PowerPoint® Lecture Slides for

MICROBIOLOGY

Lab. ٩

Gram-negative cocci

(Neisseriae)

Learning objectives:

After this lab. You must be able to:

- Differentiate between *Neissereiae spp.*
- Describe the two species microscopically and culturally.
- List types of clinical infections these organisms produce
- Predict g-ve diplococci agents causing clinical cases.
- Diagnose the *Neisseria* spp. In clinical sample.
- Know the prevention ways of each organism.

Neisseriae spp.:

The genus *Neisseriae* contain two important pathogens :

- ***N. meningitidis-*** cause meningitis and meningococcemia (the most severe form of meningococcemia is life-threatening Waterhouse-friderichsen syndrome)
- ***N. gonorrhoea-*** causes gonorrhoea, neonatal conjunctivitis (*ophthalmia neonatrum*) and pelvic inflammatory disease (PID).

Ophthalmia neonatrum

Waterhouse-Friderichsen syndrome

Important properties:

- Aerobic
- Gram –ve diplococci (each coccus is shaped like a kidney or coffee bean with concave side faced each other).
- Oxidase +
- Most catalase +ve
- Non motile
- Grow well on chocolate agar (blood heated to 80˚C), but not on blood agar because the growth is inhibited by toxic trace metal and fatty acids found in certain media including blood.

Neisseriae meningitidis:

Important properties:

- capsulated.
- Have 3 virulence factors: polysaccharide capsule, endotoxin (LPS), and IgA protease.
- Ferment maltose and glucose
- Transmitted by airborne droplets.

Laboratory diagnosis:

- Specimen: blood and CSF.
- Microscopic: presumptive diagnosis can be made if G –ve diplococci are seen in a smear of CSF inside the PMN (intracellular)
- Culture: chocolate agar at 37˚C in a 5% CO_2.
- Biochemical tests:

-Oxidase +ve.

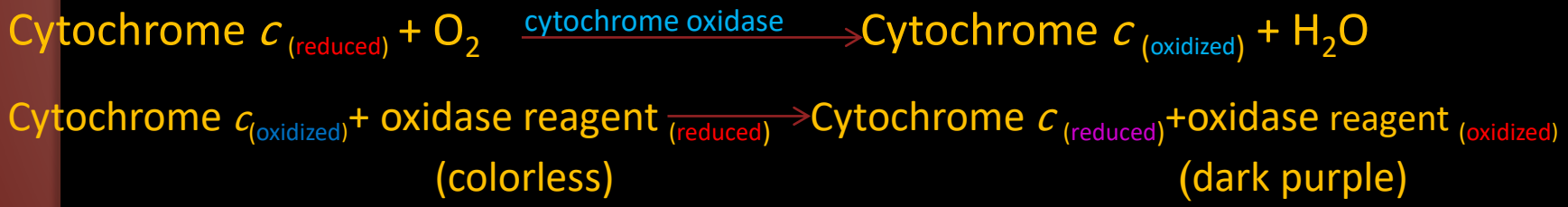
-Maltose fermentation +ve.

- Immunological assay: latex agglutination test detects capsule polysaccharide in CSF.

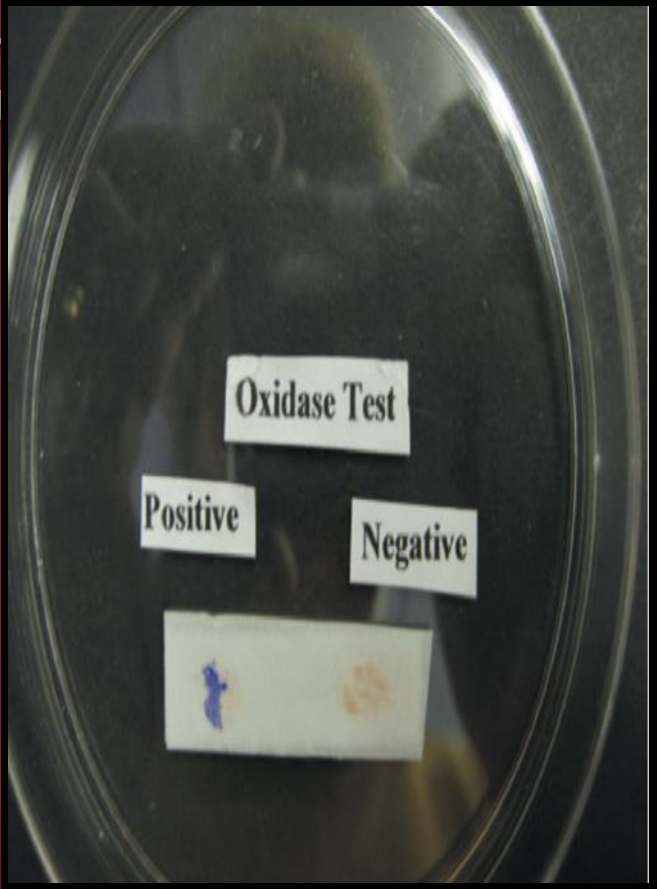
Oxidase test:

