# 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 can not 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 ($NO_3^-$) or sulphate ($SO_4^{2-}$) 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

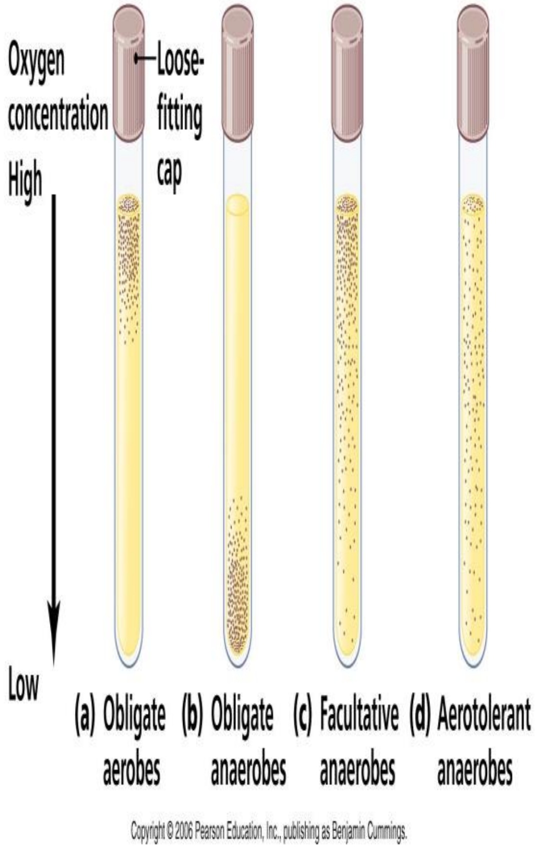
- e.g Clostridia, Bacteriodes etc....

- **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.**

| Environment | | | |
|---|---|---|---|
| **Group** | **Aerobic** | **Anaerobic** | **$O_2$ Effect** |
| Obligate Aerobe | Growth | No growth | Required (utilized for aerobic respiration) |
| Microaerophile | Growth if level not too high | No growth | Required but at levels below 0.2 atm |
| Obligate Anaerobe | No growth | Growth | Toxic |
| Facultative Anaerobe (Facultative Aerobe) | Growth | Growth | Not required for growth but utilized when available |
| Aerotolerant Anaerobe | Growth | Growth | Not required and not utilized |

# Bacteria in relation to oxygen

- Obligate aerobic -require gaseous oxygen, cannot grow without oxygen
- **Obligate anaerobic - oxygen is toxic for them. Methabolic 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

