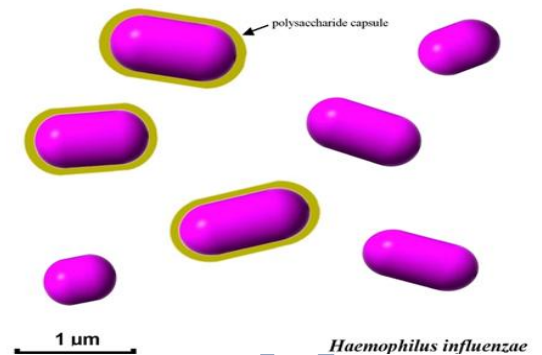


General features

- 1-Small •
- 2-Non-motile •
- 3-Gram-negative rods •
- 4-Transmitted via respiratory droplets, or direct contact with contaminated secretions •
- 5-Normal flora of the human respiratory tract and oral cavity. •
- 6-Growth require either V(NAD) or X(hemin ) factors or both . •

Haemophilus species of clinical importance1. *H. influenzae*

-type b is an important human pathogen

2. *H. ducreyi*

-sexually transmitted pathogen (chancroid)

3. Other *Haemophilus* are normal flora

- *H. parainfluenzae* – pneumonia & endocarditis

- *H. aphrophilus* – pneumonia & endocarditis

- *H. aegyptius* – pink eye (purulent conjunctivitis)

Public Typing based on capsule polysaccharide a → f •

- Polyribose-ribitol phosphate (PRP) capsule (type b) •
- Nonencapsulated (nontypeable) organisms are part of normal flora of the respiratory tract •
- 95% of invasive disease caused by type b •
- Health Aspects- *H. influenzae*
- H. influenzae* type b incidence has fallen 99% post-vaccine •
- Pre-immunization- Serotype b was the most common invasive species •

Normal habitat

- 1-Haemophilus influenza including biogroup aegypticus.....normal flora of human upper respiratory tract. •
- 2-Haemophilus ducreyi.....not part of normal flora. •
- 3-other homophiles spp. Are also part of normal flora of the upper respiratory tract

## *Haemophilus influenzae*

### Unencapsulated

Responsible for localized infections i.e.

- Otitis media
- Conjunctivitis
- Bronchitis
- Sinusitis



### Capsulated

Responsible for Invasive disease i.e.

- Meningitis
- Septicaemia
- Epiglottitis
- Pneumonia
- Septic arthritis
- Cellulitis

## LABORATORY DIAGNOSIS

### ■ Clinical Specimens

- Blood, sputum, CSF, Joint fluid

### ■ Microscopy

- Gram Stain
- Fluorescent antibody stain

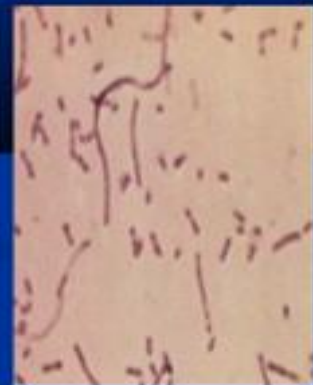
### ■ Culture

- Chocolate Agar (Factor V & X)

### ■ Serology

- Latex agglutination, Counter immunoelectrophoresis

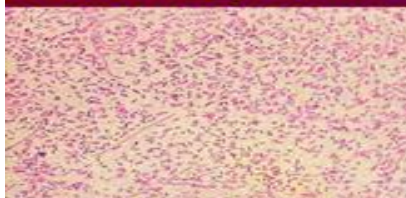
### ■ PCR



## *Haemophilus influenzae*

### Laboratory Diagnosis

- **Gram-stained smear plus culture on chocolate agar.**
- **Growth requires both factors X & V.**
- **Determine serotype** by using **antiserum** in various tests, e.g., latex agglutination.
- **Capsular antigen** can be detected in **serum** or **cerebrospinal fluid**.



