دانشگاه اصفهان

دانشکده علوم و فناوری های زیستی، گروه سلولی و مولکولی و میکروبیولوژی ،
آزمایشگاه میکروبیولوژی

آزمایشگاه باکتری شناسی ۲

بررسی خصوصیات ماکروسکوپی و میکروسکوپی و نحوه شناسایی نایسریا

The Neisseriae are Gram negative diplococci

- Pathogens are:- **N.Meningitidis**
  N.Gonorrhoeae

Neisseria Meningitidis

General characteristics

- Gram-negative, bean-shaped, diplococci
- Do not possess flagella or spores
- Capsulated and possess pili.
- Strict parasites, do not survive long outside of the host
- Aerobic
- Oxidative metabolism
- Produce catalase and oxidase
- Pathogenic species require enriched complex media and CO_2

Morphology

- Gram-negative, bean-shaped, diplococci
- Do not possess flagella or spores.
- Capsulated and possess pili.
- 0.8 x 0.6 μm in diameter.

Cultural characteristics

- Can grow in blood agar, Chocolate agar.
- Growth is improved by addition of blood or serum.
- Growth is also improved by incubation in the presence of 2- 8 % CO_2
- Growth temperature is 36-39^{0}C and pH ranges of 6-8.
- Colonies are 1-2 mm in diameter, convex, grey and transparent. No hemolysis in blood agar.

Biochemical properties

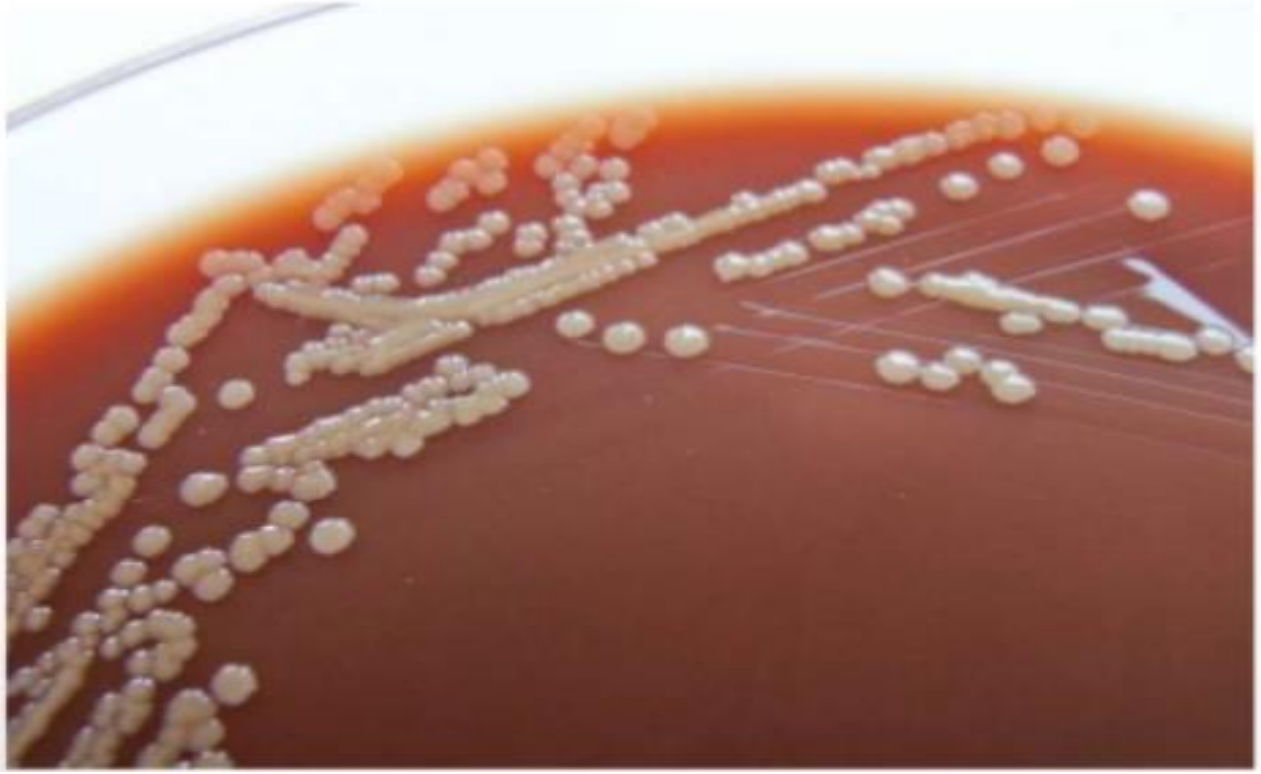
- Oxidase-positive; i.e., they possess the enzyme cytochrome and produce oxidase.
- N.Meningitidis is maltose fermenter.
- N.Meningitidis produces no beta lactamases.