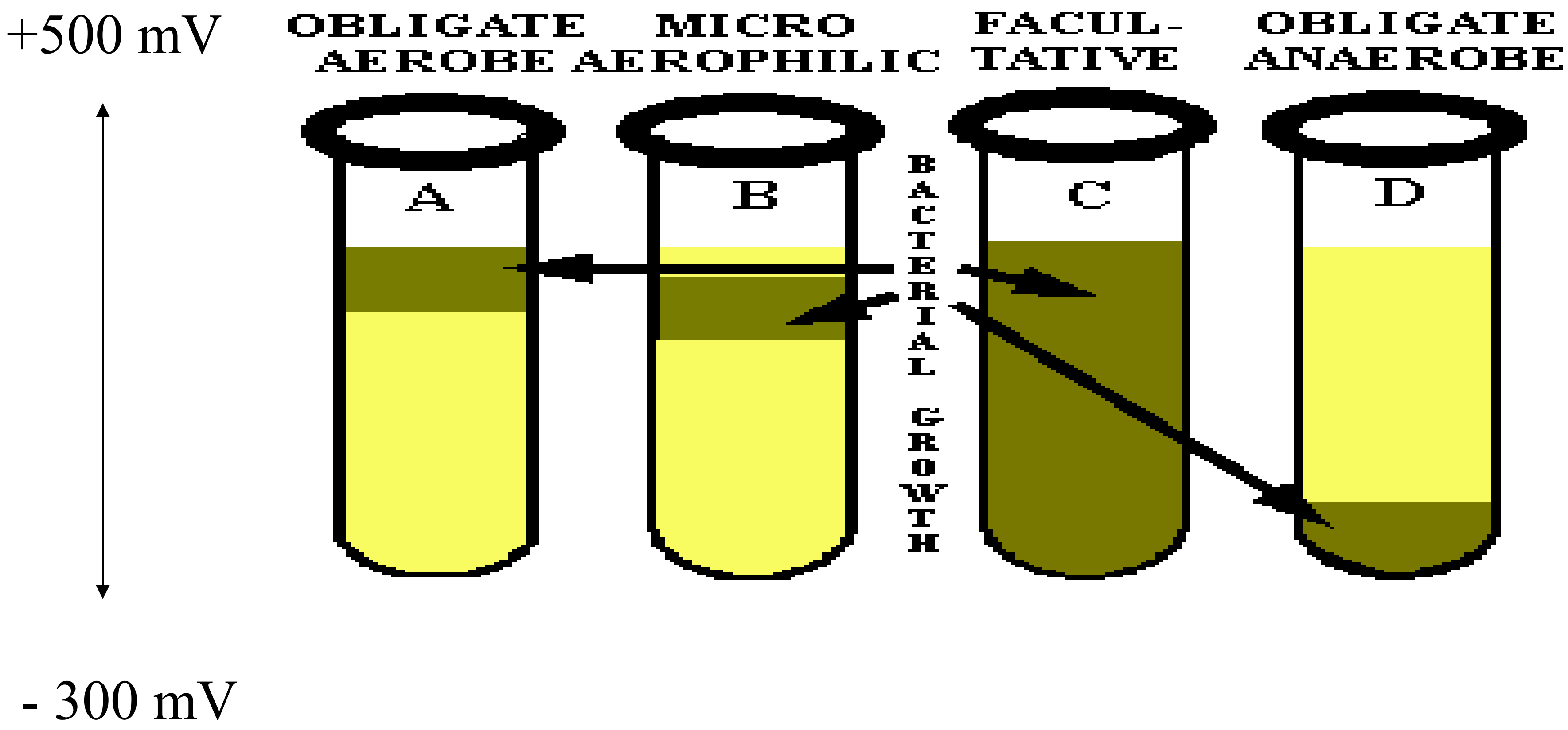
- Anaerobic environments (low reduction potential) include:
- Sediments of lakes, rivers and oceans; flooded soils, intestinal tract of animals; oral cavity of animals, ...
- Anaerobes also important in some infections, e.g. *C. tetanii* and *C. perfringens* important in deep puncture wound infections

- 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 oyxgen - colourless in its absence

# Redox potential



# Culture media

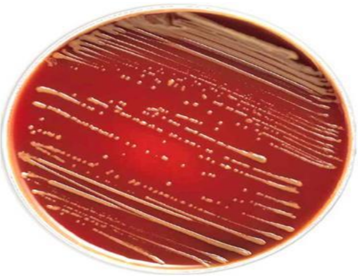
- Blood agar
- Blood agar - enriched with growths factors and antibiotics (selective, kanamycin, neomycin, aminoglycosides - to inhibit most aerobic and facultative anaerobic bacteria)
- VL agar, VF bouillon
- Should be stored in refrigerator in plastic bag - decrease the solution of oxygen in the agar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

