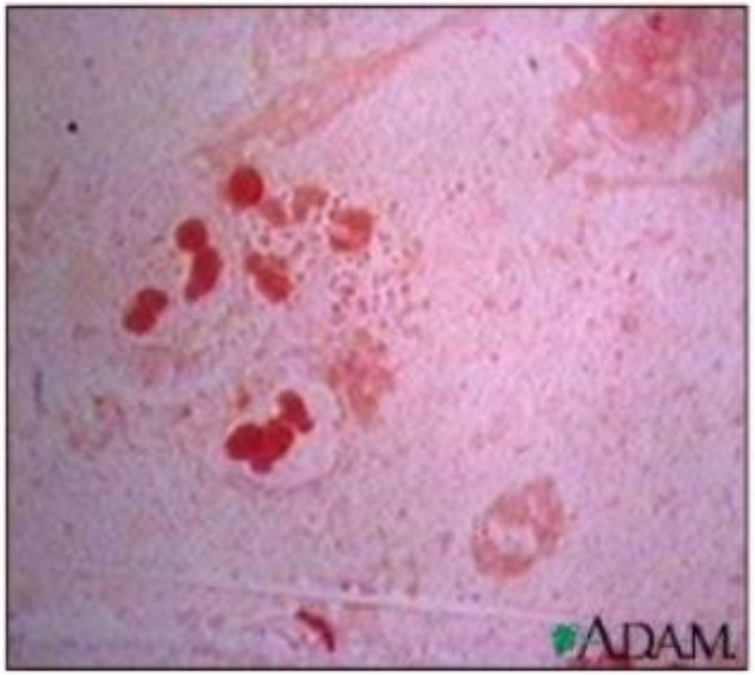
دانشگاه اصفهان
دانشکده علوم و فناوری‌های زیستی، گروه زیست‌شناسی سلولی و مولکولی، آزمایشگاه میکروبیولوژی

آزمایشگاه باکتری شناسی ۲

بررسی خصوصیات ماکروسکوپی و میکروسکوپی و نحوه شناسایی هموفیلوس

Haemophilus Influenza (Pfeiffer Bacillus)

- The size is 3 x 0.3 microns
- Gram negative
- Non Motile
- Non sporing
- Pleomorphic
- Appear as clusters or Coccobacillary forms in infected CSF
- When isolated capsulated
- Stained with Loffler's methylene blue

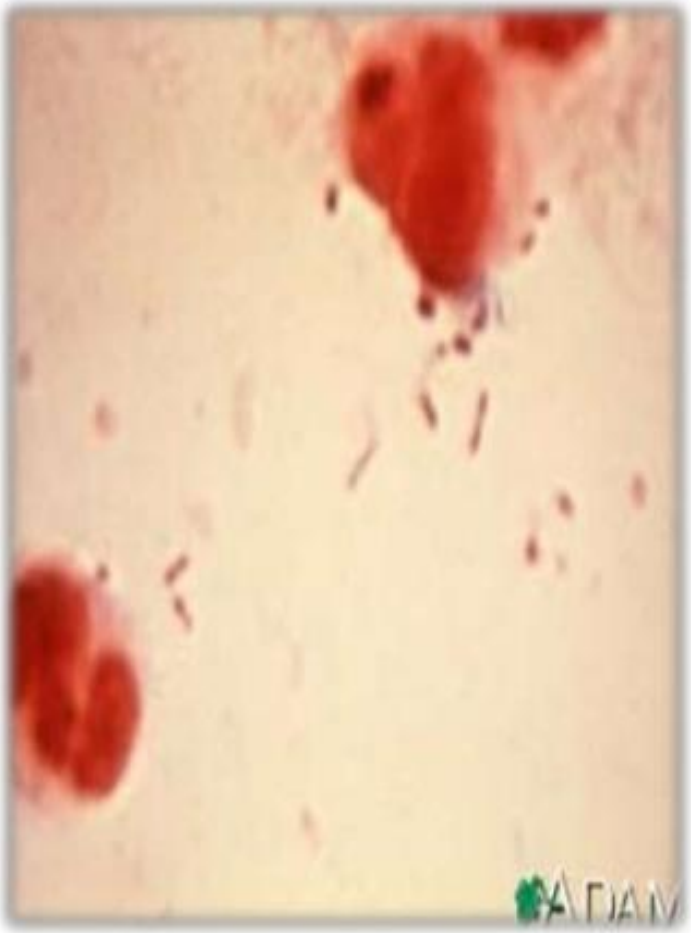


Dr.T.V.Rao MD

8

Genus Haemophilus

- Small, Non motile, Non sporing
- Oxidase test positive
- Pleomorphic
- Gram Negative



Overview- Haemophilus

- Small
- Non-motile
- Gram-negative rods
- Transmitted via respiratory droplets, or direct contact with contaminated secretions
- Normal flora of the human respiratory tract and oral cavity.

