Gram-negative cocci

Neisseria gonorrhoeae (gonococcus)

Clinical Significance

- Strictly human pathogen and **always pathogenic** when isolated and identified in a biological sample
- Causative agent of **gonorrhoea**, a sexually transmitted infection with an incubation period of 2-7 days

    - **Men**: **acute urethritis** (abundant urethral secretion and dysuria) and complications – urethral strictures, prostatitis, epididymitis
    - **Women**: **endocervicitis** (purulent vaginal secretion and dysuria) or **asymptomatic** with the possibility of further transmission to the sexual partner without noticing. Complications due to lack of or delayed treatment, such as **salpingitis** (inflammation of the fallopian tubes) + infertility, pelvic peritonitis, pelvic inflammatory disease and gonococcal arthritis + skin rash as a rare complication by haematogenous spread
    - **Newborns**: **gonococcal ophthalmia neonatorum** (from mother to newborn via infected birth canal) - may lead to blindness
      Prophylactic treatment: **erythromycin ointment** / **silver nitrate**

Bacteriological Diagnosis

Collection

- Women: **endocervical secretion**
- Men: **urethral secretion**

Microscopic examination

Gram-stained smears of *N. gonorrhoeae* in men are highly sensitive and specific for the diagnosis of gonorrhoea.

Intra- and extracellular, encapsulated gram-negative reniform (kidney-shaped) cocci arranged in pairs (**diplococci)**.

Culture media and Identification

N. gonorrhoeae is a fastidious organism and requires complex cultivation conditions, i.e. a mixture of blood, amino acids and vitamins. A media that provides these requirements is the **Thayer Martin media**, containing **chocolate agar** and **antibiotics (to inhibit the growth of other germs,** making it a selective media):

- **Vancomycin** (inhibits **gram-positive cocci**)
- **Colistin** (inhibits **gram-negative bacilli**)
- **Trimethoprim** (inhibits **Proteus spp.**)
- **Nystatin** (inhibits **fungi**)

Incubation should be performed at **35°C – 37°C** in **5% CO2** atmosphere and **humidity** for 24-72 hours (readings should be performed at 24, 48 and 72 hours)

Identification is based on:

- **Colonial characters:** round, transparent to grey, shiny colonies with a diameter of 0.5 – 1 mm
- **Gram-stained smears** performed from these colonies reveal Gram-negative, kidney-shaped cocci

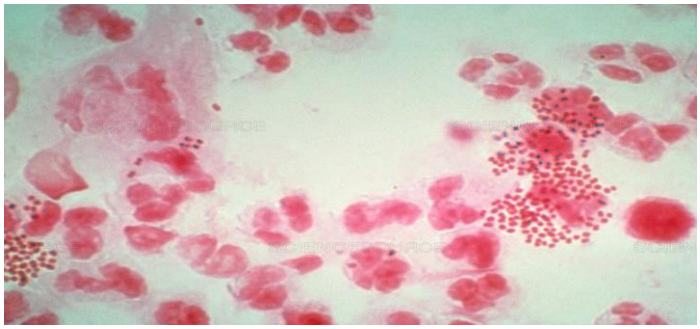


Figure 20. *Neisseria gonorrhoeae:* Microscopic examination from biological specimens

- **Biochemical tests:**
  - Oxidase test: **positive**
  - Catalase test: **positive** (principle: see *S. aureus*)
  - Sugar oxidation test (Utilisation of **sugars** and consequent **acid production**)**:** positive for **glucose**, negative for **maltose, lactose** and **sucrose.**
  - Serogroup identification (agglutination with antisera)

Oxidase test

- Principle: **cytochrome c oxidase** is an enzyme that, as the name suggests, catalyses a reaction leading to the oxidation of cytochrome c, a constituent of the electron transport chain. When the reagent TMPD (Tetra-Methyl-Phenylen-Diamin) is put into contact with a bacterial colony, if the bacteria in that colony possess the enzyme cytochrome c oxidase then the TMPD is oxidised to indophenol. As a result a **dark purple** end product is formed. If the enzyme is absent, addition of the reagent TMPD does not lead to any colour change.
- Procedure: A filter paper is saturated with one drop of TMPD reagent, after which a colony is picked up from the culture plate with a sterile loop and smeared on the filter paper. If the test is positive, the colour will change to dark purple after 10-30 seconds.

- Use: differentiation of Gram-negative bacteria

Antimicrobial susceptibility testing (Antibiogram)

Antimicrobial susceptibility testing is required due to strains exhibiting resistance against penicillins and spectinomycin. In case of penicillin resistance, cephalosporins or fluoroquinolones are prescribed.

