allergies, Anatomical abnormalities, Immunodeficiency

CLINICAL MANIFESTATIONS - INVASIVE

1. **MENINGITIS (Most serious)**
   - **Age:** 6 months - 2 years (historically)
   - **Symptoms:** Fever, headache, neck stiffness, altered consciousness
   - **Sequelae:** Deafness (15-30%), learning disabilities, seizures
   - **Mortality:** 3-6% despite treatment
2. **BACTEREMIA/SEPTICEMIA**
   - High fever, chills
   - May lead to septic shock
3. **EPIGLOTTITIS**
   - Life-threatening emergency
   - Drooling, stridor, dysphagia, **"thumb sign"** on X-ray
   - Risk of airway obstruction → requires intubation/tracheostomy

CLINICAL MANIFESTATIONS - INVASIVE (Continued)

4. **PNEUMONIA**
   - More common in adults
   - Often with underlying lung disease
   - Lobar or bronchopneumonia pattern
   - May be complicated by empyema
5. **SEPTIC ARTHRITIS**
6. **CELLULITIS**
   - Head and neck region in children
   - Periorbital/buccal cellulitis

CLINICAL MANIFESTATIONS - NON-INVASIVE

1. **OTITIS MEDIA**
   - **Most common** NTHi infection
   - 2nd most common cause after *S. pneumoniae*
   - Earache, fever, hearing loss
   - Recurrent/chronic cases in children
2. **SINUSITIS**
   - Acute or chronic
   - Facial pain, purulent discharge
   - All age groups
3. **CONJUNCTIVITIS**
   - Purulent discharge
   - Often mild, self-limiting

CLINICAL MANIFESTATIONS - NON-INVASIVE (Continued)

4. **ACUTE EXACERBATIONS OF COPD**
   - Common in adults with chronic lung disease
   - Increased purulent sputum
   - Worsening dyspnea
   - May require hospitalization
5. **BRONCHITIS**
   - Both acute and chronic forms
   - Productive cough
6. **Special Populations:**
   - **Neonates:** Sepsis, pneumonia (usually biotype IV)
   - **Pregnant women:** Puerperal sepsis (rare)
   - **Immunocompromised:** More severe, disseminated disease

LABORATORY DIAGNOSIS - SPECIMENS

Specimen Collection:

- **Invasive Disease:**
  - **Blood** → Blood cultures
  - **CSF** → Lumbar puncture
  - **Joint fluid** → Arthrocentesis
  - **Pleural fluid** → Thoracentesis
  - **Other sterile sites**
- **Non-invasive Disease:**
  - **Sputum** → Respiratory infections
  - **Middle ear fluid** → Tympanocentesis (rarely done)
  - **Sinus aspirate**
  - **Conjunctival swabs**
- **Important:**
  - Transport rapidly (organism is fastidious)
  - Maintain viability (dies quickly outside host)

LABORATORY DIAGNOSIS - MICROSCOPY

- **Gram Staining:**
  - **Appearance**: Small gram-negative coccobacilli
  - **Pleomorphic**: Variable shapes

LABORATORY DIAGNOSIS - CULTURE

- **Culture Media:**
  - **Chocolate agar** (primary medium)
  - **Incubation:** 35-37°C, 5-10% CO_2, 24-48 hours
- **Colony Characteristics:**
  - Small, grey, translucent colonies
  - Smooth, convex
  - Distinctive musty odor
- **Satellitism Test:**
  - Streak on blood agar with *S. aureus*
  - Colonies grow around *S. aureus* (provides Factor V)

LABORATORY DIAGNOSIS - IDENTIFICATION

- **Growth Factor Requirements Test:**
  - **X strip, V strip, XV strip** on nutrient agar
  - **H. influenzae:** Requires both X and V
  - Definitive identification
- **Biochemical Tests:**
  - Oxidase: Positive
  - Catalase: Positive

LABORATORY DIAGNOSIS - SEROTYPING

- **1. Slide Agglutination:**
  - Using specific antisera (a-f)
  - Identifies capsular type
  - Quick and simple
- **2. Quellung Reaction:**
  - Capsular swelling with specific antisera
  - Direct from clinical specimens
- **3. PCR-based Capsular Typing:**
  - Molecular method
  - More sensitive and specific
  - Can detect capsular genes
- **4. Whole Genome Sequencing:**
  - Most comprehensive
  - Research/epidemiological tool

TREATMENT

- Cephalosporines as cefotaxime or ceftriaxone.

