

Practical No.11

Neisseria

The Neisseria are a large family of commensal bacteria that colonize the mucosal surfaces of many animals. Of the eleven species that colonize humans, only two are pathogens. *N. meningitidis* and *N. gonorrhoeae* often cause asymptomatic infections, a commensal-like behavior. Most gonococcal infections are asymptomatic and self-resolving, and epidemic strains of the meningococcus may be carried in >95% of a population where systemic disease occurs at <1% prevalence. Neisseria are a genus of aerobic to facultatively anaerobic bacteria containing Gram-negative bacteria. *Neisseria* are diplococci that resemble coffee beans when viewed microscopically. The genus includes:

- *N. gonorrhoeae* (also called the *gonococcus*), which causes gonorrhoea, a sexually transmitted disease (STD).
- *N. meningitidis* (also called the *meningococcus*), one of the most common causes of bacterial meningitis and the causative agent of meningococcal septicaemia.

All the medically significant species of *Neisseria* are positive for both [catalase](#) and [oxidase](#).

N. gonorrhoeae (*gonococcus*)

Very delicate and fastidious, requiring increased carbon dioxide tension (3% to 10%) for cultivation. Narrow optimal temperature range, between 35° and 37°C and fails to grow much above or below these temperatures. Extremely sensitive to drying though it may remain viable in dried pus for several weeks.

Diagnosis;

Swab sample. A swab sample from the part of the body likely to be infected (cervix, urethra, penis, rectum, or throat) can be sent to a lab for testing:

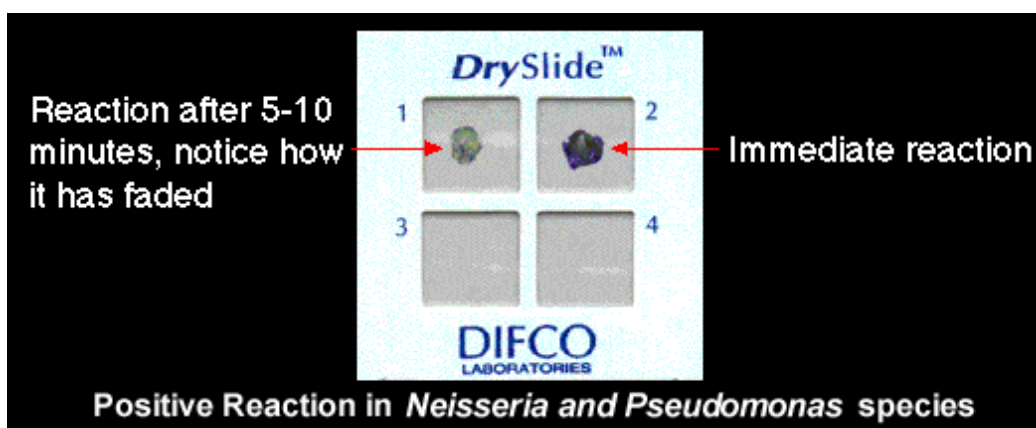
- 1- **Gram stain.** This is done right in a clinic or doctor's office. A sample from the urethra or a cervix is placed on a slide and stained with Gram stain. This test works better for men than for women. The cells appear as **Gram negative** cocci form masses with adjacent cells (i.e. in diploid forms), having a bean-shape, a coffee bean-shape, or Kidney-shape. These cells are seen intracellularly (inside polymorphonuclear leucocytes), or extracellularly.
- 2- **Urine test.** Gonorrhoea in the cervix or urethra can also be diagnosed with a urine sample sent to a lab.
- 3- **Oxidase Test** - It is a hallmark test for the *Neisseria*.