Haemophilus species of clinical importance

1. ***H. influenzae***

   -**type b is an important human pathogen**

2. ***H. ducreyi***

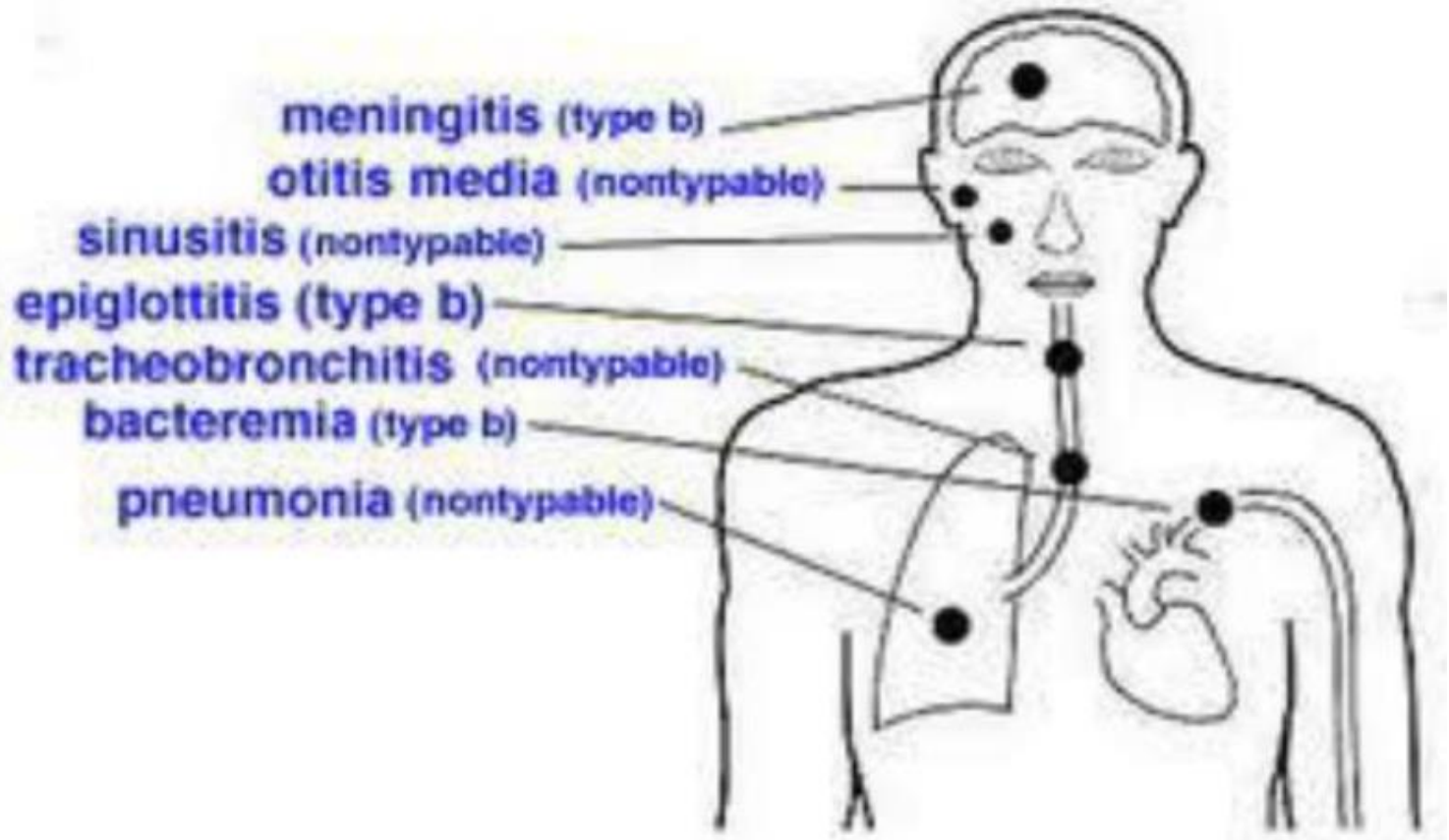
   -**sexually transmitted pathogen (chancroid)**