Laboratory diagnosis

- It is frequently isolated from samples such as blood, CSF.
- Different methods for laboratory diagnosis are:
  - Gram staining
  - Culture
  - Oxidase test
  - Fermentation tests
  - Latex agglutination test

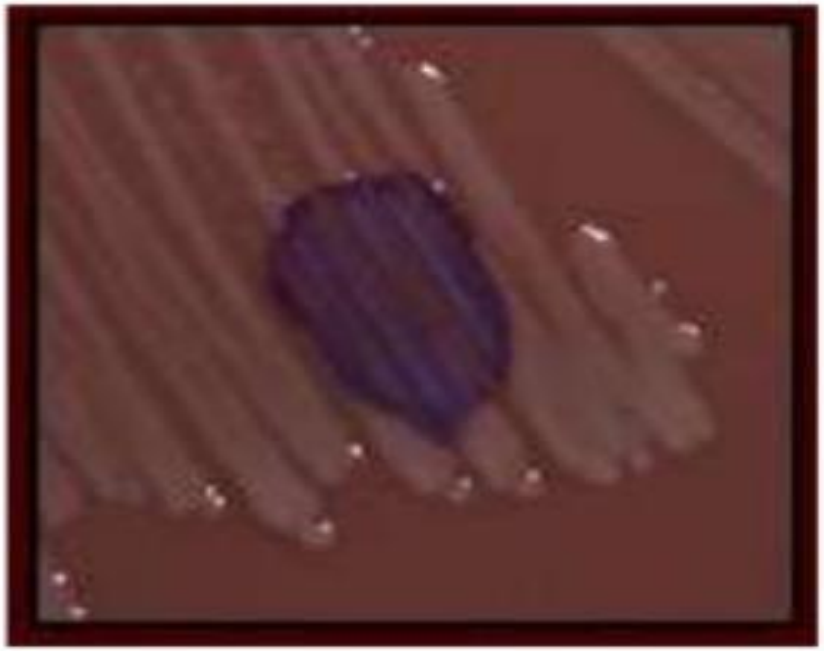
Culture

The organism is cultured on blood agar or chocolate agar incubated at 37°C in a 5% CO2 atmosphere. Colonies are 1-2 mm in diameter, convex, grey and transparent. No hemolysis



- **Oxidase test**: Determines the presence of cytochrome oxidase. It is Positive in N.Meningitidis.

Grow the isolate(s) to be tested for 18-24 hours on a blood agar plate at 35-37°C with ~5% CO_2. Dispense a few drops of Kovac's oxidase reagent. Tilt the plate and observe colonies for a color change to purple. Positive reactions will develop within 10 seconds in the form of a purple color.



- **Manitol fermentation**: N. Meningitidis ferment manitol.
- **Maltose fermentation:** N. Meningitidis ferment maltose.
- **Latex agglutination test**, which detects capsular polysaccharide in the spinal fluid.

Neisseria Gonorrhoeae

- N. Gonorrhoeae causes gonorrhea, neonatal conjunctivitis (ophthalmia neonatorum) and pelvic inflammatory disease (PID).

Morphology

- Oval shaped
- Gram negative diplococci
- Size is 0.6 to 0.8 μm.
- Occurs in pair
- Non motile
- Capsulated and have pilli

Cultural characteristics

- Can grow in enriched media such as chocolate agar.
- Growth is also improved by incubation in the presence of 5- 10% CO_2
- Growth temperature is 37^0C and no growth if the temperature is less than 25^0C or more than 38.5^0C
- pH ranges of 7.2-7.6.

Biochemical properties

The virulence factors are.

1. **Pili**: Most important virulence factors.

   Piliated gonococci are usually virulent, whereas non piliated strains are avirulent.

2. Two virulence factors in the cell wall

a) **Lipooligosaccharride (LOS)** (a modified form of endotoxin). Endotoxin of gonococci is weaker than that of meningococci.

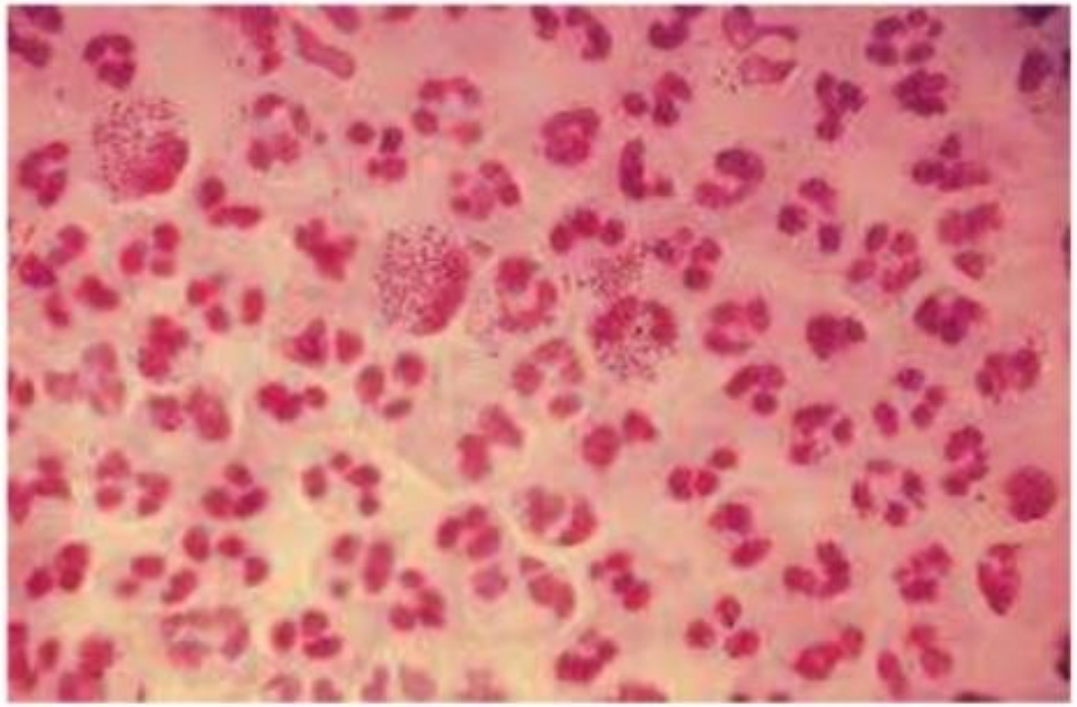
b) **Outer membrane proteins**(OMP) : OMP cause attachment of bacteria to epithelial cells of the urethra, rectum, cervix, pharynx, or conjunctiva, like pilli.