**A-specimen**

1-specimen are collected according to the types of infection . •

2-**Haemophilus spp.** are very susceptible to drying and temperature extremes ,therefore, specimens suspected of containing these organisms must be plated immediately if they don't put into a suitable transport medium

3-for recovery of **H.ducreyi** ,from genital ulcer, special measures are necessary because of the bacterium fastidious natures .a cotton swab premoistened with phosphate-buffered saline is then used to collect material from the base of the ulcer and swab should be plated to special selective media within 10 minutes of collection

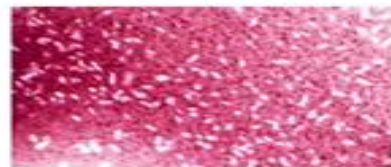
**B-Direct detection methods****directs observation** •

- Gram stain is generally used for the direct detection of Haemophilus in clinical material .However, •  
in some instances the acridine orange stain is used to detect smaller numbers of organisms than may be detected by Gram stain.

-To increase sensitivity of Gram stain of body fluids e.g.csf,the specimen usually are centrifuged ,and the smear is prepared from the resulting pellet .This concentration the sensitivity for 5-10 fold

## Microscopy

- - Gram staining- Haemophilus influenzae - G - rods ( coccoid to filamentous



- Capsule detection in H.influenzae – Burri method,
  - quellung reaction- aplication of specific antiserum (anti a, b, c, d, e or f) to the testing culture and staining sc Burri or in native smear growing – magnification of capsule
  - agglutination with specific antiserum - agglutination and clearing of suspension after application of specific antiserum to the colony on slide

**Appearance on Gram stain**

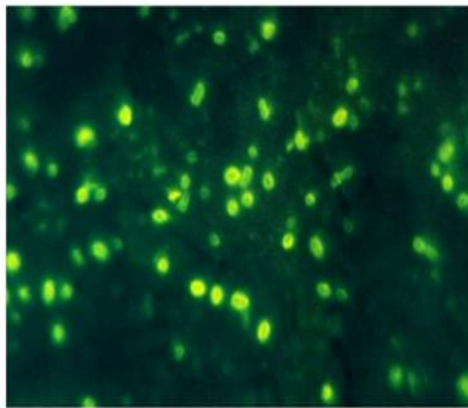
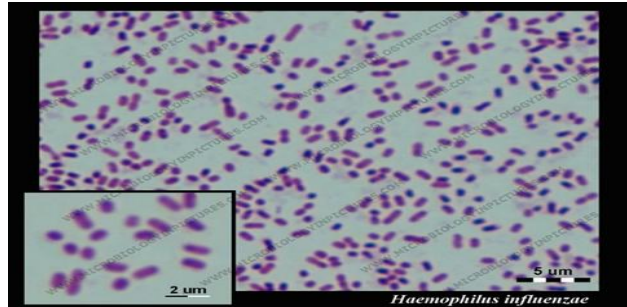
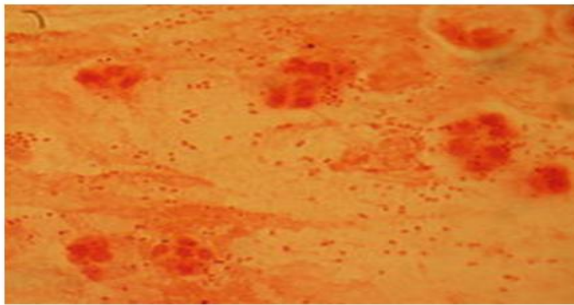
1-Haemophilus spp.stain pink and may be difficult to detect in the pink background of proteinaceous material that is often found •  
in patient specimen.

2-H.influenza appear as coccobacilli or as small rods. •

3-H-influenza biotypes aegypticus usually appear as long ,slender rods •

4-H-haemolyticus are small coccobacilli or short rod. •





H. influenzae in immunofluorescence microscopy

### Antigen detection.

Detection of H. influenzae type b capsular polysaccharide directly in clinical specimens such as csf and urine

Direct antigen detection

- The capsular polysaccharide antigen may be present in CSF in meningitis and in urine in systemic infection.
- Its demonstration by latex particle agglutination is useful in rapid diagnosis.