Essential to remember:

- Other name: gonococcus
- Causative agent for the STD **gonorrhoea** (women can be asymptomatic and infect sexual partner)
- Gram-negative diplococcus
- Fastidious organism (Thayer Martin media)
- **Oxidase positive**
- **Glucose positive** (lactose, maltose and sucrose negative)
- Penicillin and Spectinomycin resistance

Neisseria meningitidis (meningococcus)

Clinical Significance

- Strictly human pathogen
- Transmission via contaminated respiratory droplets
- May cause severe infections termed “meningococcal disease”:
    - Bloodstream invasion leads to the manifestations of meningococcemia (*N. meningitidis* within bloodstream), meningitis (meningococcal meningitis), petechiae and eventually fulminant meningococcemia (Waterhouse-Friedrichsen syndrome)
- **Indigenous microflora**: may colonize the human oro- and nasopharynx of some people without signs of disease

Bacteriological Diagnosis

Collection

- CSF through a spinal tap before antibiotic treatment - **turbid aspect** suggests bacterial (possibly meningococcal) meningitis
- Blood for haemocultures
- Nasopharyngeal exudate

Transport to the laboratory should be performed as soon as possible, as well as emphasis on protection from light and extreme temperature variations.

Microscopic examination

Microscopic examination is performed after CSF has been centrifuged.

Intra- and extracellular, encapsulated gram-negative cocci with a **reniform** shape, arranged in pairs (**diplococci)**.

Culture media and Identification

- **CSF sediment** inoculated on**:** Blood or chocolate agar – non-haemolytic, grey, transparent, smooth colonies of 1mm in diameter
- **Blood** inoculated on liquid media: examination and reinoculation during 5-7 days
- **Nasopharyngeal exudate** inoculated on Müller-Hinton agar, Selective media with antibiotic content of vancomycin

The identification is based on:

- Colonial characters
- Gram-stained smear from suspected colonies
- Biochemical tests:
    - Oxidase test**: positive**
    - Sugar oxidation test: **positive** for **glucose** and **maltose, negative** for **lactose** and **sucrose**
    - **Serogroup identification** (agglutination with antisera); there are 12 serogroups according to the biochemical composition of the capsular polysaccharide; however, only 6 serogroups (A, B, C, W, X, and Y) are responsible for the majority of disease

Antimicrobial susceptibility testing (Antibiogram)

Most of the strains isolated in Europe are sensitive to beta-lactams, thus antimicrobial susceptibility testing is not routinely performed in diagnostic laboratories.

Prophylaxis

- **Immunization**: Effective anti-meningococcal vaccines have been developed for serogroups A, B, C, W, and Y and have been used to successfully prevent disease due to these serogroups.
- **Chemoprophylaxis**: based on two days of **rifampicin**

Essential to remember:

- Other name: meningococcus
- May cause severe infection: **meningococcal disease**
- Gram-negative diplococcus
- **Oxidase positive**
- **Glucose and maltose positive** (lactose and sucrose negative)

Gram-positive bacilli

Corynebacterium diphtheriae

Clinical Significance

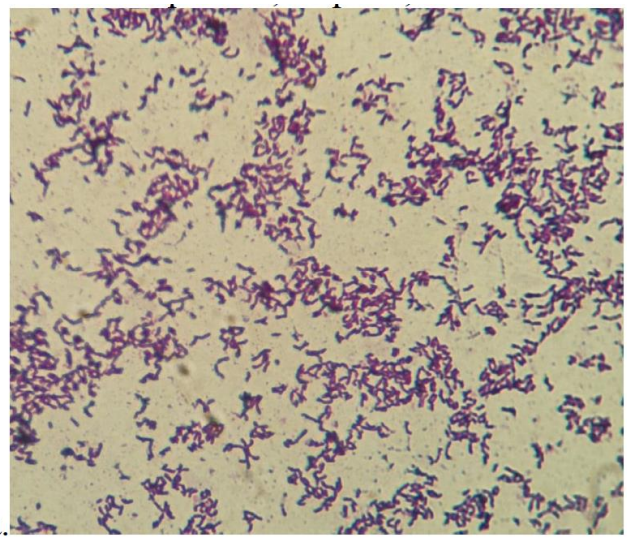
- High pathogenicity
- Direct transmission from human to human
- Causative agent for **diphtheria**, due to production of the **diphtheric toxin**
    - Manifestations at entry gate: pharyngitis (sore throat) and dysphagia, formation of an **adherent membrane** (pseudomembrane) in the throat that may lead to obstruction of the airway
    - Systemic manifestations: fever, myocarditis, neuritis and kidney damage due to the haematogenic diffusion of the diphtheric toxin

Bacteriological Diagnosis

Collection

- Throat swab
- Nasal swab
- Wound swab

Microscopic examination

a. 

b. 