Laboratory diagnosis

- It is frequently isolated from samples such as blood, urethral discharge in men, cervical discharge in females.
- Different methods for laboratory diagnosis are:
  - Gram staining
  - Culture
  - Oxidase test
  - Fermentation tests

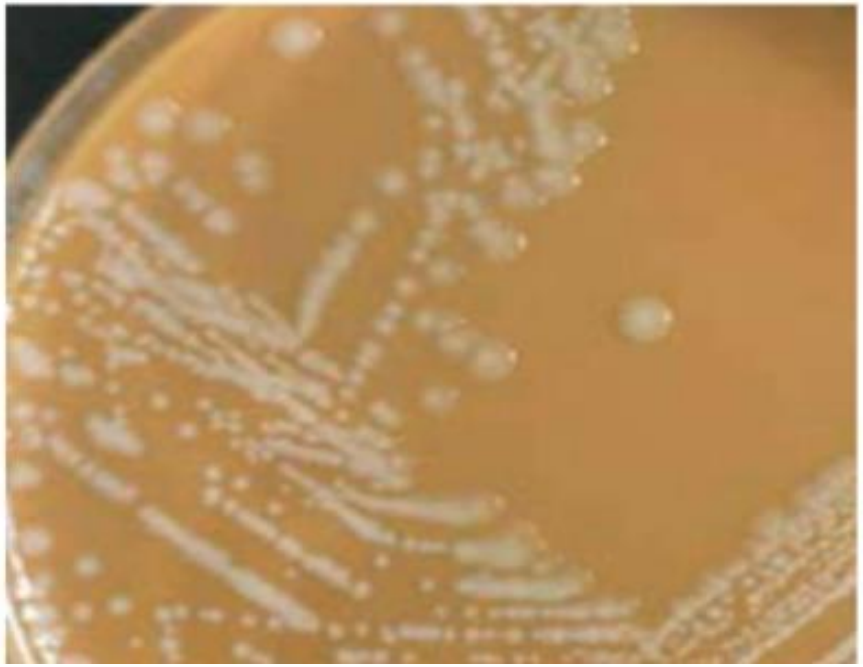
Gram staining

The diagnosis is suggested by the finding of gram negative bacteria bean shaped capsular diplococci.



Culture

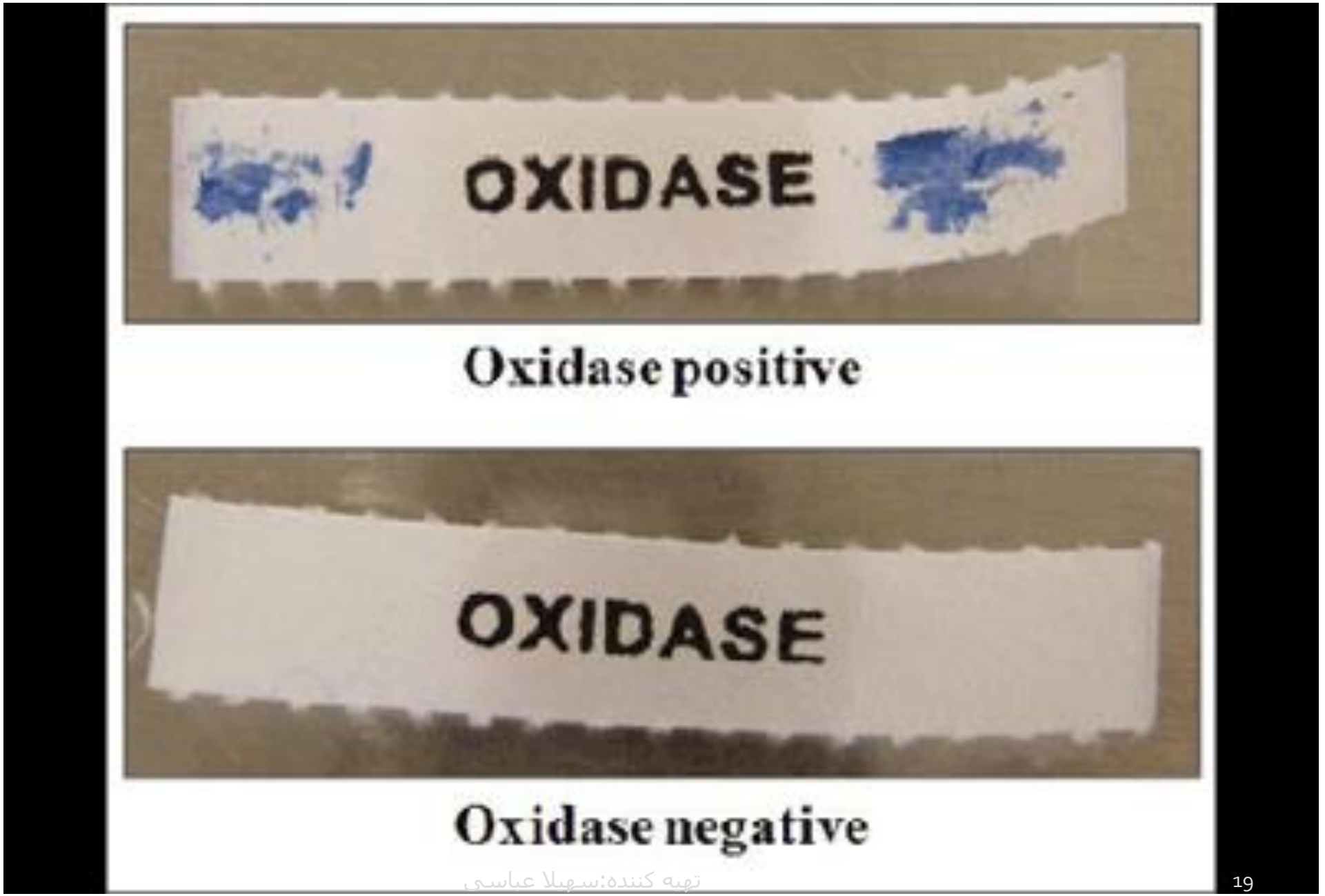
The organism is cultured on Thayar - Martin Agar or Mueller-Hinton agar (chocolate Agar) incubated at 37°C in a 5% CO2 atmosphere. Colonies are 1-2 mm in diameter, grey and transparent. N. gonorrhea grows rapidly producing small, No hemolysis.