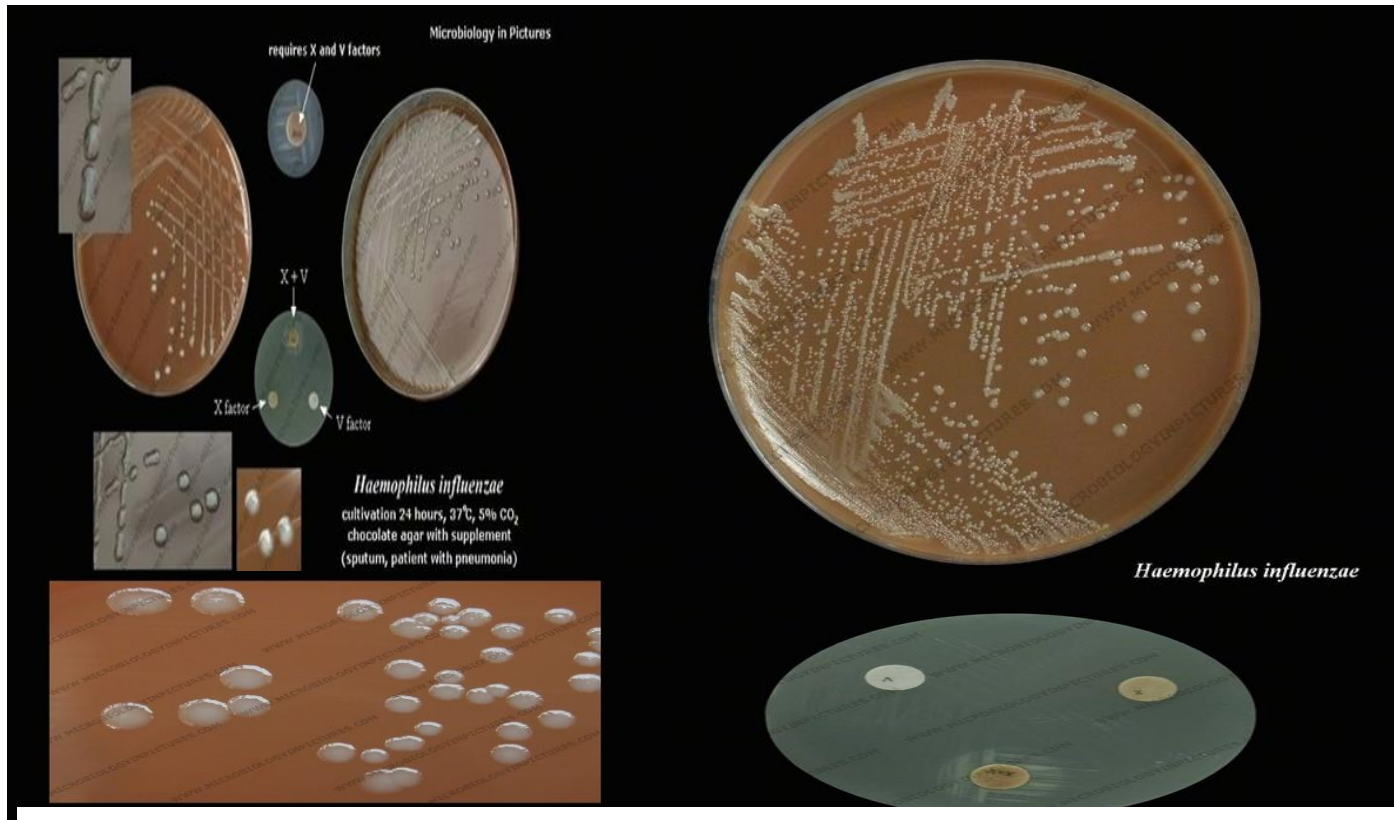
### C-cultivation.