Figure 21. Microscopic examination for *C. diphtheriae*

Microscopic examination for *C. diphtheriae* is of low diagnostic value due to the morphological similarities to other commensal corynebacteria (diphtheroid bacilli) in the throat. Gram-staining will reveal slightly curved Gram-positive bacilli that are disposed in a fashion that resemble "Chinese letters".

Culture media and Identification

Cultivation is performed on:

- Blood agar: non-haemolytic, white-grey colonies with **striated margins**
- Selective media with tellurite (Tinsdale media): small, black colonies with brown halo due to reaction between H_2S produced by bacteria and potassium tellurite within the media
- Highly selective Löffler medium: white, creamy colonies on slant

Identification is based on:

- Colonial characters
- Biochemical tests:
    - Urease test: **negative** result
    - Elek's test: **positive** result

The challenge in the diagnosis of *C. diphtheriae* is first to differentiate between *C.* ***diptheriae*** and **diphtheroid bacilli** (that pertain to the indigenous microflora) and further to discriminate between **toxigenic** and **non-toxigenic** strains of *C. diphtheriae* (as only the toxigenic strains cause diphtheria). The **urease test** is performed to differentiate *C. diphtheriae* from diphtheroid bacilli, while **Elek's test** is used to differentiate the toxigenic from the non-toxigenic strains of *C. diphtheriae*.

Urease Test

For the urease test a liquid medium containing urea and a phenol red indicator is used. The principle is based on an enzyme called **urease** that decomposes the urea within the culture medium, leading to a change in colour of the medium to pink, when this reaction takes place (phenol red indicator).

Results:

- **Positive test** = colour change to pink **diptheroid bacilli**
- **Negative test** = no colour change ***Corynebacterium diphtheriae***

Elek's test

Elek agar is inoculated with streaks of the bacterial culture to be tested. Then, a strip of sterile filter paper that is impregnated with a diphtheric antitoxin is placed on the surface of the culture medium perpendicular to the bacterial streaks. After an incubation period of 24 hours, the result is read. A positive result means that precipitation lines radiating in a 45° fashion out of the area where the bacterial culture meets the strip impregnated with diphtheric antitoxin are visible, indicating a toxigenic strain.

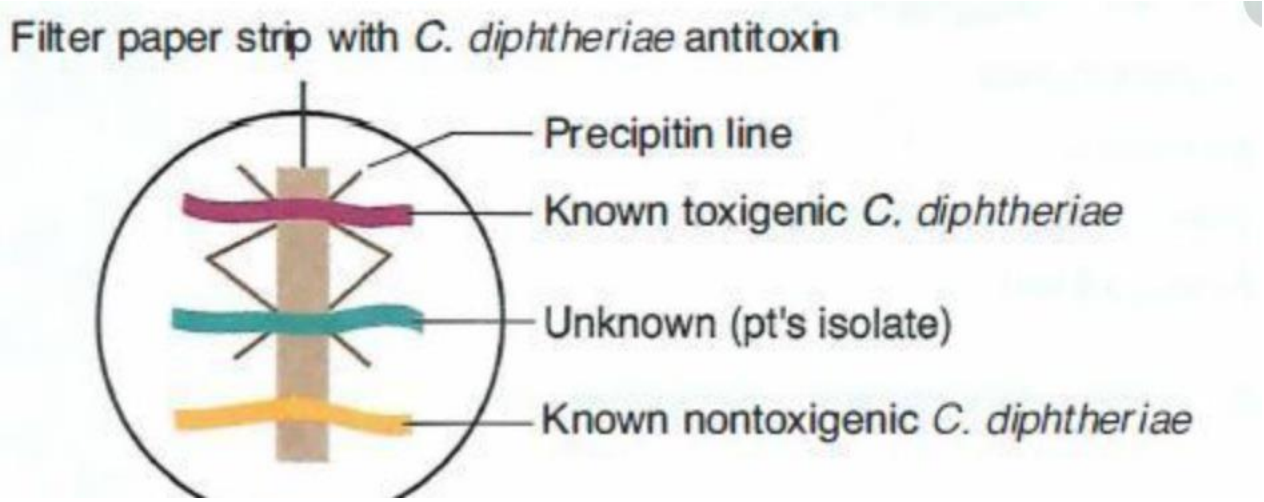


Figure 22. Elek Test

Antimicrobial susceptibility testing (Antibiogram)

C. diphtheriae is sensitive to penicillin, vancomycin and erythromycin that are given as soon as a clinical suspicion on diphtheria exists – even before the laboratory has confirmed the diagnosis. The treatment necessarily includes administration of diphtheric antitoxin. Delay or lack of treatment leads to death of the patient due to the effects of the diphtheric toxin, which inhibits protein synthesis organ necrosis: heart, liver, kidney and neurologic lesions.