HIB VACCINE - HISTORY & IMPACT

- **Vaccine Development:**
- **1985: First Hib vaccine (pure polysaccharide PRP)**
  - Poor immunogenicity in children <2 years
  - No immunologic memory
- **1990: Conjugate vaccine introduced**
  - PRP linked to **protein carrier**
  - Converts T-independent → T-dependent response
  - Excellent immunogenicity in infants
  - Long-lasting immunity

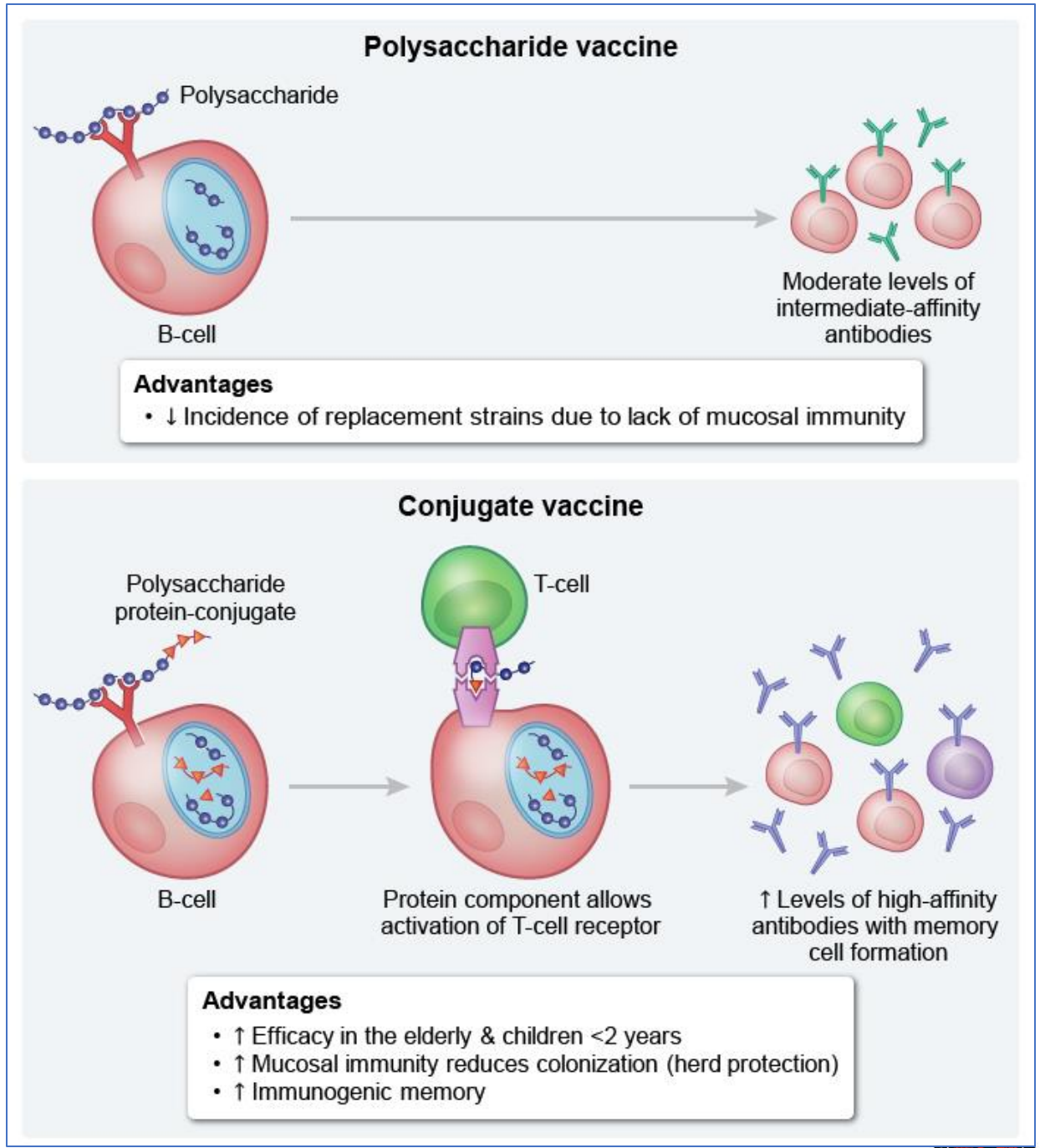
Impact:

>99% reduction in invasive Hib disease

One of most successful vaccines in history

Eliminated Hib meningitis in vaccinated populations

Immunogenicity of polysaccharide vs conjugate vaccines		
Immunology	**Polysaccharide vaccine**	**Polysaccharide-protein conjugate vaccine**
Response type	B cell	B cell & T cell
Memory cell response	No	Yes
Relative duration of immunity	Short	Long
Immunogenicity in infancy	No	Yes