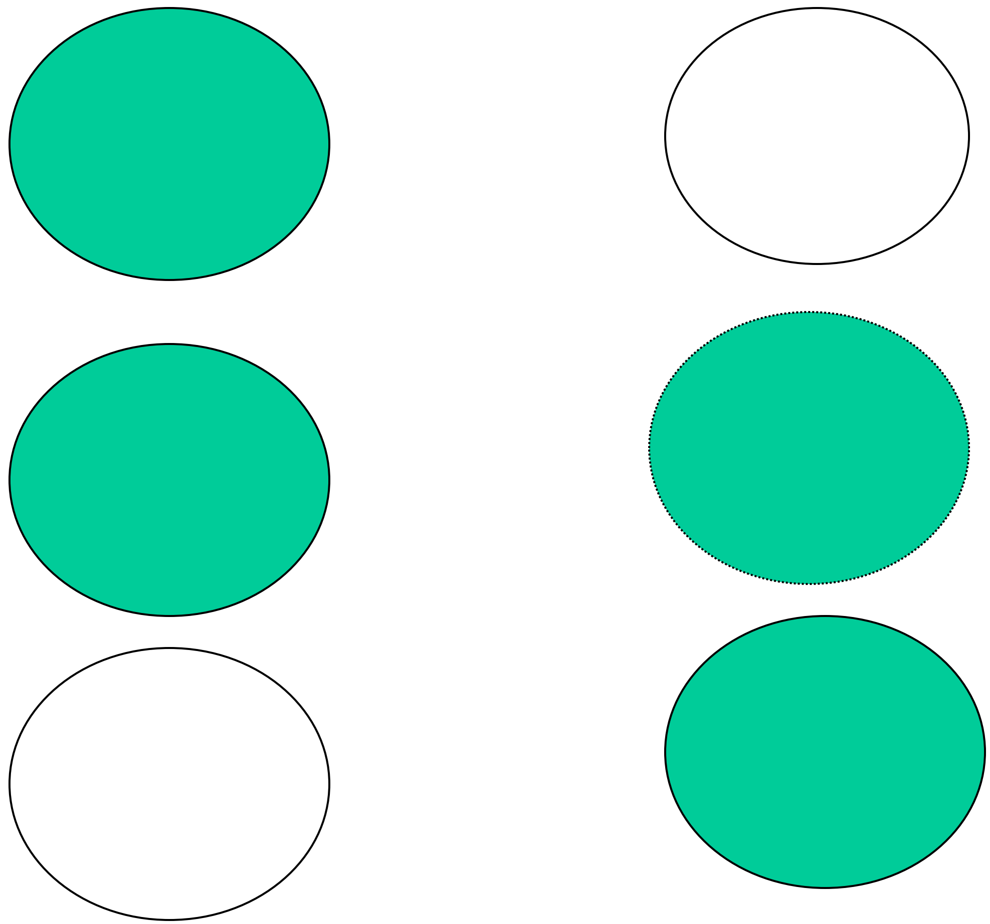
# Innoculation, isolation

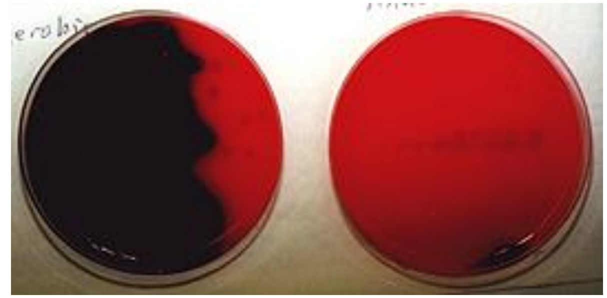
- Innoculation without delay
- Innoculation of anaerobic and aerobic culture (anaerobic jar, candle jar or anaerobic incubator)
- 48hrs
- Often mixed infection
- Comparison of aerobic and anaerobic culture
- Gram staining: principal is the presence of anaerobic infection G+coccus, G+rod, G-coccus, G-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



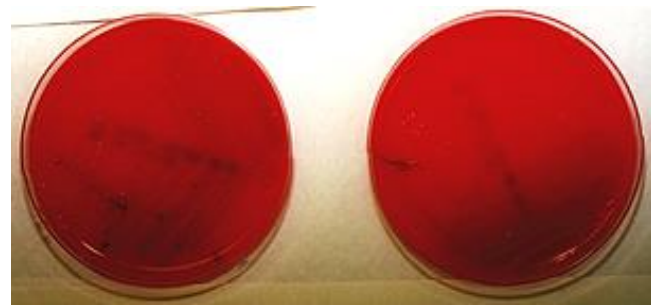
# Algorythm

- anaerobic jar aerobic incubator
- anaerobic
- facultatively anaerobic
- aerobic