3. Other *Haemophilus* are normal flora

   - *H. parainfluenzae* – pneumonia & endocarditis
   - *H. aphrophilus* – pneumonia & endocarditis
   - *H. aegyptius* – pink eye (purulent conjunctivitis)

- Scientific classification
- Kingdom: Bacteria
- Phylum: Proteobacteria
- Class:Gamma Proteobacteria
- Order:Pasteurellales
- Family:Pasteurellaceae
- Genus:Haemophilus
- Species:H. influenzae
- Binomial name Haemophilus influenzae

Haemophilus influenzae infections



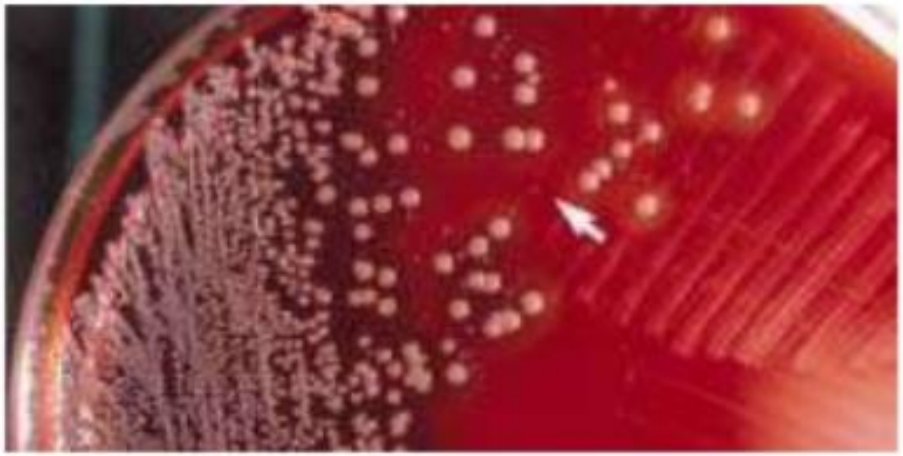
Laboratory Diagnosis

- On Microscopy Gram Negative Pleomorphic organisms are seen
- Capsulated with polysaccharide antigen in CSF
- Urine also detect Antigen

Haemophilus Influenza

- Aerobic gram-negative bacteria
- Polysaccharide capsule
- Six different serotypes (a-f) of polysaccharide capsule
- 95% of invasive disease caused by type b (Hib)

Haemophilus Species



H. influenzae satellitism around and between the large, white, hemo lytic staphylococci

Haemophilus species require hemoglobin for growth:

X-factor (hemin): Heat-stable substance

V-factor (NAD): Heat- labile, coenzyme I, nicotinamide adenine dinucleotide, found in blood or secreted by certain organisms