HIB VACCINE - TYPES

- **Available Conjugate Vaccines:**
- **Protein Carriers:**
  - 1. **PRP-T:** the conjugated protein is tetanus toxoid
  - 2. **PRP-OMP:** *N. meningitidis* outer membrane protein
  - 3. **PRP-CRM197:** Diphtheria toxin mutant
- **Combination Vaccines:**
  - DTaP-IPV-Hib (Pentacel®)
  - DTaP-IPV-Hib-HepB (Vaxelis®)
  - **Hib-MenCY:** Combined Hib and meningococcal
- **All are equally effective**
  - Choice based on schedule and availability
- The vaccine is given at 2,4,6 months and at 12-15 month.

HIB Passive immunity

Passive immunity from maternal antibodies protects infants until vaccination. The peak incidence is at ages **6-18 months** when children lose maternal antibodies but have not yet been vaccinated.

HIB VACCINE - SPECIAL POPULATIONS

- **High-Risk Groups Requiring Additional Doses:**
- **Indications for Additional Vaccination:**
  - Asplenia (functional or anatomical)
  - Sickle cell disease
  - HIV infection
  - Immunoglobulin deficiency
  - Hematopoietic stem cell transplant
  - Chemotherapy/radiation therapy

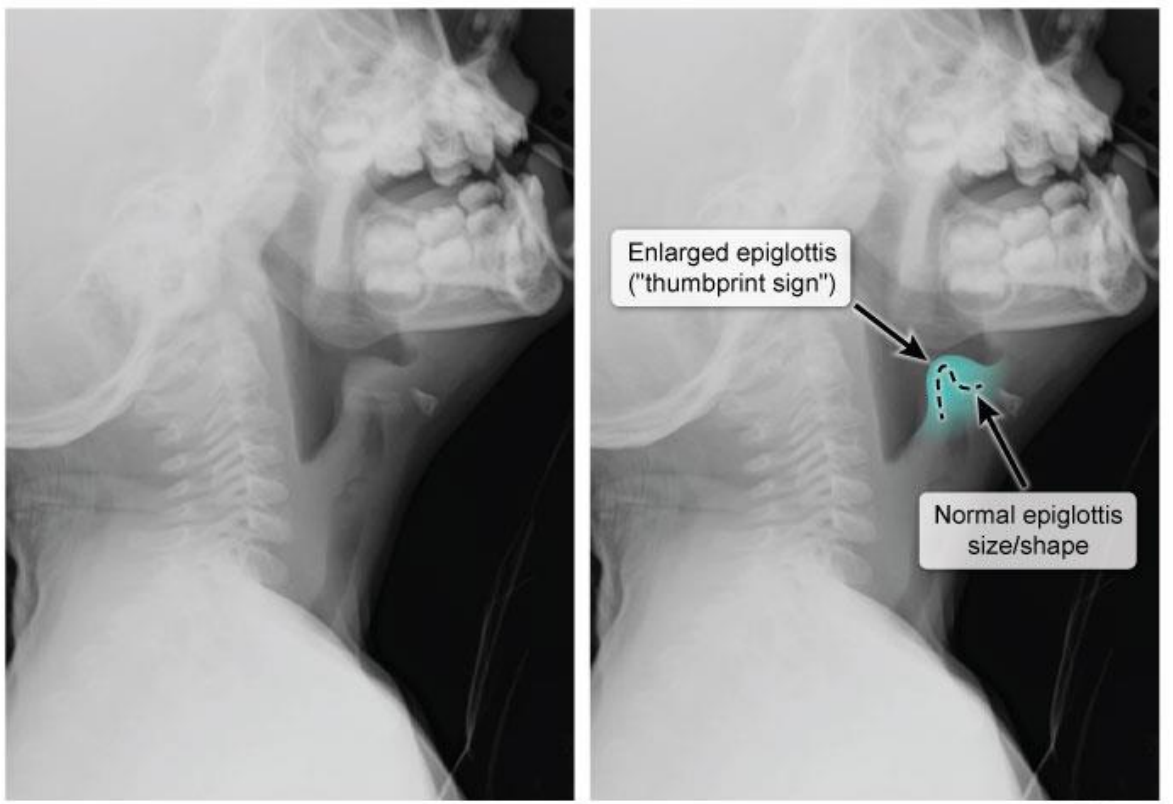
CURRENT CHALLENGES

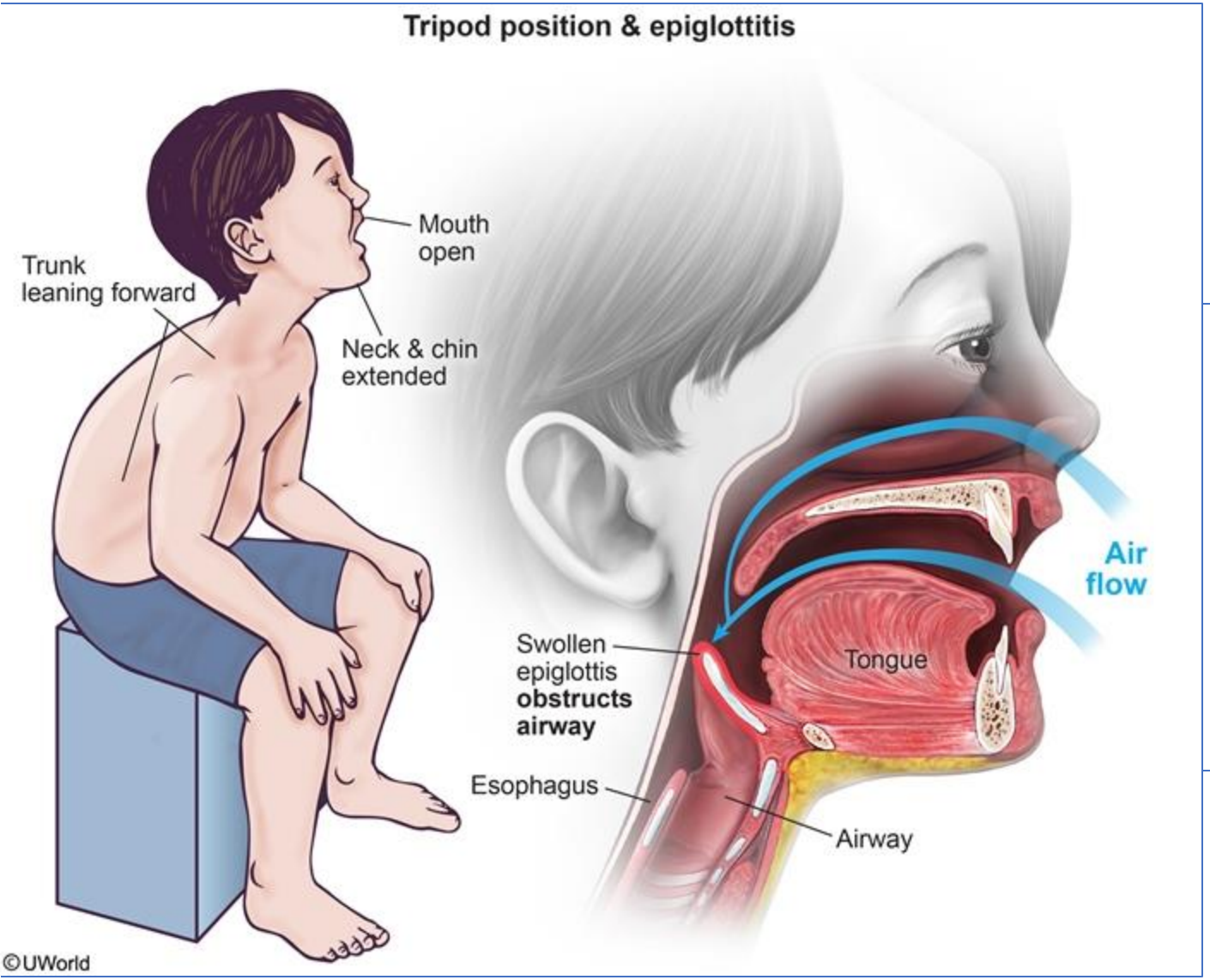
- **1. Emerging Serotypes:**
  - **Hia (type a):** Increasing in Alaska Native/First Nations populations
  - **Hif (type f):** Cases reported globally
  - Current vaccine doesn't protect against these
- **2. Nontypeable H. influenzae (NTHi):**
  - Now predominant cause of disease
  - No available vaccine
  - Causes:
    - Otitis media (most common)
    - COPD exacerbations
    - Occasional invasive disease
- **3. Antibiotic Resistance:**
  - β-lactamase production (13-50%)
  - BLNAR strains (>50% in some Asian countries)
  - Limits treatment options

CURRENT CHALLENGES (Continued)

- **4. Global Vaccine Coverage:**
  - Not all countries have Hib vaccine in programs
  - Coverage varies:
    - High-income: >90%
    - Low-income: <70%
  - Continued burden in developing nations
- **6. Unvaccinated Populations:**
  - Vaccine hesitancy
  - Underserved communities
  - Risk of outbreaks

