# **Neisseria Species (Gram-Negative Diplococci)**

**Characteristics:**

* *Neisseria* are **Gram-negative diplococci**, typically appearing as kidney-bean shaped pairs with adjacent sides flattened.
* Two primary pathogens are **Neisseria gonorrhoeae** and **Neisseria meningitidis**, while others (commensal Neisseria) are normal flora of mucous membranes.
* **oxidase positive** and **catalase positive**.
* Pathogenic Neisseria are fastidious: they require enriched media and often CO₂.

**Key Identification Tests:**

* **Oxidase Test:** All Neisseria give a **positive oxidase** reaction (Note: *Moraxella catarrhalis* is also oxidase positive, so oxidase alone doesn’t confirm Neisseria, but in context of diplococci it’s suggestive).
* **Catalase**: positive
* **Sugar Utilization (Carbohydrate Fermentation Tests):** Classic definitive test for Neisseria species. They ferment different sugars producing acid:
  + *N. gonorrhoeae* ferments **Glucose only** (often remembered as “Gonococcus = Glucose”).
  + *N. meningitidis* ferments **Glucose and Maltose** (remember “MeninGococcus = Maltose and Glucose”).
  + *N. lactamica* ferments **Glucose, Maltose, and Lactose** (the only Neisseria that ferments lactose; also ONPG positive).
  + *N. sicca* ferments **Glucose, Maltose and** **Sucrose**
  + These tests are performed on cystine-tryptic digest agar with individual sugars (CTA sugars) or by rapid enzyme-based methods. A typical lab will have a set of four sugars (glucose, maltose, lactose, sucrose) to differentiate the common Neisseria.
* **Growth Requirements:** - *N. gonorrhoeae* is fastidious: it **requires enriched media** like chocolate agar and will **not grow on blood agar**, especially if non-CO₂ conditions. It often needs selective media (Thayer-Martin or NYC agar) to isolate from clinical specimens due to its sensitivity and the presence of normal flora. - *N. meningitidis* can grow on blood agar (somewhat less fastidious) but better on chocolate; it grows in 5% CO₂ but can survive without CO₂ better than gonococci. - Commensal Neisseria (e.g., *N. sicca, N. subflava*) grow on nutrient agar at room temperature and may produce pigmented colonies (yellow or whitish). They are less fastidious. -
* **Colony Morphology:** *N. gonorrhoeae* colonies are typically small, grayish and translucent. *N. meningitidis* colonies are larger, mucoid if encapsulated (especially in clinical isolates from CSF). Colony appearance is not definitive but encapsulated *N. meningitidis* may be slimy due to the polysaccharide capsule.
* **Rapid Tests:** Nowadays, confirmatory tests include monoclonal antibody agglutination tests or coagglutination for *N. gonorrhoeae*, and latex agglutination for *N. meningitidis* capsular antigens (e.g., in CSF, but culture is confirmatory). NAAT (nucleic acid amplification tests) are commonly used for gonorrhea diagnosis from clinical samples, but in terms of culture identification, sugar utilization remains a classic method.
* **Additional Differentiation:** *N. gonorrhoeae* is usually **superoxol positive** (30% H₂O₂ drop causes immediate vigorous bubbling – this is an older confirmatory test using a strong catalase reaction unique intensity in gonococci). Also, *N. gonorrhoeae* can be identified by DNA probe or MALDI in modern labs.

**Pathogen-specific notes:**

* *Neisseria gonorrhoeae:*
  + Often identified from urethral, cervical, or pharyngeal specimens. A Gram stain showing **intracellular Gram-negative diplococci** inside polymorphonuclear leukocytes is diagnostic in male urethral exudate (in symptomatic men).
  + Culture on Thayer-Martin
  + oxidase positive colonies
  + ferment glucose (only) confirms gonococcus.
  + Betalactamase production can be tested (many strains penicillin-resistant). All isolates should be tested for antibiotic susceptibility due to rising resistance.
* *Neisseria meningitidis:*
  + If isolated from CSF or blood, it’s a critical find.
  + It ferments both glucose and maltose.
  + Serogrouping (A, B, C, W, Y) is done by agglutination tests on cultures.
  + Colonies may be transparent or mucoid.
  + Identification is via the sugar pattern and often a **latex agglutination** on CSF (though PCR is common now).
* **Commensal Neisseria:** 
  + These include *N. lactamica, N. sicca, N. subflava, N. elongata* (which is rod-shaped actually), etc.
  + They are usually differentiated by broader sugar patterns but are generally of low virulence.
  + *N. lactamica* is noteworthy as it can colonize infants and is ONPG positive (hydrolyzes lactose).
  + Commensals are often identified to ensure they are not misidentified as pathogenic Neisseria. For example, *N. lactamica* (glucose/maltose/lactose +) could be mistaken for N. meningitidis if only glucose/maltose tested; lactose utilization clears that up.