Cultural Characteristics

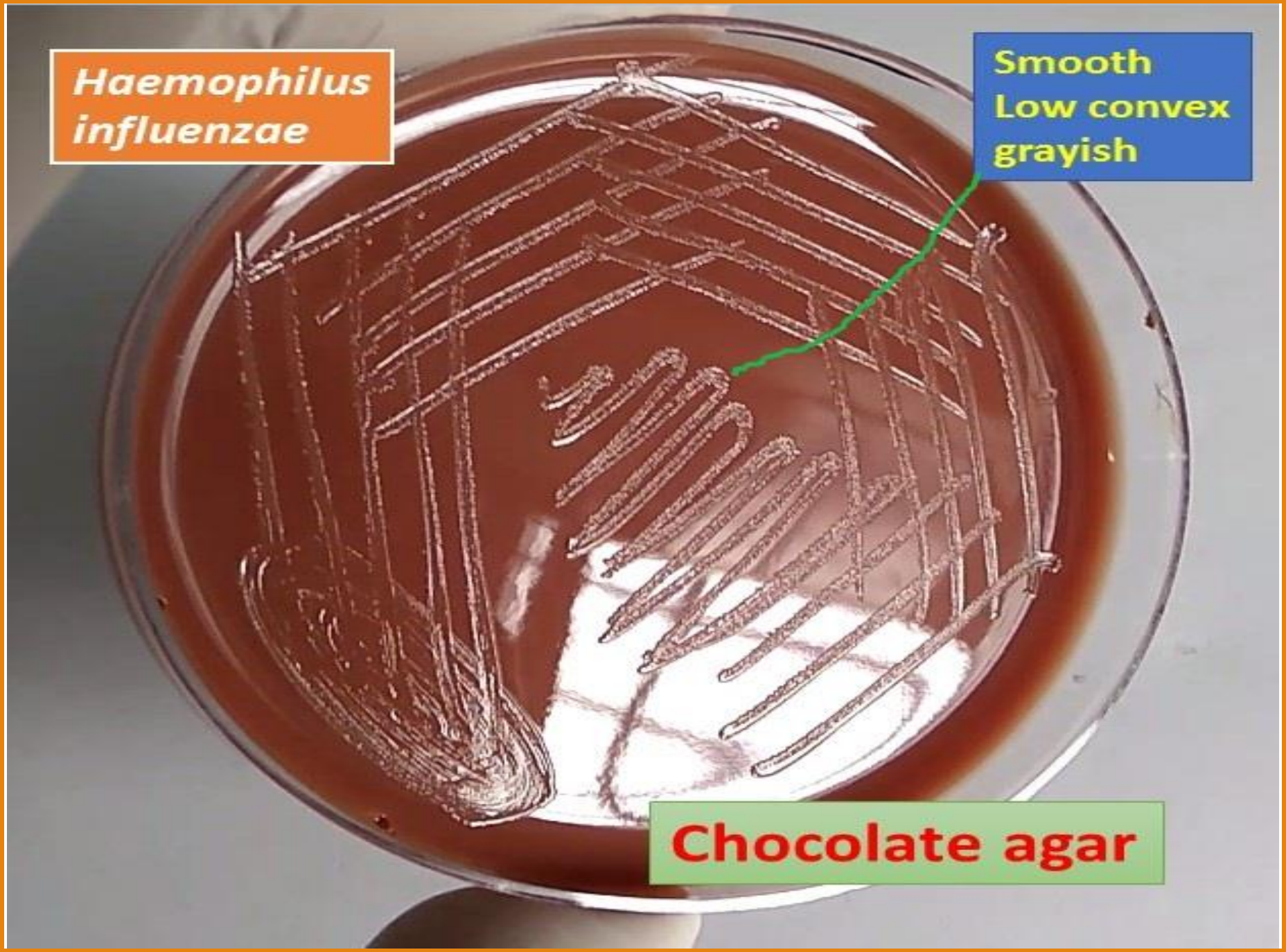
- Fastidious growth requirements
- Factors X and V are essential for growth
- X is Hemin heat stable
- Porphyrins for synthesis of Cytochromes
- V factor Coenzyme Nicotinamide adenine dinucleotide or NAD phosphate acts as hydrogen acceptor
- Aerobic 37^0c
- **Grows in Blood agar**