Epiglottitis	
Microbiology	• *Haemophilus influenzae* type b (Hib)
Clinical features	• Distress (tripod position, sniffing position, stridor) • Dysphagia, dysphonia • Drooling • High fever
X-ray	• **"Thumb sign"** (enlarged epiglottis)
Management	• Endotracheal intubation • Antibiotics
Prevention	• Immunization against Hib



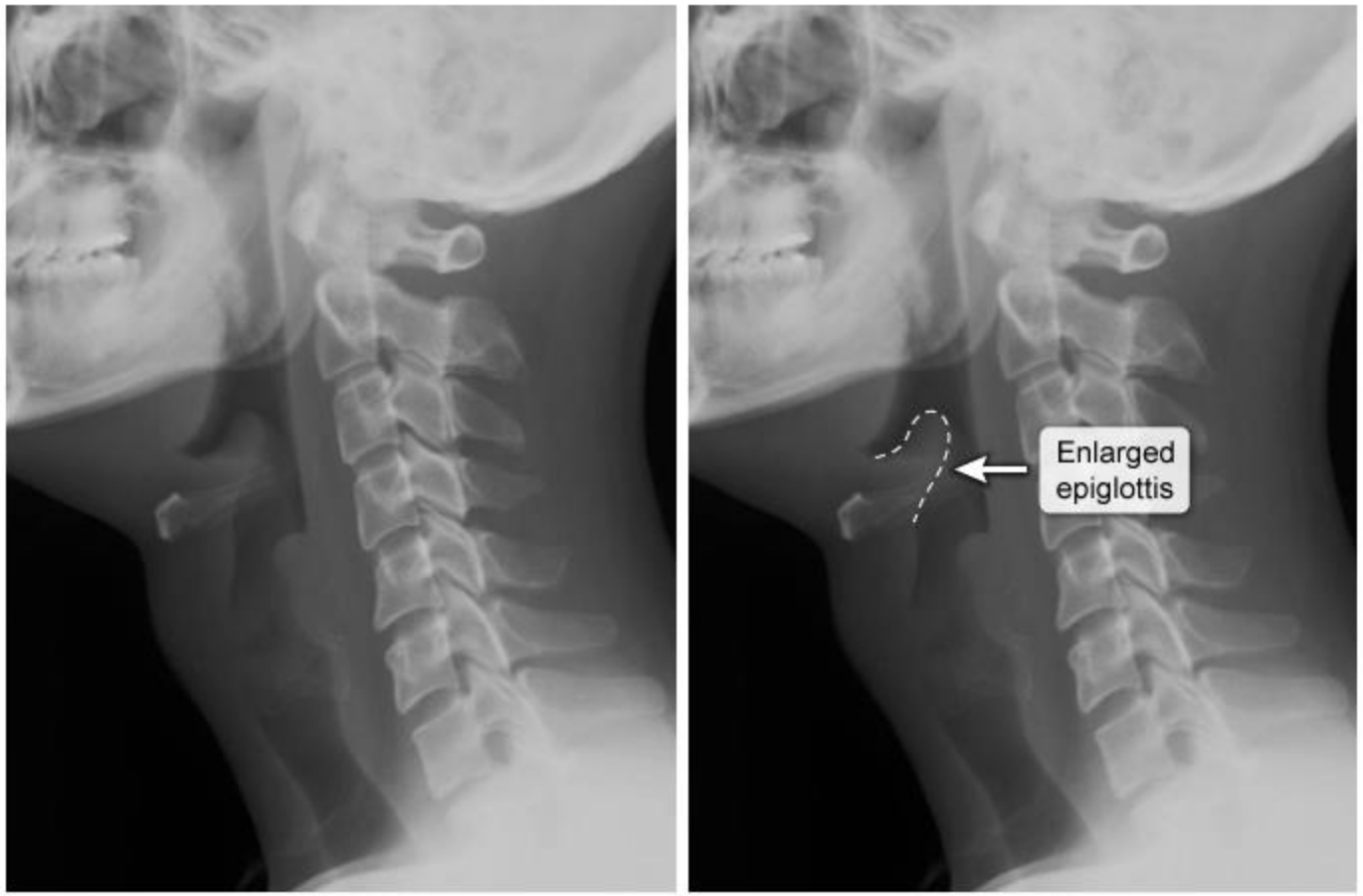


Patients classically assume **tripod positioning** (ie, leaning forward with the neck extended) to maximize airflow

Tripod positioning (extending neck and chin to the sniffing position) pulls the tongue forward to partially open the laryngeal airway.

Edema of the epiglottis can cause laryngeal obstruction that leads to inspiratory stridor.

Epiglottitis



Epiglottitis