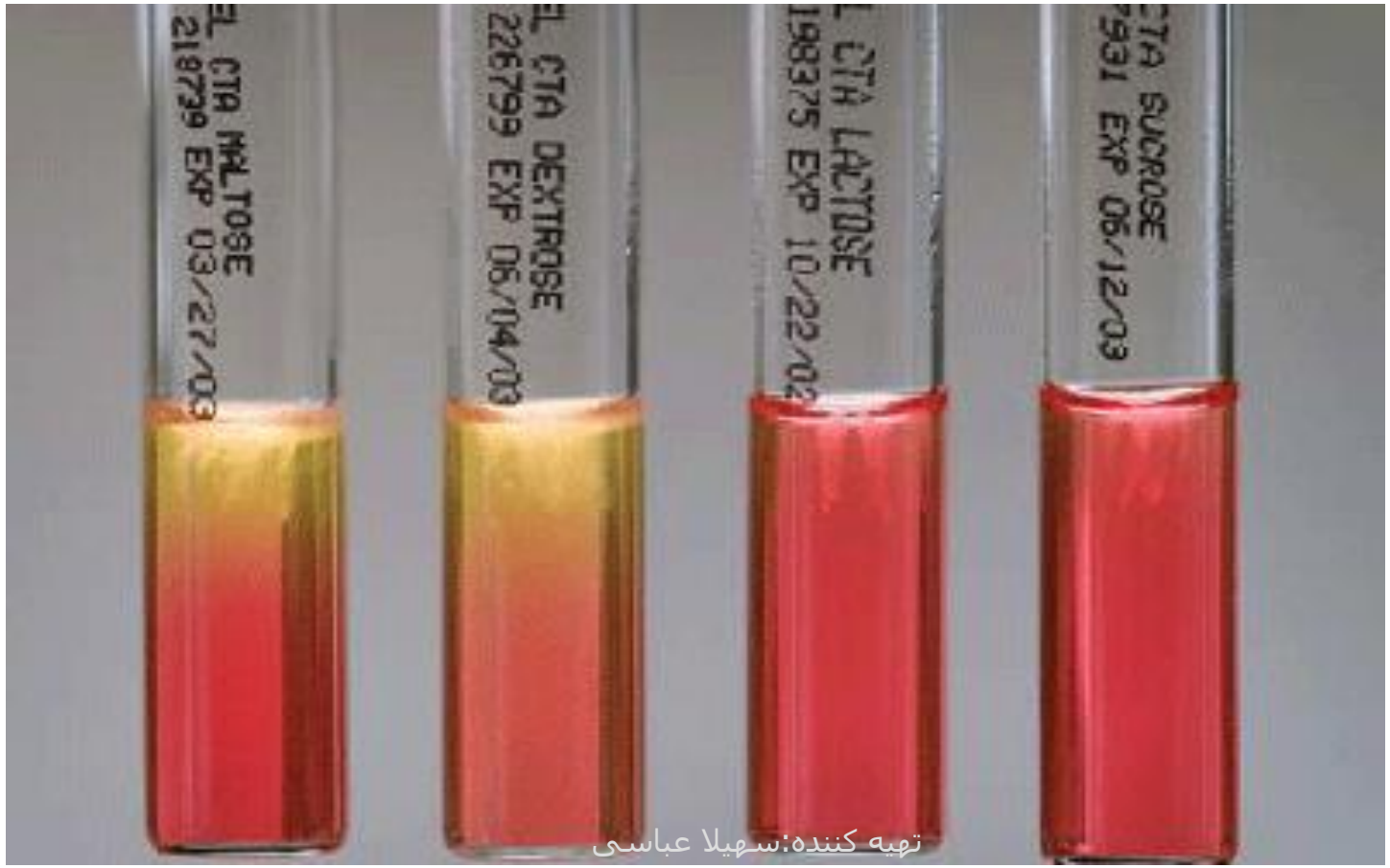
Oxidase Test

- Test on filter paper or directly on plate
- Oxidase reagent =Dimethyl or tetramethyl oxidase reagent
- Violet-purple color indicates a positive result.

- **Manitol fermentation**: N. Gonorrhea ferment manitol.
- **Maltose fermentation:** N. Gonorrhea do not ferment maltose.