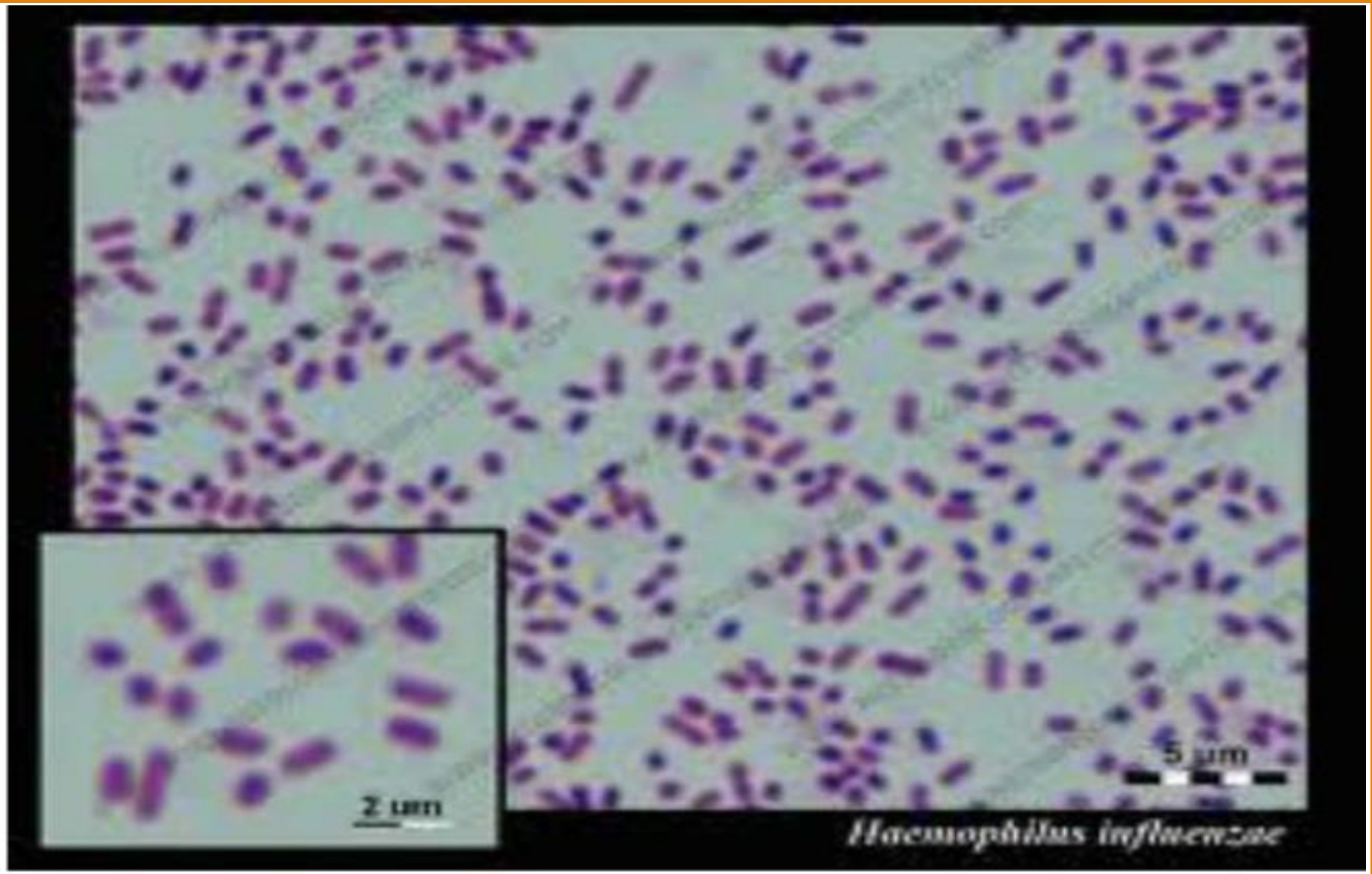
Resistance

- Heating at 55^0c for 30mt destroys
- Drying and Disinfectants destroy

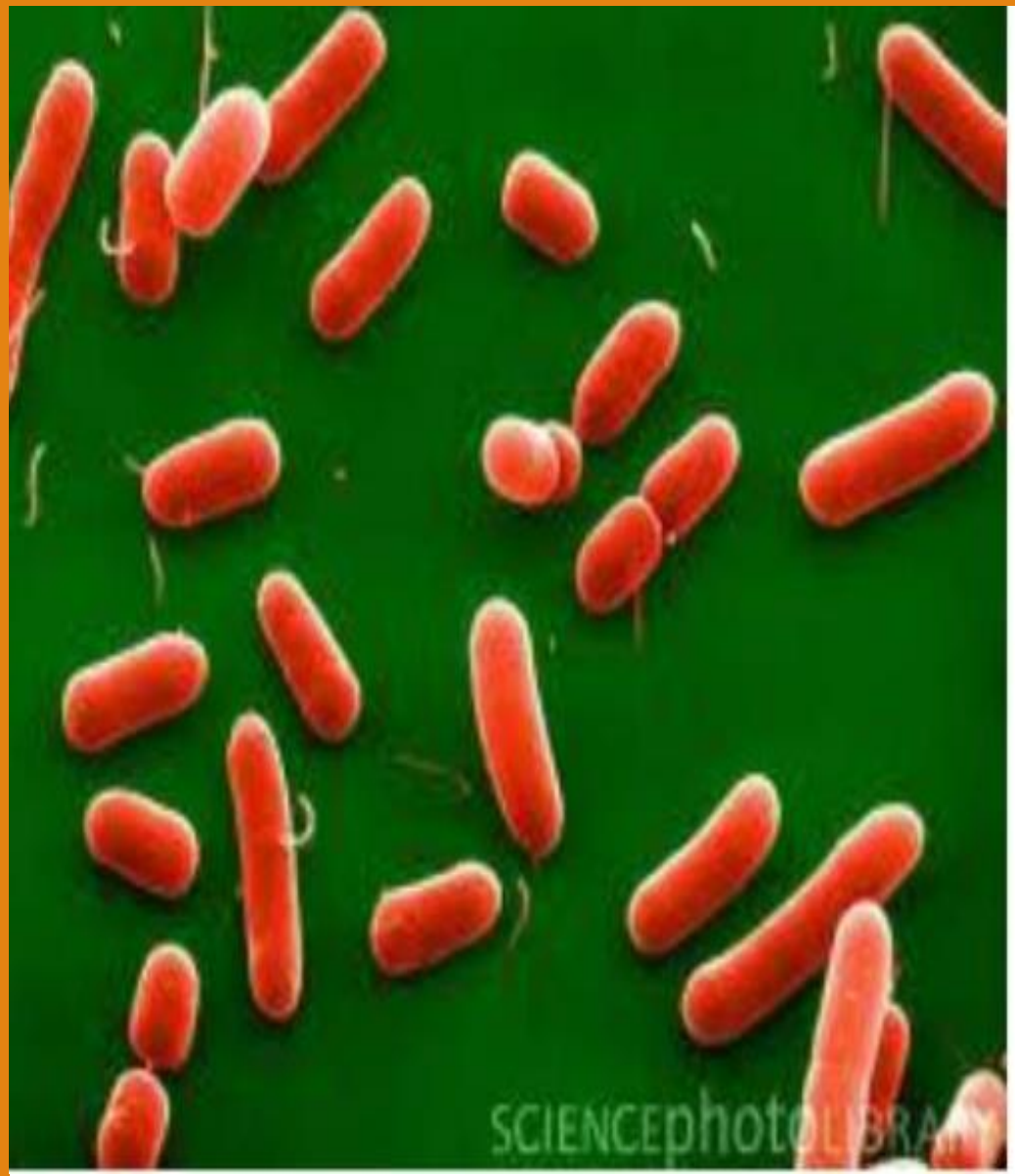
Culturing and Isolation

- Can be grown on Blood agar and Chocolate agar
- Need 5 – 10 % carbon dioxide
- A streak of Staphylococcus should be streaked across the plate at 37^0c
- Opaque colonies appear shows as Satellitisim
- Iridescence Demonstrates on Leviathan medium
- Blood culture


