Practical 7 ST
anaerobic cultivation

• Bacteria in relation to oxygen
• Anaerobic infections - sampling and transport
• Cultivation
  - establishing of anaerobic environment
  - anaerobic culture media,
  - procedure: inoculation, isolation
Anaerobes

- are the predominant components of the skin’s and mucous membranes normal flora - cause infections of endogenous origin
- mixed infections caused by numerous aerobic and anaerobic bacteria are often observed in clinical situations.
Anaerobic infections

• Usually endogenous bacteria - disturbance of the balance of normal flora (ATB and colon microflora) or unusual anatomic sites (perforation of colon - anaerobic peritonitis)

• Exogenous bacteria - wound contamination

• Exudate, pus, abscess

• Sampling and transport in anaerobic conditions - aspirate in the syringe with the needle capped or protected with rubber. Surgical sampling can be required. Never use the dry swab!
Anaerobic bacteria can be divided into:

- strict anaerobes that cannot grow in the presence of more than 0.5% oxygen
- moderate anaerobic bacteria that are able of growing between 2 to 8% oxygen.
- Anaerobic bacteria usually do not possess catalase
- can generate superoxide dismutase which protects them from oxygen.
• **Facultative anaerobes** - can grow in the presence or absence of oxygen

• Obtain energy by both respiration and fermentation

• Oxygen not toxic, some use nitrate \((\text{NO}_3^-)\) or sulphate \((\text{SO}_4^{2-})\) as a terminal electron acceptor under anaerobic conditions
• **Obligate (strict) anaerobes** - oxygen is toxic to these organisms, do not use oxygen as terminal electron acceptor

• e.g Clostridia, Bacteriodes etc....
• Microaerophilic organisms - require low levels of oxygen for growth, but cannot tolerate the levels present in the atmosphere

• Aerotolerant Anaerobes: Metabolism is anaerobic but they are unaffected by the presence of oxygen.

<table>
<thead>
<tr>
<th>Group</th>
<th>Aerobic</th>
<th>Anaerobic</th>
<th>O₂ Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Obligate Aerobe</td>
<td>Growth</td>
<td>No growth</td>
<td>Required (utilized for aerobic respiration)</td>
</tr>
<tr>
<td>Microaerophile</td>
<td>Growth if level not too high</td>
<td>No growth</td>
<td>Required but at levels below 0.2 atm</td>
</tr>
<tr>
<td>Obligate Anaerobe</td>
<td>No growth</td>
<td>Growth</td>
<td>Toxic</td>
</tr>
<tr>
<td>Facultative Anaerobe (Facultative Aerobe)</td>
<td>Growth</td>
<td>Growth</td>
<td>Not required for growth but utilized when available</td>
</tr>
<tr>
<td>Aerotolerant Anaerobe</td>
<td>Growth</td>
<td>Growth</td>
<td>Not required and not utilized</td>
</tr>
</tbody>
</table>
Bacteria in relation to oxygen

- Obligate aerobic - require gaseous oxygen, cannot grow without oxygen
- Obligate anaerobic - oxygen is toxic for them. Metabolic pathway used for gaining energy is fermentation with production of foul-smelling end products.
- Facultative anaerobic - able to adapt to aerobic or anaerobic
- Microaerophilic - require reduced oxygen tension
Oxygen Toxicity

• Oxygen is used by aerobic and facultatively anaerobic organisms as its strong oxidising ability makes it an excellent electron acceptor.

• During the stepwise reduction of oxygen, which takes place in respiration toxic and highly reactive intermediates are produced reactive oxygen species (ROS).
The clinically important anaerobes

• 1. Gram-negative rods (*Bacteroides*, *Prevotella*, *Porphyromonas*, *Fusobacterium*),
• 2. Gram-positive cocci (primarily *Peptostreptococcus* spp.);
• 3. Gram-positive spore-forming (*Clostridium* spp.) and nonspore-forming bacilli (*Actinomyces*, *Propionibacterium*, *Eubacterium*, *Lactobacillus* and *Bifidobacterium* spp.);
• 4. Gram-negative cocci (mainly *Veillonella*)
Anaerobic environments

- Anaerobic environments (low reduction potential) include:
  - Sediments of lakes, rivers and oceans; flooded soils, intestinal tract of animals; oral cavity of animals, ...
  - Anaerobes also important in some infections, e.g. *C. tetanii* and *C. perfringens* important in deep puncture wound infections
• Culture of anaerobes is extremely difficult due to the need to exclude oxygen, slow growth and complex growth requirements
Culture methods

- Most common adaptation of media is the addition of a reducing agent, e.g. thioglycollate, cysteine.

- Acts to reduce the oxygen to water, brings down the redox potential -300mV or less.

- Can add a redox indicator such as rezazurin, pink in the presence of oxygen - colourless in its absence.
Redox potential

+500 mV

OBLIGATE AEROBE
MICRO AEROPHILIC
FACULTATIVE
OBLIGATE ANAEROBE

BACTERIAL GROWTH

- 300 mV
Culture media

- Blood agar
- Blood agar - enriched with growths factors and antibiotics (selective, kanamycin, neomycin, aminoglycosides - to inhibit most aerobic and facultative anaerobic bacteria)
- VL agar, VF bouillon
- Should be stored in refrigerator in plastic bag - decrease the solution of oxygen in the agar - use freshly prepared media
Establishing of anaerobic conditions

- Candle jar - microaerophilic, facultative anaerobic
- Anaerobic jar with atmosphere generator (foil envelopes): release hydrogen and carbon dioxide - after addition of water.
- Biological method - cultivation with *Serratia marcescens*
- Tubes of broth cultures (thioglycolate, cooked meat) - their formulations contain reducing substances - anaerobic conditions at the lower part - regeneration - 15 minutes in boiling water bath
Innoculation, isolation

- Innoculation without delay
- Innoculation of anaerobic and aerobic culture (anaerobic jar, candle jar or anaerobic incubator)
- 48hrs
- Often mixed infection
- Comparison of aerobic and anaerobic culture
- Gram staining: principal is the presence of anaerobic infection G+coccus, G+rod, G-coccus, G- rod, sporulation
Algorythm

- anaerobic jar
- aerobic incubator
- anaerobic
- facultatively anaerobic
- aerobic
P. aeruginosa  Strict aerobe

Enterococcus  Facultative
Grows aerobic or anaerobic.

Bacteriodes fragilis
Anaerobic jar

(a) Clamp
Air-tight lid
Chamber
Envelope containing chemicals to release $\text{CO}_2$ and $\text{H}_2$
Petri plates

(b) Palladium pellets to catalyze reaction removing $\text{O}_2$
Methylene blue (anaerobic indicator)

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Biological method

• Half of medium in the Petri´s dish is inoculated with the tested sample, the other with Serratia marcescens - bacteria able to produce anaerobic environment by the consumption of oxygen. Petri dishes is sealed with the wax or parafin and introduced to the not oxygen - free incubator.