**Differentiating Neisseria from Similar Genera:**

* *Moraxella catarrhalis* (see below) is also Gramnegative diplococci and oxidase positive, but it is **asaccharolytic** (ferments no sugars). Also DNase positive and butyrate esterase positive (Neisseria are DNase negative and butyrate esterase negative).
* *Kingella denitrificans* can resemble Neisseria (Gram-negative coccoid rods that may be in pairs); it is catalase negative (Neisseria are catalase positive) and reduces nitrates (Neisseria do not). This is occasionally mentioned as a mimicker of gonococcus in older literature.
* *Acinetobacter* can appear as Gram-negative cocci (often plump coccobacilli that resist decolorization and may resemble cocci in smears) but Acinetobacter is **oxidase negative**, which immediately separates it from Neisseria.
* *Psychrobacter* and *Enhydrobacter* are mentioned in literature as rare oxidative Gramnegative cocci that could be mistaken, but these are extremely uncommon clinically.
* *Veilonella* – are anaerobic

**Laboratory Confirmation:** Definitive ID of Neisseria in the lab may involve **MALDI-TOF** or **genetic probes** nowadays, but understanding the sugar utilization is a fundamental classic approach.

**Safety Note:** Pathogenic Neisseria (especially *N. meningitidis*) can be a lab hazard via aerosol. Work under a biological safety cabinet is recommended when manipulating cultures (because of meningococcal sepsis risk to lab workers).

# **Moraxella catarrhalis and Similar Organisms**

**Characteristics:**

* *Moraxella catarrhalis* is a Gram-negative diplococcus often indistinguishable morphologically from Neisseria on Gram stain.
* It is a common cause of otitis media, sinusitis, and respiratory infections (especially in COPD patients). *I*t frequently produces beta-lactamase, so amoxicillin alone may fail (need inhibitor or another antibiotic). However, *M. catarrhalis* is usually susceptible to many oral antibiotics (macrolides, SXT, etc.).

**Identification:**

* *M. catarrhalis* grows well on blood and chocolate agar (unlike *N. gonorrhoeae*).
* Colonies are opaque, gray-white, and sometimes **hockey puck** in texture (can be slid across the agar in one piece).
* **oxidase positive** and **catalase positive, but nitrate positive (unlike Neisseria)**
* A defining test is the **Butyrate esterase test (Tributyrin hydrolysis, blue-green)**
* **DNase positive** (it can hydrolyze DNA in a DNase agar test), whereas Neisseria are DNase negative.
* Carbohydrate utilization: *M. catarrhalis* is **asaccharolytic** – it does **not ferment any sugars** in the CTA tests (glucose, maltose, etc. all negative).
* Beta-lactamase production is common (most *M. catarrhalis* produce beta-lactamase, causing resistance to penicillin).

**Other “Morphologically Similar” Organisms:**

**Practical Work-Up for Oxidase-Positive Gram-Negative Diplococci:**

* If isolated from ear/sinus or sputum of adult, consider *M. catarrhalis*. Perform **tributyrin test** – a positive result confirms *Moraxella catarrhalis*.
* If isolated from CSF or blood, consider *N. meningitidis* – do sugar tests (glucose + maltose).
* If from genital tract (and especially if inside PMNs on smear), consider *N. gonorrhoeae* – do sugar test (glucose only) or a rapid NAAT.
* If oxidase-positive cocci but **no sugars utilized** and tributyrin negative, consider unusual strains or misidentified Acinetobacter (recheck oxidase and Gram – Acinetobacter oxidase neg).
* MALDI-TOF can accurately distinguish these, if available.