Staining of Bacteria

- Bacteria cells are almost colorless and transparent
- A staining technique is often applied to the cells to color them
- Their shape and size can be easily determined under the microscope.

Types of Stain

SIMPLE STAIN.
- One dye.
- Study morphology & structure of organisms.
- Types: Positive & Negative stains.

DIFFERENTIAL STAIN.
- More than 2 dyes.
- Differentiate the organisms.
- Gram stain.

SPECIAL STAIN.
1. Acid fast (Ziehl-Neelsen stain).
2. Capsular Stain.

Composition.
1. Methyl (crystal) violet 1%, 1¹⁷ stain.
2. Iodine solution, mordant, CV-I.
3. Alcohol 95%, decolorizer, organic solvent; acetone/ethanol or both.
4. Diluted carbol fuchsin 0.1%/Safranin, counter basic stain.

Gram Stain
- It is the most important differential stain used in bacteriology because
- it classified bacteria into two major groups

<table>
<thead>
<tr>
<th>Gram positive</th>
<th>Gram negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Appears violet after Gram’s stain</td>
<td>Appears red after Gram’s stain</td>
</tr>
</tbody>
</table>

Gram –ve bacilli
Non specific arrangement

Gram +ve bacilli
Chain arrangement

Gram +ve cocci
Clusters arrangement
THE GENUS NEISSERIA

The genus Neisseria contains

<table>
<thead>
<tr>
<th>Pathogenic neisseria</th>
<th>Non pathogenic (commensal)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N. gonorrhoae: causes gonorrhoea</td>
<td>Neisseria occurs in normal flora</td>
</tr>
<tr>
<td>N. meningitides: causes acute cerebrospinal meningitis.</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Temp.</th>
<th>Room temp.</th>
</tr>
</thead>
<tbody>
<tr>
<td>37</td>
<td></td>
</tr>
<tr>
<td>Ordinary media</td>
<td>Grow</td>
</tr>
<tr>
<td>Thyer Martin medium</td>
<td></td>
</tr>
<tr>
<td>Colony pig</td>
<td>May</td>
</tr>
<tr>
<td>Reaction with antimeningococcal sera</td>
<td>No</td>
</tr>
</tbody>
</table>

PATHOGENIC NEISSERIA

N. gonorrhoea and N. meningitidis are very similar in their morphology & cultural characters so can be described together.

<table>
<thead>
<tr>
<th>MORPHOLOGY OF PATHOGENIC NEISSERIA</th>
<th>CULTURAL CHARACTERS OF PATHOGENIC NEISSERIA</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Gram negative</td>
<td>Aerobic or facultative anaerobe</td>
</tr>
<tr>
<td>• Kidney shaped diplococci (flat/concave sides adjacent)</td>
<td>Media used for isolation</td>
</tr>
<tr>
<td>• Non motile &amp; non spore forming.</td>
<td></td>
</tr>
<tr>
<td>• N. meningitidis have polysaccharide capsule.</td>
<td></td>
</tr>
<tr>
<td>- Opt temp: 37 °C, w narrow temp range (30-38 °C)</td>
<td>No growth on ordinary media</td>
</tr>
<tr>
<td>- No growth at 22°C.</td>
<td>Grow on chocolate agar.</td>
</tr>
<tr>
<td>- 10% Co2 &amp; moist atmosphere are required for growth.</td>
<td>Thayer-Martin media is selective media for Neisseria.</td>
</tr>
</tbody>
</table>

Microscopic Pictures Of Neisseria (Gram-negative Diplococci)

Rectal Specimen (Testing for Neisseria gonorrhoea)

Chocolate Medium Overgrowth  Thayer-Martin Medium Neisseria Only
NEISSERIA GONORRHEA (GONOCOCCI)

CLINICAL MANIFESTATIONS OF GONORRHEA

It is a sexually transmitted disease.