- Principle:

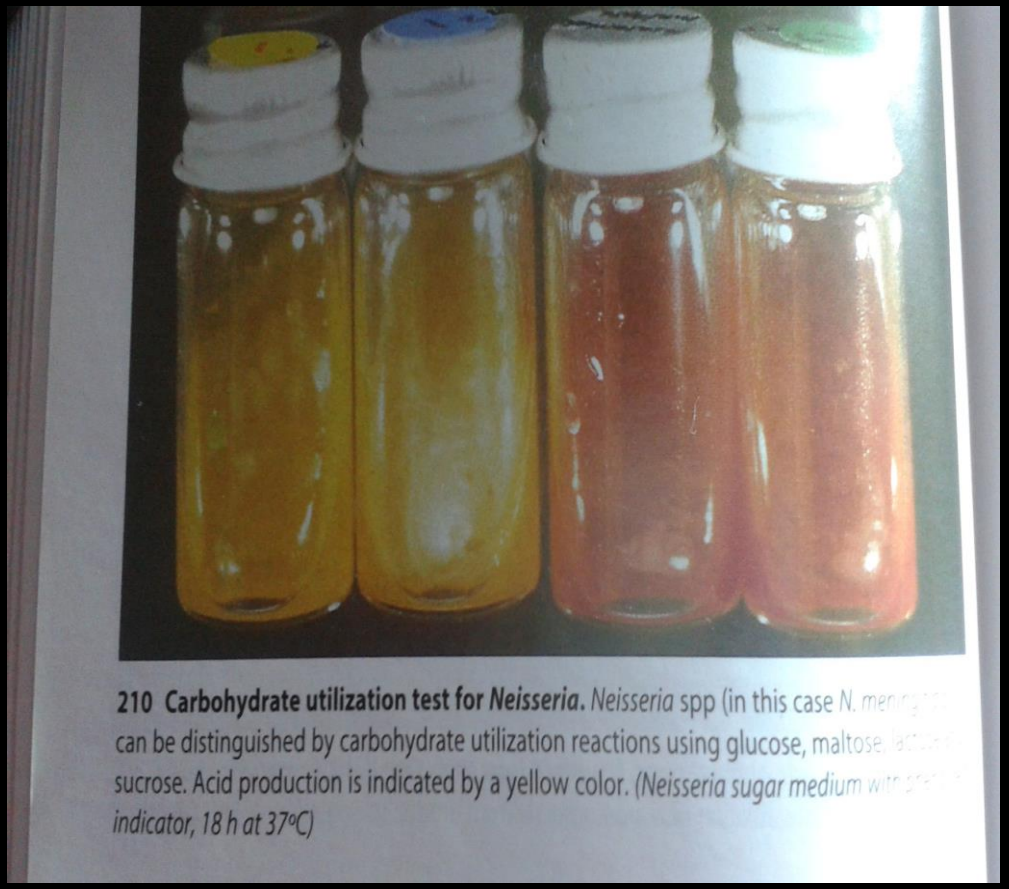
The oxidase test is used to identify bacteria that produce cytochrome c oxidase, an enzyme of the bacterial electron transport chain. When present, the cytochrome c oxidase oxidizes the reagent to purple color end product. When the enzyme is not present, the reagent remains reduced and is colorless.



Oxidase test



Maltose and glucose fermentation



210 Carbohydrate utilization test for *Neisseria*. *Neisseria* spp (in this case *N. meni*[illegible] can be distinguished by carbohydrate utilization reactions using glucose, maltose, [illegible] sucrose. Acid production is indicated by a yellow color. *(Neisseria sugar medium with* [illegible] *indicator, 18 h at 37°C)*

Treatment:

- Penicillin is the treatment of choice

Prevention:

- Chemoprophylaxis: rifampin or ciprofloxacin
- Immunization:

- Conjugated (the four capsular polysaccharide types conjugated to a carrier; diphtheria toxoid)

- Non-conjugated: only the four capsular polysaccharide without the carrier.

Neisseriae gonorrhoea:

- Important properties:

- Have no capsule.
- Have 3 virulence factors: pili, endotoxin (LOS; a modified form of endotoxin), and IgA protease.
- Ferment glucose but not maltose.
- Transmitted by sexual contact (killed by drying).