Haemophilus influenzae
Smooth
Low convex
grayish
Chocolate agar



H. influenzae is a small non-motile Gram negative coccobacillus or short rod



coccobacillus or short rod

appearance of *H. influenzae*

by Electron Microscope

Biochemical Characters

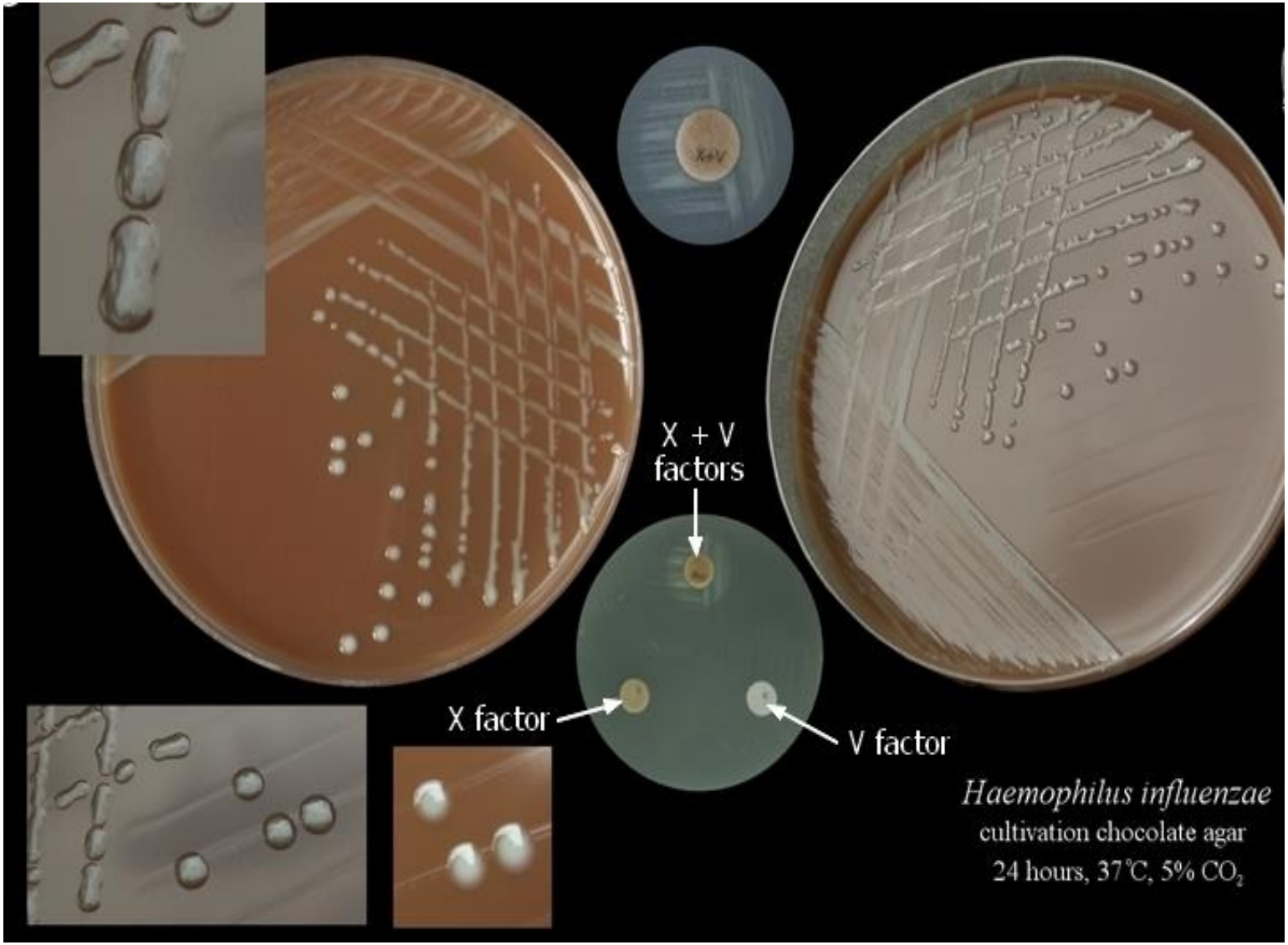
- Glucose +
- Xylose +
- Lactose –
- Sucrose –
- Mannitol –
- Nitrites reduced
- Indole differs on
- B type causes Meningitis

- Media used to grow *H. influenzae* must contain haemin or other iron-containing porphyrin and nicotinamide adenine dinucleotide (NAD) or its phosphate (NADP).

-The porphyrin requirement is referred to as growth factor X and the NAD or NADP requirement as growth factor V.

- Factor X is used by *H. influenzae* to produce essential respiratory enzymes such as cytochromes, catalase, and peroxidase.

- Factor V is used as an electron carrier in the organism's oxidation-reduction system..


X + V
factors
X factor
V factor
Haemophilus influenzae
cultivation chocolate agar
24 hours, 37°C, 5% CO_2

in a moist carbon dioxide atmosphere, capsulated *H. influenzae* strains produce mucoid colonies, 1.5 mm or more in diameter. Cultures have a distinctive smell.

H. influenzae grows well on chocolate agar because it contains factors X and V. Heating blood agar to 75 °C inactivates serum NADase and releases extra factor V from the red cells.

Addition of bacitracin (300 mg/litre) provides a selective medium to recover *H. influenzae* from sputum. This is NOT needed when culturing c.s.f ?!?!?

H. influenzae produces very small colonies on horse or rabbit blood agar (colonies may appear *beta-haemolytic*).

There is usually no growth on sheep blood agar. If, however, *S. aureus* which produces factor V in excess of its own needs, is cultured on a blood agar plate with *H. influenzae*, the factor V and the haemin released by staphylococcal haemolysins help the growth of *H. influenzae*.

-This 'help' given by *S. aureus*, forms the basis of the **satellitism test** which is a simple way of recognizing *H. influenzae* .

- *S. pneumoniae* also produces factor V and causes *H. influenzae* to show **satellitism**

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.

3- Streak a pure culture of *S. aureus* across each of the inoculated plates.

4- Incubate both plates in a carbon dioxide enriched atmosphere at 35–37 °C overnight.

5- The following morning examine the cultures for growth and satellite colonies.