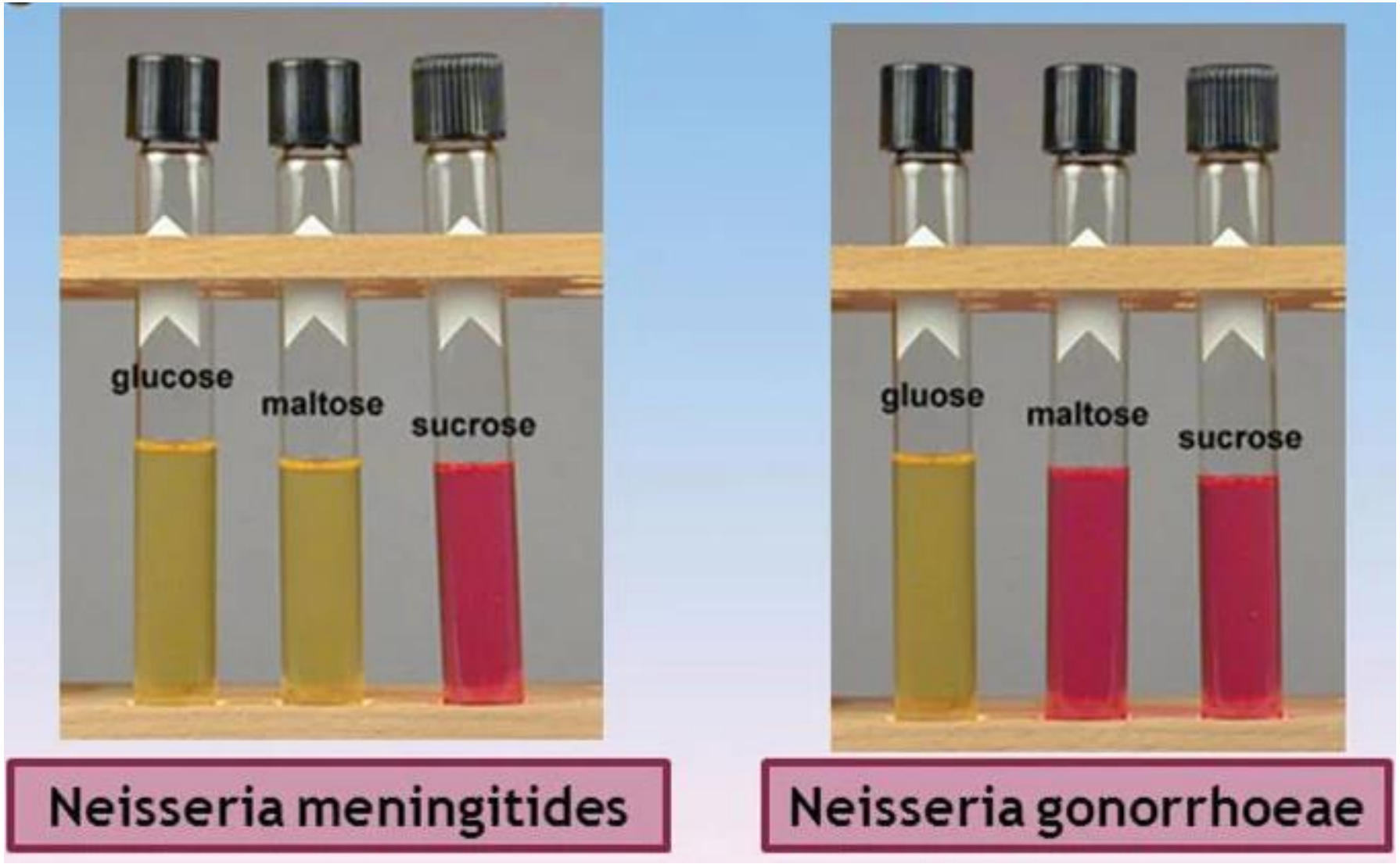

OXIDASE
Oxidase positive
OXIDASE
Oxidase negative

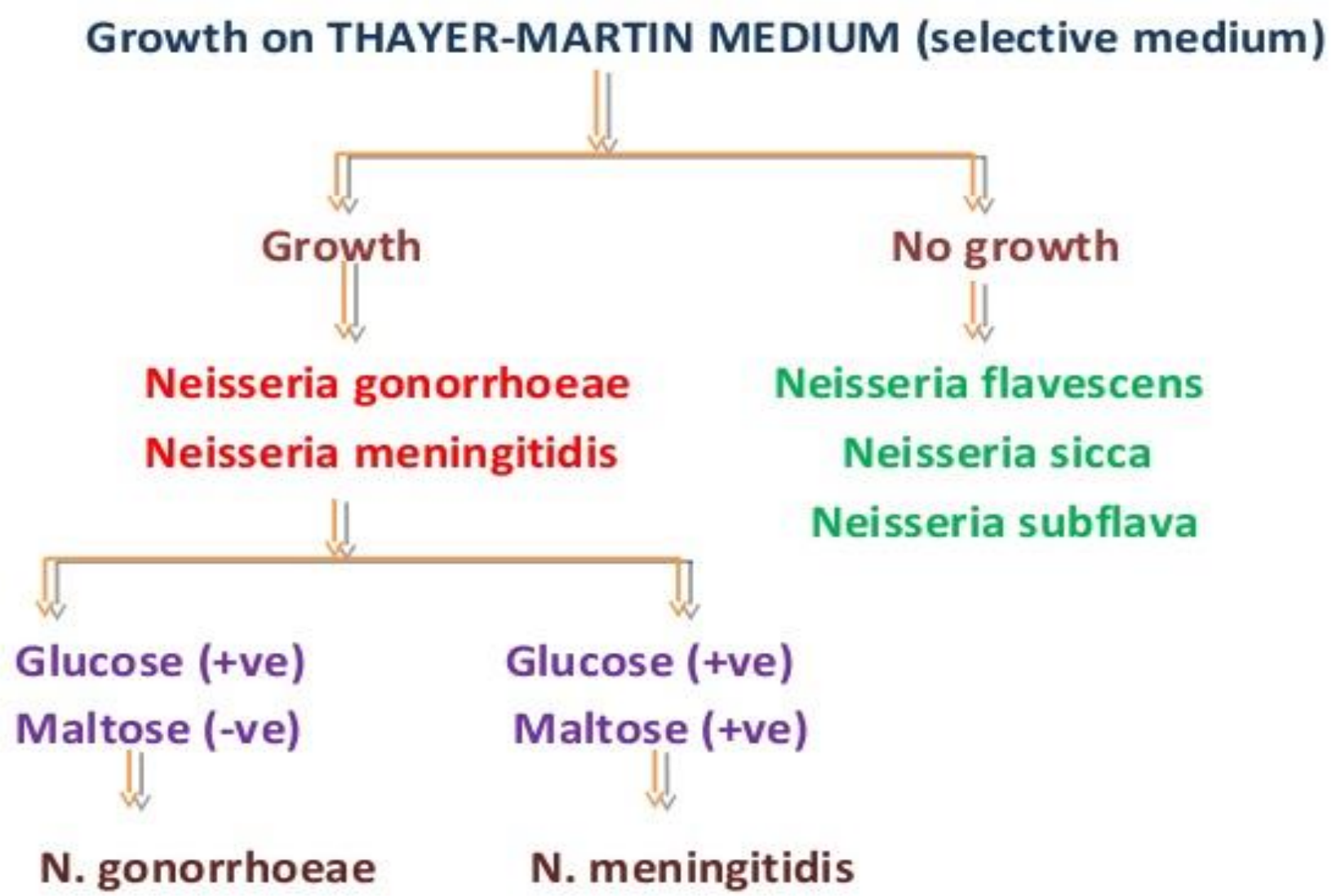
sugar reactions for *N. meningitidis* with utilization of glucose (dextrose) and maltose, indicated by acid production (color change to yellow), and no utilization of lactose or sucrose