Laboratory diagnosis:

- Specimen: urethral discharge and cervical swab.
- Microscopic: in men the finding of G –ve diplococci within PMNs in a urethral discharge specimen is sufficient for diagnosis but in women culture should be done.
- Culture: Thayer-Martin medium (chocolate agar containing antibiotics; vancomycin, colistin, trimethoprim, and nystatin to suppress the normal flora) at 37 ˚C in a 5% CO_2.
- Biochemical tests:

-Oxidase +ve

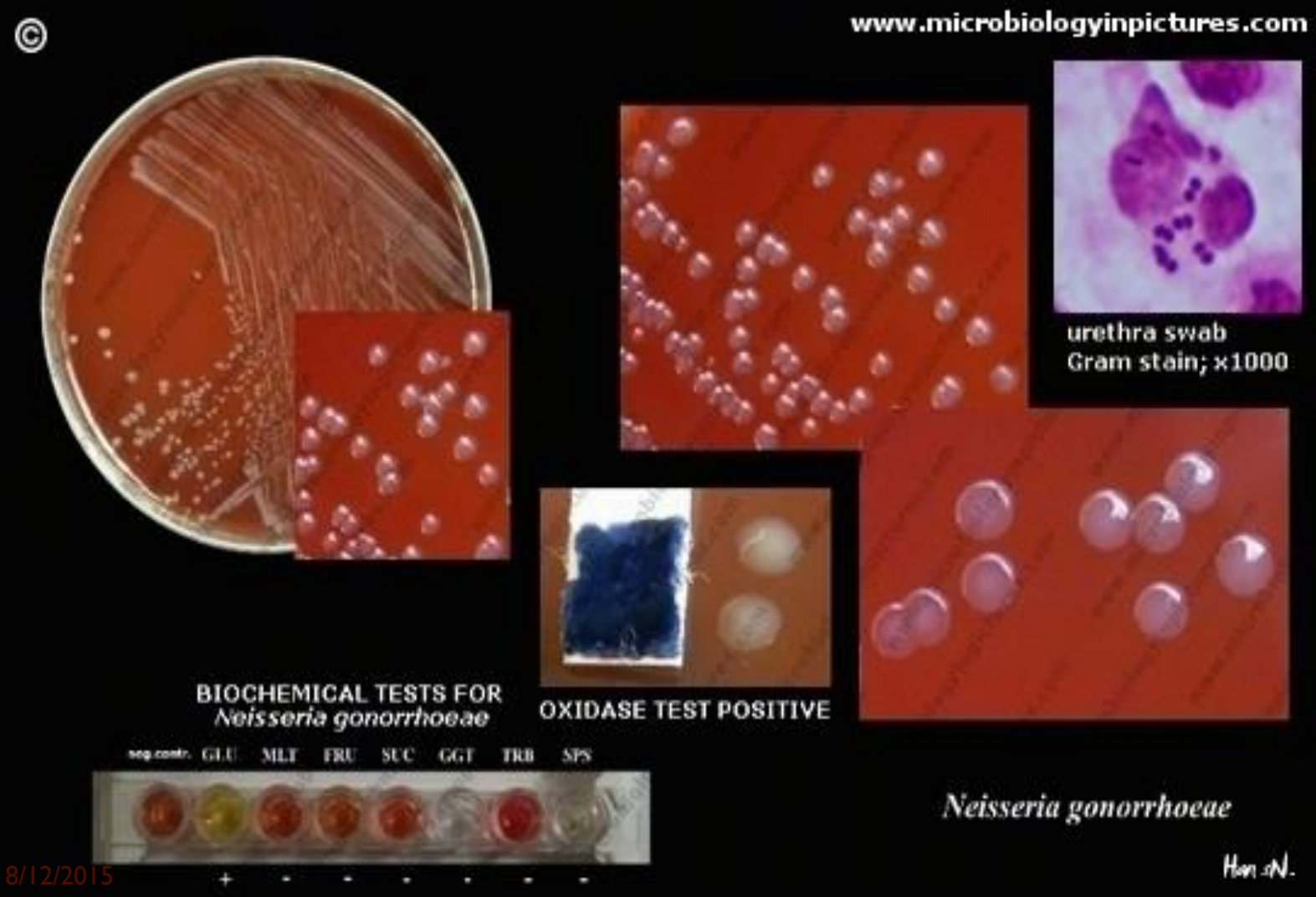
-Maltose fermentation -ve

- Nucleic acid based tests.