second most common venereal disease

<table>
<thead>
<tr>
<th>Asymptomatic infection</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>See in 10%</td>
<td>&gt;30%</td>
</tr>
<tr>
<td></td>
<td>↑ risk of developing disseminated disease</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Act as reservoir for transmission.</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Primary disease</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute urethritis: characterized by purulent discharge</td>
<td>cervicitis characterized by vaginal mucopurulent discharge</td>
<td>Vulvovaginitis: Occurs in girls 2-8 years of age</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Invasion from the primary disease focus</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>spread to</td>
<td>may result in</td>
<td>resulting in</td>
</tr>
<tr>
<td>1. posterior urethra</td>
<td>• fibrosis</td>
<td>• endometritis</td>
</tr>
<tr>
<td>2. prostrate</td>
<td>• urethral</td>
<td>• salpingitis</td>
</tr>
<tr>
<td>3. epididymis</td>
<td>strictures</td>
<td>• finally</td>
</tr>
<tr>
<td></td>
<td>• permanent</td>
<td>peritonitis</td>
</tr>
<tr>
<td></td>
<td>sterility.</td>
<td>may cause</td>
</tr>
<tr>
<td></td>
<td></td>
<td>permanent</td>
</tr>
<tr>
<td></td>
<td></td>
<td>sterility &amp; ectopic pregnancy.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Disseminated Gonococcal Infection (DGI)</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>may occur in approx. 1% of either males or females infected, usually those that are asymptomatic.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Gonococcal bacteremia may lead to dermatitis-arthritis syndrome, endocarditis &amp; meningitis which are rare.</td>
<td></td>
</tr>
</tbody>
</table>

TREATMENT

The recommended treatment for uncomplicated infections is:
third-generation cephalosporin or fluoroquinolone.
NEISSERIA MENINGITIDIS (MENINGOCOCCUS)
- resides in man only (mostly young children)
- outbreaks
  1. adults
  2. crowded conditions
e.g. army barracks
- 1-4 days
- Second most common meningitis (pneumococcus)
- Fatal if untreated
- Responds well to antibiotic therapy (penicillin)

ANTIGENIC COMPOSITION
- Meningococcal capsular polysaccharides provide the basis for grouping these organisms.
- 12 serogroups have been identified (A, B, C, H, I, K, L, X, Y, Z, 29E, and W135).
The most important serogroups associated with disease in humans are A, B, C, Y, and W135.

PATHOGENECITY
- Humans are the only reservoirs.
- Source of infection is case or nasopharyngeal carrier.
- Transmitted by droplet infection to nasopharynx, to blood stream by the way of cervical lymph nodes causing bacteremia with fever, malaise & skin rash, then cross the blood brain barrier & infect meninges.

LABORATORY DIAGNOSIS OF ACUTE CEREBROSPINAL MENINGITIS (MENINGOCOCCAL MENINGITIS)

<table>
<thead>
<tr>
<th>Laboratory diagnosis</th>
<th>Identification of culture</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample</td>
<td>Colonies</td>
</tr>
<tr>
<td>Direct smear</td>
<td>Film</td>
</tr>
<tr>
<td>Culture</td>
<td>BR</td>
</tr>
<tr>
<td>Special</td>
<td>Animal pathogenicity</td>
</tr>
</tbody>
</table>

A) DIAGNOSIS OF A CASE

SAMPLES
Meningococci are found in CSF, blood, skin rash & in nasopharynx early in the disease.

1. Aseptically to prevent introduction of m.o into the CNS.
2. Sterile, wide-bore needle (4) 4th and 5th lumbar vertebrae
3. Collect in 3 containers sequentially
   N. 1: 1ml for culture
   N. 2: 2-3ml for other tests (Smear, protein, glucose)
   N. 3: preserve

CSF
- Obtained by lumbar puncture under complete aseptic precautions.
- Turbid & under tension due to presence of pus cells.
- Lumbar puncture under complete aseptic precautions.