The reaction is dependent upon the presence of an intracellular cytochrome oxidase system that catalyzes the oxidation of **cytochrome c** by molecular oxygen, which then serves as the terminal electron acceptor in

the organism's electron transport system. The reagent is colorless in its reduced state, while in the oxidized state the reagent is dark purple. In essence, the oxidase test determines the presence or absence of cytochrome c. Organisms containing cytochrome c as part of their respiratory chain are **oxidase positive** and turn the reagent purple; organisms lacking cytochrome c as part of their respiratory chain do not oxidize the reagent, leaving it colorless within the time limits of the test, and are **oxidase negative**.

Procedure:

Add a drop of oxidase reagent (1% solution of N,N-dimethyl, p-phenylenediamine monohydrochloride) to a piece of filter paper. Apply a visible quantity of bacteria to the paper using a sterile applicator stick. Colonies producing oxidase will become pink, changing to violet and finally become black.



Oxidase test

Culture; Thayer-Martin agar (or **Thayer-Martin medium**) is a Mueller-Hinton agar with 5% chocolate sheep blood and antibiotics; (VCN) Vancomycin, Colistin and Nystatin which is used to inhibit normal flora and aid in the recovery of the gonococcus. This medium is used for culturing and primarily isolating *Neisseria* bacteria, including *Neisseria gonorrhoeae* and *Neisseria meningitidis*, as the medium inhibits the growth of most other microorganisms. It produces small, convex, round, grayish-white colonies in 48 hours. Prompt cultivation in a candle jar or carbon dioxide incubator is essential.

A report of "**typical gram-negative intracellular, oxidase-positive diplococcus isolated**" should be made if the organism is suspected to be *N. gonorrhoeae*



Neisseria meningitidis (Meningococcus)

Meningococcus is similar to the gonococcus in morphology and staining reactions. *Neisseria meningitidis* is a gram-negative diplococcal bacterium, fastidious in its growth requirements and may be cultivated on blood agar or chocolate agar on which it develops relatively large, bluish gray, smooth, raised colonies. No hemolysis is produced on blood agar. Extremely sensitive to temperature and dehydration.

Neisseria meningitidis is the Causative agent of meningococcal meningitis, meningococemia, and bacterial endocarditis. It is an obligate human pathogen.

Diagnosis;

Specimen; The gold standard of diagnosis is isolation of *N. meningitidis* from sterile body fluid. Blood, CSF specimen is sent to the laboratory immediately for identification of the organism. It may be isolated from nasopharynx, joint fluid, and from petechiae of the skin.

- 1- **Gram stain;** show adjacent coffee bean-shape, Gram negative cells (diplococci) with flattened surfaces facing each other.
- 2- **Culture;** culturing the organism on Thayer-Martin agar.
- 3- **Oxidase test;** (all *Neisseria* show a positive reaction)
- 4- **Sugar fermentation tests;** carbohydrates; maltose, sucrose, and glucose test in which *N. meningitidis* will oxidize (utilize) the glucose and maltose.
- 5- **Serology;** determines the group of the isolated organism.

If the organism reaches the circulation, then blood cultures should be drawn and processed accordingly. **PCR**; polymerase chain reaction tests can be used to identify the organism even after antibiotics have begun to reduce the infection.

DIFFERENTIATION OF THE *NEISSERIAE*

Different *Neisseria* species can be identified by the sets of sugars from which they will produce acid. For example, *N. gonorrhoea* makes acid from only glucose, however *N. meningitidis* produces acid from both glucose and maltose.

Neisseriae	Growth Characteristics	Sugar fermentation			
		Glucose	Maltose	Sucrose	Lactose
<i>N. gonorrhoea</i>	Growth on enriched media at 37°C in CO2 jar; colonies similar to <i>N. meningitidis</i>, but smaller and grayish white	+	-	-	-
<i>N. meningitidis</i>	Growth on enriched media at 37°C in CO2 jar; colonies smooth, glistening, translucent, soft, or bluish gray	+	+	-	-

Polysaccharide capsule; *N. meningitidis* has a polysaccharide capsule that surrounds the outer membrane of the bacterium and protects against soluble immune effector mechanisms within the serum. It is considered to be an essential virulence factor for the bacteria.