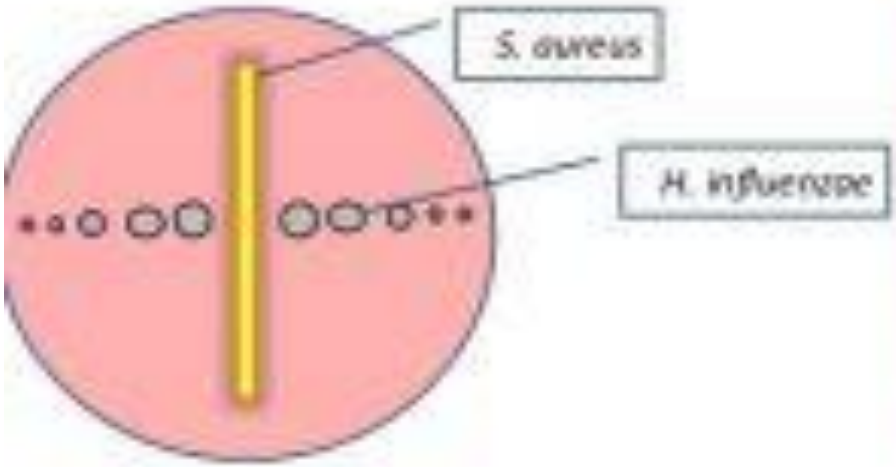
H. Influenzae

shows growth on the blood agar plate but not on the nutrient agar plate,

and the colonies near the column of *S. aureus* growth are larger than those furthest from it.

Satellitism

- BA – only X is available – V inside RBCs – hence small colonies
- *S. aureus* streak – provides V – large colonies near the streak and smaller as we move farther



Identification of *H. influenzae* using X, V, XV discs

1- Make a saline suspension (approx. 0.5 McFarland turbidity) of the test organism from a primary culture.

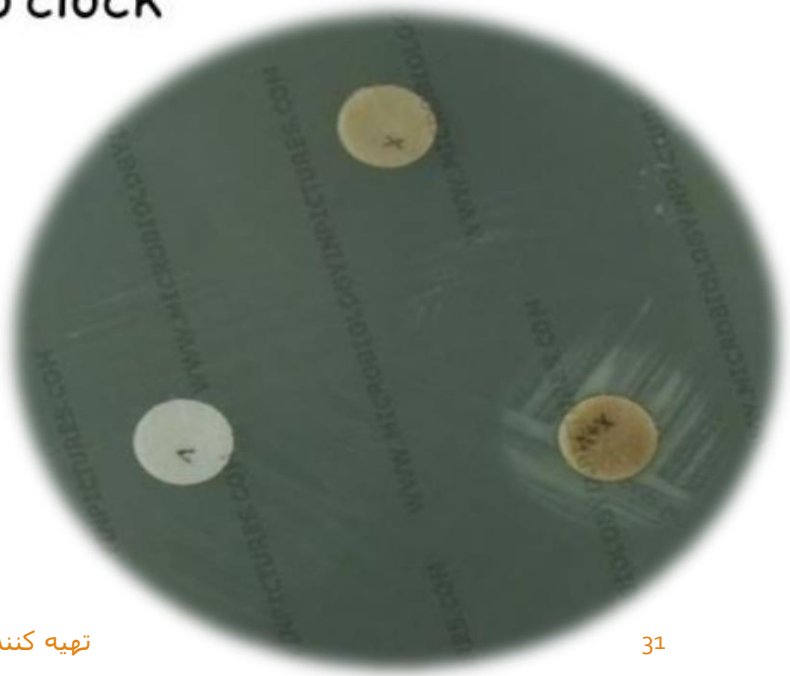
Using a swab, inoculate the suspension on a plate of nutrient agar.

2- Place the factor tablets or discs 10–20 mm in from the side of the plate, positioning each disc as follows:

Factor X. . . . . at '12 o'clock'

Factor V. . . . . at '4 o'clock'

Factor XV . . . . . at '8 o'clock'



Serology

- *H. influenzae* organisms are divided into six serogroups, a–f.

-The majority of strains that cause meningitis belong to invasive serogroup b. Very occasionally meningitis is caused by groups a, e, and f.

- Most of the strains that cause chronic bronchial disease are non-capsulated.

Slide coagglutination reagents are commercially available for the rapid immunological detection of specific polysaccharide *H. influenzae b* antigen in c.s.f.

rapid

Easy to perform

specific

sensitive

با تشکر از توجه شما