4. No delay in examination of the sample/reporting the result esp. the Gram smear
5. If exam will be delayed → Do not refrigerate, keep at 35-37

- If the patient was given antibiotic, it will be more difficult to detect bacteria in Gram-stained smear & to isolate pathogen from culture.
- others: S. pneumoniae, H. influenza,
- E. coli, other coliform, S. agalactiae, L. monocytogenes ‘neonates’
- TB “if suspected → do ZN smear”
- Intra-cellular/ extra-cellular due to ruptured pus cells during spreading the smear or heat-fixation so, use methanol for fixation.
DIRECT FILM
- Stained with Gram stain.
- Gram negative
- Kidney shaped diplococcic
- Capsulated
- Non-motile and non-spore forming
- Present inside pus cells.

CULTURE
- Aerobic or facultative anaerobe.
- Optimum temperature: 37 °C, with narrow temperature range (30-38°C), no growth at 22°C.
- 10% Co2 and moist atmosphere are required for growth. (48hrs)
- It cannot be grown on ordinary media, can grow on chocolate agar.
- Thayer-Martin media is selective media for Pathogenic Neisseria

Culture
1. Culture characters
   - OT : 37C
   - CO2: 5-10% (48h)
   - O2: arobe
   Mac Conkey ‘newborn’ for E. Coli
   - Blood for S. pneumoniae

2. Media
   - Enriched Hemolysis
   - Selective
   - Ordinary (x growth)
   Blood agar
   - enriched medium for isolation of fastidious organisms
   - indicator or differential medium

3. Colony
   i. Characters ‘describe’
   ii. B.Rs : oxidase & sugar fermentation
   iii. Gram stained film
   - because it can differentiate the species of the organism acc. to its effect on the RBCs
   - e.g: S. aureus is beta- hemolytic due to production of hemolysin toxin.

IDENTIFICATION OF THE OBTAINED GROWTH
1. Colony morphology.
2. Gram stained films.

Rapid diagnosis of meningitis: by detection of meningococcal antigen in CSF.

4. Biochemical reactions:
<table>
<thead>
<tr>
<th>Oxidase test</th>
<th>Sugar fermentation</th>
</tr>
</thead>
<tbody>
<tr>
<td>- all pathogenic Neisseria are oxidase positive.</td>
<td></td>
</tr>
<tr>
<td>- They produce oxidase enzyme, which can reduce oxidase reagent to a deep purple color.</td>
<td></td>
</tr>
<tr>
<td>- N. gonorrhoeae ferment glucose with acid production only.</td>
<td></td>
</tr>
<tr>
<td>- N. Meningitidis ferment glucose &amp; maltose with acid production</td>
<td></td>
</tr>
</tbody>
</table>
B) DIAGNOSIS OF CARRIER

- Nasopharyngeal swab is examined as in diagnosis of case.
- The obtained growth should be differentiated from commensal neisseria.

PREVENTION OF ACUTE CEREBROSPINAL MENINGITIS

i. Capsular polysaccharide vaccines

- ACWY capsular polysaccharide vaccines are available.
- Polysaccharide vaccines are ineffective in young children (under 1 year old) & the duration of protection is limited in children vaccinated at 1 to 4 years of age.
- Routine vaccination is not currently recommended because risk of infection is low.
- Group B capsular polysaccharide is a homopolymer of sialic acid & is not immunogenic in humans.
- A group B meningococcal vaccine consisting of outer membrane protein antigens has recently been developed.

ii. Chemoprophylaxis

- Sulfonamides were the chemoprophylactic agent of choice until the emergence of sulfonamide-resistant meningococci.
- Rifampicin is the chemoprophylactic agent of choice.

TREATMENT: (NEISSERIA MENINGITIDIS)

- Penicillin is the drug of choice.
- Penicillin does not penetrate the normal blood-brain barrier, but can penetrate the blood-brain barrier when the meninges are acutely inflamed.
- Chloramphenicol or a third-generation cephalosporin such as cefotaxime is used in persons allergic to penicillin.