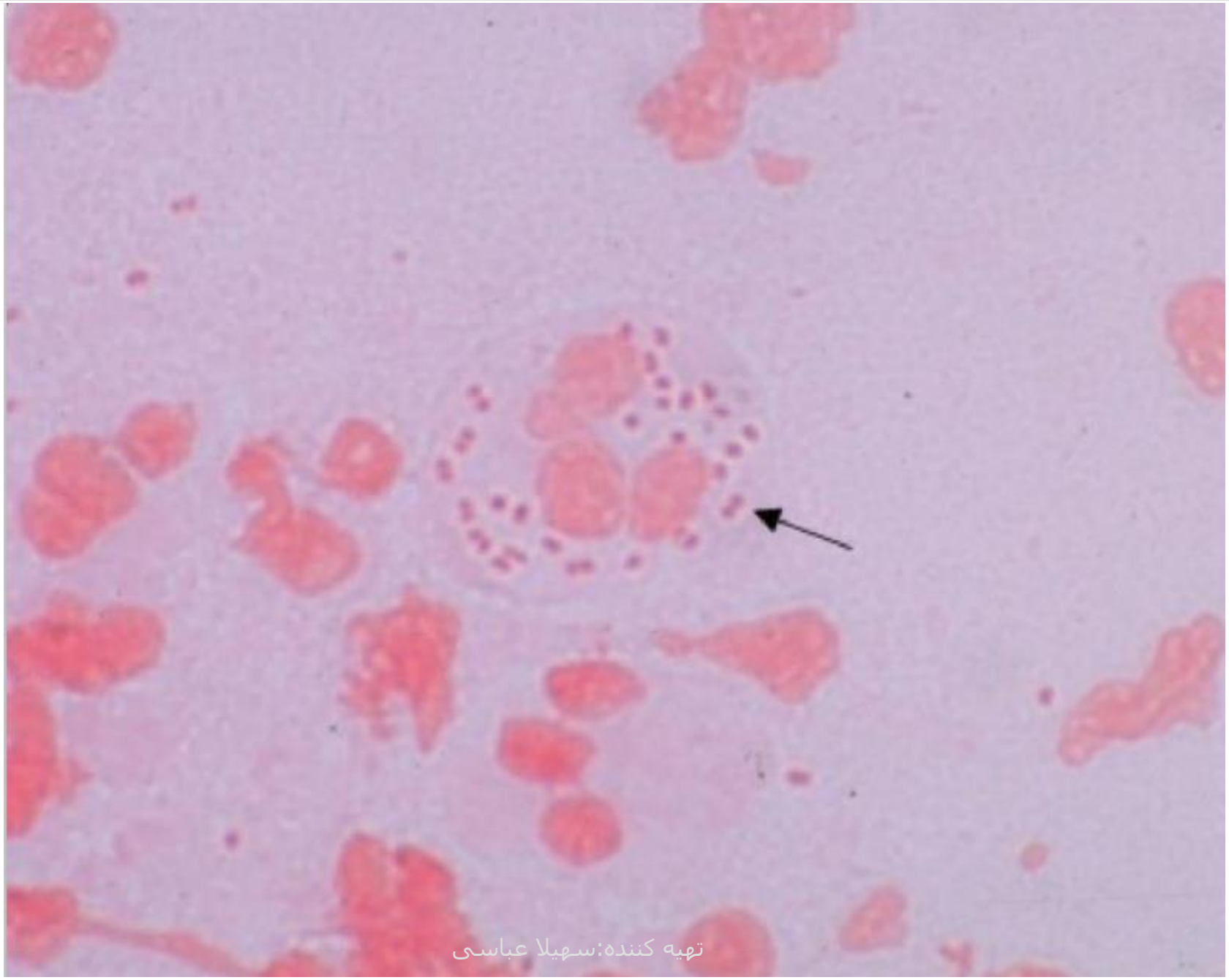


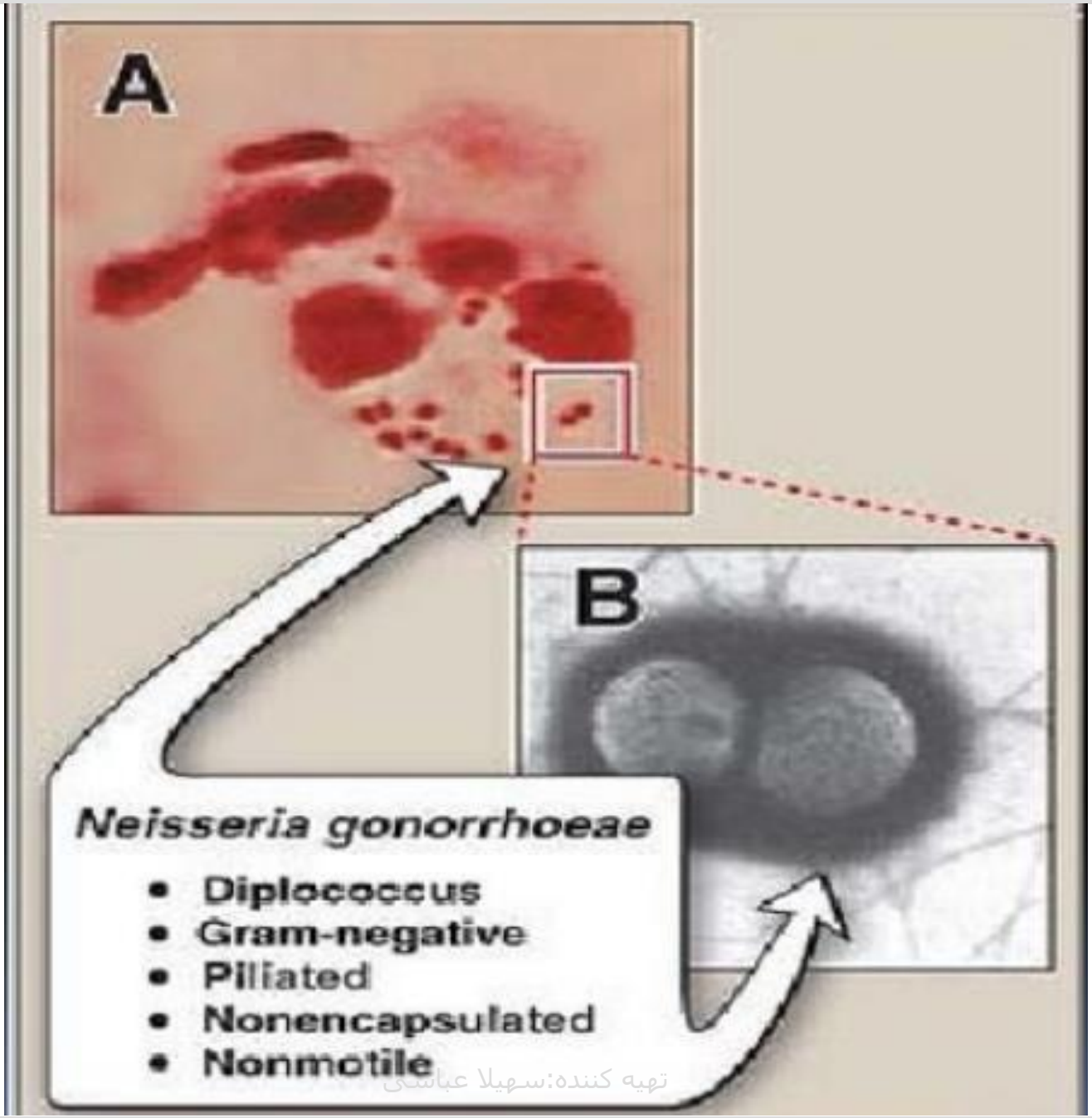
Acid Production from:

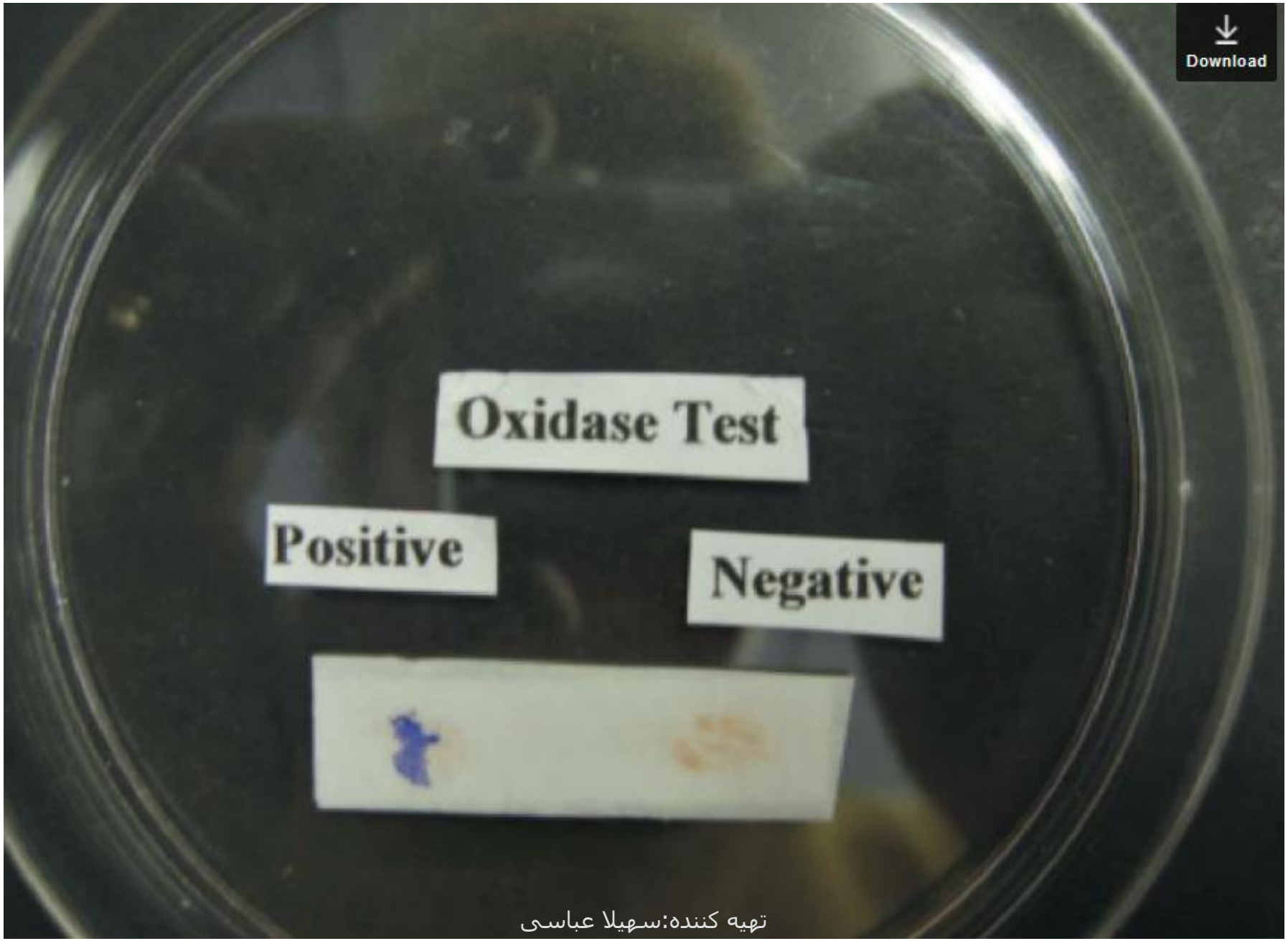
Organism	Glucose[1]	Maltose	Lactose	Sucrose
Neisseria meningitidis	+	+	–	–
Neisseria lactamica	+	+	+	–
Neisseria gonorrhoeae	+[2]	–	–	–
Neisseria sicca	+	+	–	+
Moraxella catarrhalis	–	–	–	–


glucose
maltose
sucrose
Neisseria meningitides
gluose
maltose
sucrose
Neisseria gonorrhoeae


Growth on THAYER-MARTIN MEDIUM (selective medium)
Growth
No growth
Neisseria gonorrhoeae
Neisseria meningitidis
Neisseria flavescens
Neisseria sicca
Neisseria subflava
Glucose (+ve)
Maltose (-ve)
Glucose (+ve)
Maltose (+ve)
N. gonorrhoeae
N. meningitidis




A
B
Neisseria gonorrhoeae
Diplococcus
Gram-negative
Piliated
Nonencapsulated
Nonmotile


Download
Oxidase Test
Positive
Negative

Laboratory diagnosis

- Specimens:
  - Neisseria meningitidis:
    - C.S.F.
    - Blood.
    - Nasopharyngeal swab.
  - Transport media is Aimies or Stuart transport media.

Laboratory diagnosis

- Specimens:
  - N.gonorrhoeae: (avoid using cotton or calcium alignament swab use Rayon or Dacron swab)
    - Urethral swab.
    - Endocervical swab.
    - Eye swab.
    - Throat swab and Rectal swab.

- **Direct Gram stain:**
  - G ram Negative kidney shape diplococci intra and extracellular.

Culture:

- Chocolate agar with a 5-10% CO_2.
- Blood Agar.

Blood cultures

Meningococci grow well in

- **Columbia diphasic** medium Because sodium polyanethol sulphonate (SPS) may be inhibitory to meningococci.
- add sterile gelatin (1% final concentration) to neutralize the effect of SPS.
- Subculture a positive blood culture onto chocolate agar and incubate in a carbon-dioxide enriched atmosphere

Culture:

- Chocolate agar with a 5-10% CO_2.
- Selective media for gonococci:
  - Thayer-Martin media chocolate agar contain:
    - Vancomycin for G+ve
    - Colistin for G-ve.
    - Nystatin for fungi and Yeast.
  - Modified Thayer-Martin media:
    - Addition of Trimethoprim which kill swarming proteus species.

- Martin-Lewis:
  - Contain Anisomycin instead of Nystatin
- Modified New York City media contain:
  - Vancomycin.
  - Colistin.
  - Amphotericin B
  - Trimethoprim

Incubation:

- At 37°C in candle jar for 24-48 hrs.
- Colonial morphology:
  - small, gray, translucent and raised.
- Biochemical reaction:
  - Oxidase +ve.
  - Catalse +ve.

Incubation:

- At 37°C in candle jar for 24-48 hrs.
- Colonial morphology:
  - small, gray, translucent and raised.
- Biochemical reaction:
  - Oxidase +ve.
  - Catalse +ve.


Culture of sample from cervix on plain chocolate agar allows growth of contaminating organisms.
Culture on Thayer-Martin chocolate agar medium (containing vancomycin, colistin, trimethoprim, and nystatin) allows selective growth of *Neisseria*.


THANK YOU