Very importantly, diphtheria is a **vaccine preventable disease**. Vaccination protocols include trivalent vaccines for Diphtheria, Tetanus and Pertussis or pentavalent vaccines for *Haemophilus influenzae* type B, Diphtheria, Tetanus, Pertussis and Hepatitis B.

Essential to remember:

- Toxigenic strains causative agents for **diphtheria**
- Gram-positive bacilli with “Chinese letter” disposition
- Tinsdale medium: small, black colonies with brown halo
- **Urease negative**
- **Elek’s test positive**
- **Vaccine preventable disease**

Bacillus anthracis

Clinical Significance

- Aetiological agent of **anthrax**: zoonosis (infection of animals and humans)
- High pathogenicity
- Produces endospores survive in soil for many years
- Clinical forms (depending on site of entry)
    - **Cutaneous anthrax** (most common, 99%): spores enter body via skin lesions
    - **Pulmonary anthrax**: inhalatory infection
    - **Digestive anthrax**: ingestion of undercooked meat with spores
        ➔ Pulmonary and digestive forms more serious than cutaneous anthrax
- May be used as a **biological weapon**
- Habitat: widespread in nature (soil, water) and animals (cows, goats, horses, sheep)
        ➔ Patients at risk: contact with infected animals or their products, e.g. meat, wool.

Bacteriological Diagnosis

Collection

Depending on the site of entry and infection:

- Sputum
- Vomit
- Skin biopsy

Microscopic examination

Microscopic examination reveals **large**, **Gram-positive** bacilli (10µm long, 1.5µm wide) with straight cut ends that are often disposed in chains. Furthermore, **central-to-terminal spores** should be visible during examination.

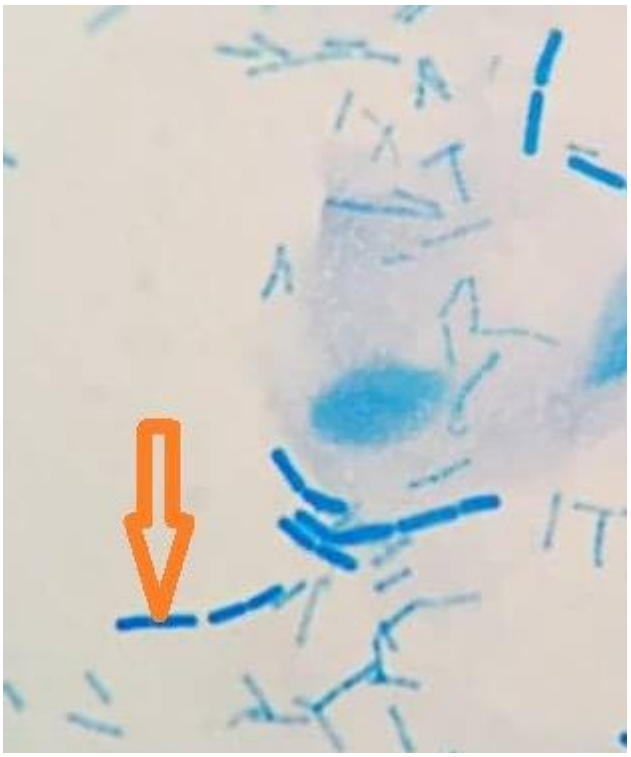


Figure 23. *Bacillus* sp. - Microscopic examination from a biological specimen- slide stained with methylene blue

Culture media

B. anthracis is a non-fastidious germ, meaning that it grows well on **blood agar**.

- Colonial characters: large, flat and irregular (2-5mm) non-haemolytic white or grey colonies with irregular margins and ground-glass aspect, sometimes with comma shape.

Antimicrobial susceptibility testing (Antibiogram)

Treatment: Penicillin G, Erythromycin and Tetracycline

Essential to remember:

- Causative agent of anthrax
- Large, Gram-positive bacilli with straight cut ends often disposed in chains
- Central-to-terminal spores
- Irregular margins on blood agar