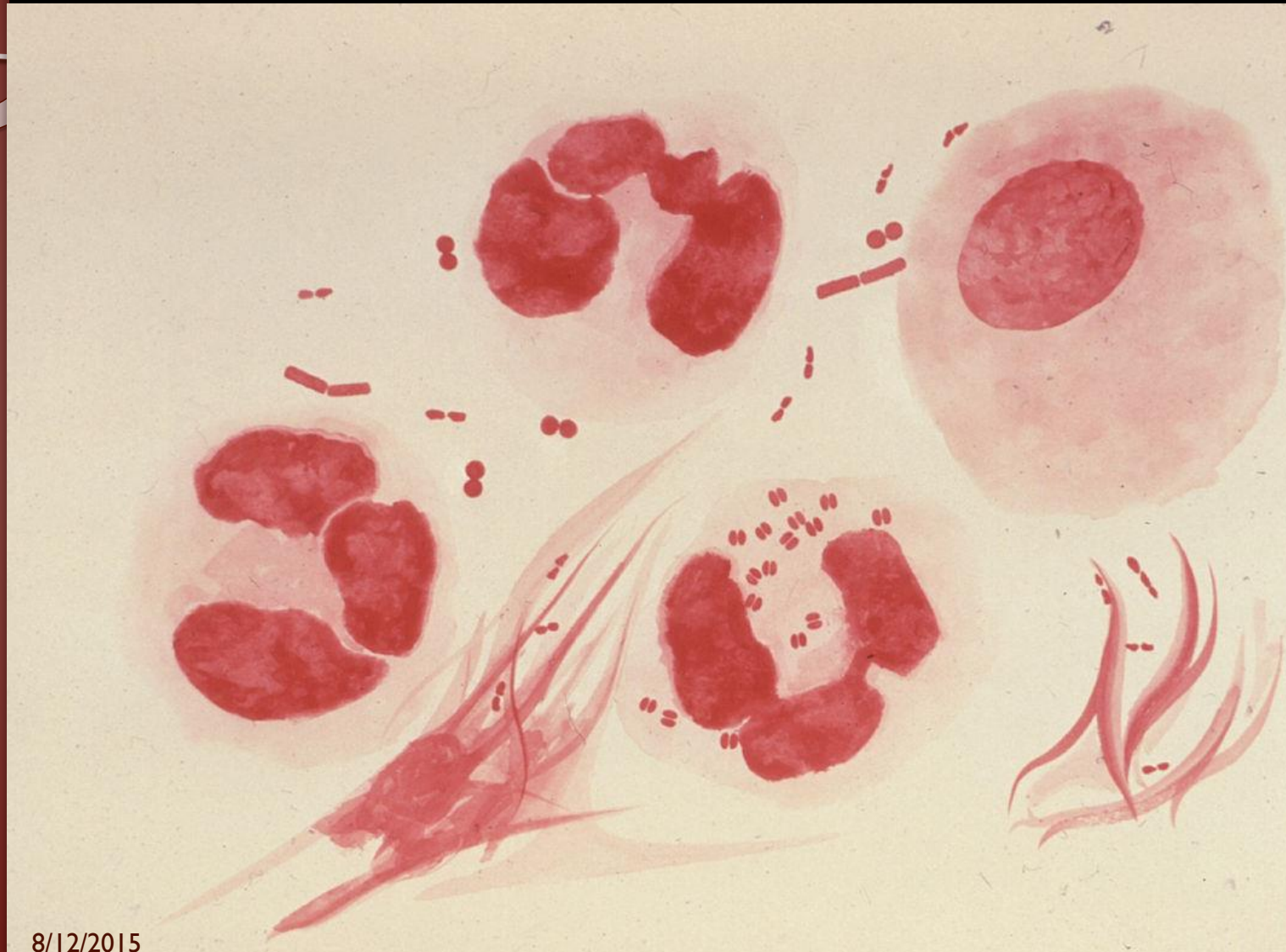
Glucose fermentation

211 Carbohydrate utilization test for *Neisseria*. *Neisseria gonorrhoeae* ferments only glucose. *(Neisseria sugar medium with phenol red indicator, 18 h at 37°C)*

Laboratory diagnosis:



Neisseria gonorrhoea in a urethral discharge



N. gonorrhoea on Thayer-Martin agar

Treatment:

- Penicillin is no longer drug of choice due to:

-Chromosomally-mediated resistance because of (1- reduced uptake of the drug, 2- altered binding site)

-Plasmid-encoded beta-lactamase production (penicillinase-producing *N. gonorrhoea- PPNG*)

- Cefitrixone is the treatment of choice in uncomplicated cases
- In combination of tetracycline, doxycycline or azithromycin in dual infection with *C. trachomatis*
- follow-up.

Prevention:

- Chemoprophylaxis of newborns against ophthalmia neonatrum with 1% silver nitrate, 0.5% erythromycin eye ointment.

- Measures to limit epidemic include education, aggressive detection, uses of condoms, and the prompt treatment of symptomatic patients and their partners


Thank you