#### 1-Media of choice.

-chocolate agar provides the factors ,that is hemin (x factors) and NAD (V factors) •

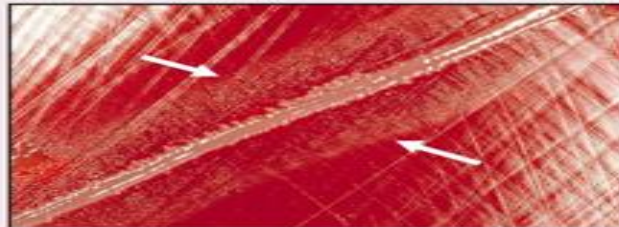
-Most strain will not grow on 5% sheep blood agar , which contain hemin but not NAD. •

-Several bacterial species, including staphylococcus aureus ,produce NAD as metabolic byproduct. so •  
Haemophilus spp .may be seen growing on sheep blood agar very close to colonies of bacteria that can produce v factor ,this phenomenon is known as satellitism



## SATELLITISM

- Some bacteria, such as *S. aureus*, produce V factor and tiny *Haemophilus* can be seen growing on sheep blood agar very close to the bacteria producing the V factor



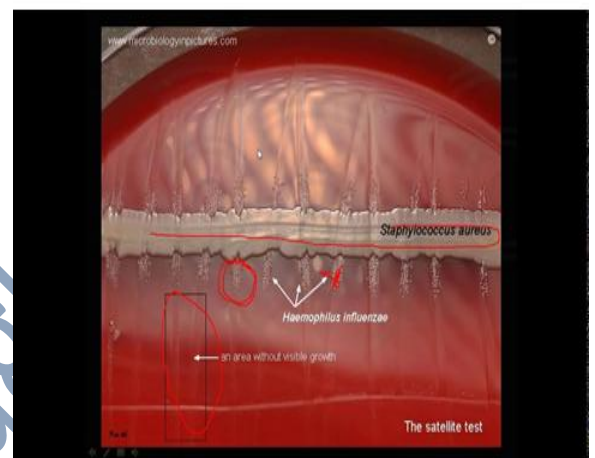
### Station 5



### How to perform satellitism test ??

1- Mix a loopful of suspect *Haemophilus* growth in about 2 ml of sterile physiological saline or sterile peptone water. Make sure none of the chocolate agar medium is transferred.

2- Using a sterile swab, inoculate the organism suspension on a plate of nutrient agar, and a plate of blood agar.



-**Haemophilus ducreyi** :requires special media to grow such media are

1-**Mueller –Hinton-based chocolate agar**: supplemented with 1% isovitalex and 3ug ml vancomycin .The vancomycin inhibits G+ve organism normally colonizing the genital tract

2-**Heart infusion –based agar**. Supplemented with 10%fetal bovine serum and 3ug ml vancomycin .the vancomycin inhibits G+ve organism normally colonizing the genital tract

#### Colonial appearance

1-H.influenzae → uncapsulated strain are small,smooth,and translucent at 24 hour, encapsulated strains from larger, more mucoid colonies, all are Nonhemolytic.

2-H.ducreyi → small ,flat, smooth ,and translucent to opaque at 48-72 hrs.

3-H.aphrophilus → round ,convex with opaque zone near center.



## Diagnosis of *Haemophilus*

### • **Satellitism:**

- Blood agar contains much X factor and little V factor
- *S. aureus* produces V factor
- A lawn of test bacteria is plated onto a blood agar plate
- *S. aureus* is placed on plate & the culture is incubated
- *H. influenzae* will grow in the hemolytic zone of *S. aureus*
- The hemolysis of cells by *S. aureus* releases nutrients
- *H. influenzae* will not grow outside the hemolytic zone of *S. aureus* due to the lack of nutrients in these areas

### • **Capsule swelling:**

- Specific antiserum added to a slide of the organism allows swelling of the bacterial capsule thus permitting rapid diagnosis of *H. influenzae* in sputum

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**Key biochemical and physiological characteristic of *Haemophilus* spp.**

organism	X-factor	V-factor	Beta - haemolysis	Lactose fermentation
<i>H. influenzae</i>	+	+	–	–
<i>H. Influenzae</i> biotypes <i>aegypticus</i>	+	+	–	–
<i>H. haemolytic</i>	+	+	+	–
<i>H. parahaemolytic</i>	–	+	+	–
<i>H. parainfluenzae</i>	–	+	–	–
<i>H. ducreyi</i>	+	–	–	–
<i>H. aphrophilus</i>	–	–	–	+