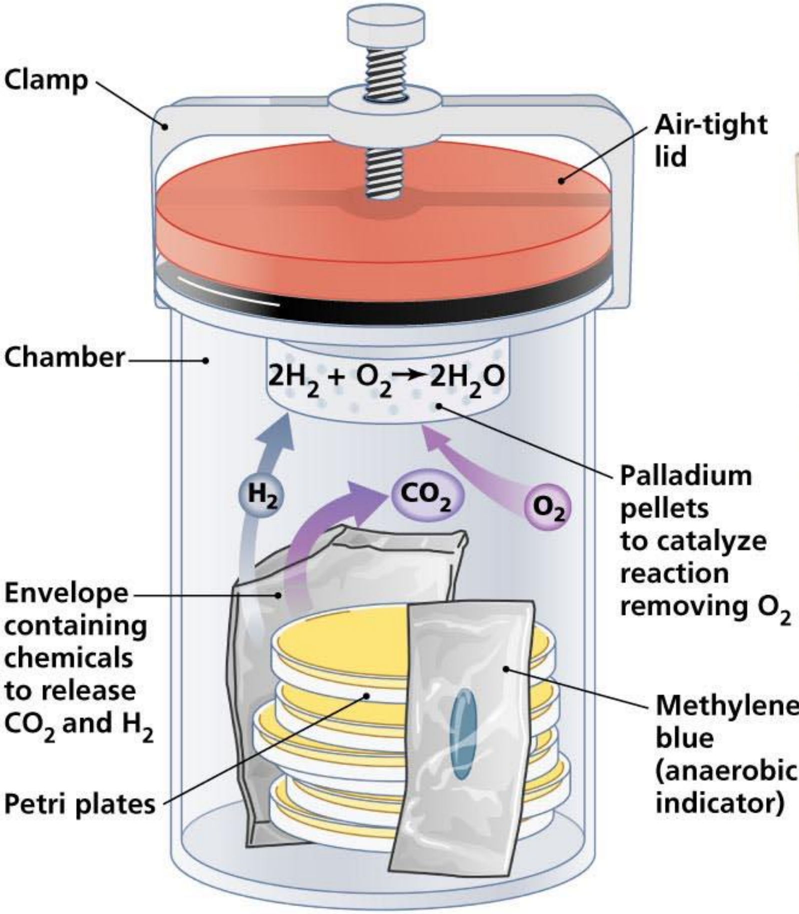
*P. aeruginosa* Strict aerobe



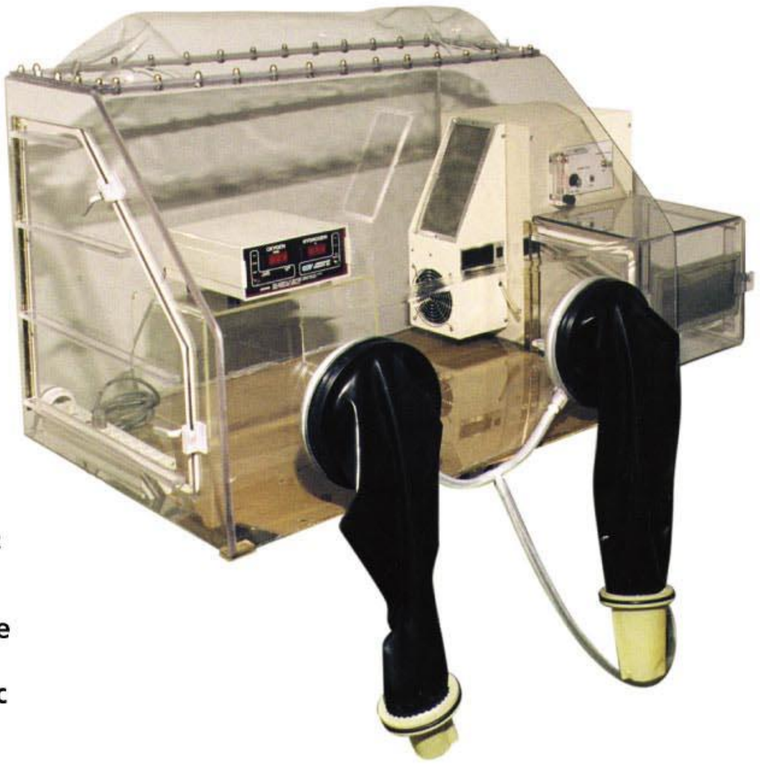
*Enterococcus* Facultative
Grows aerobic or anaerobic.

*Bacteriodes fragilis*

# Anaerobic jar



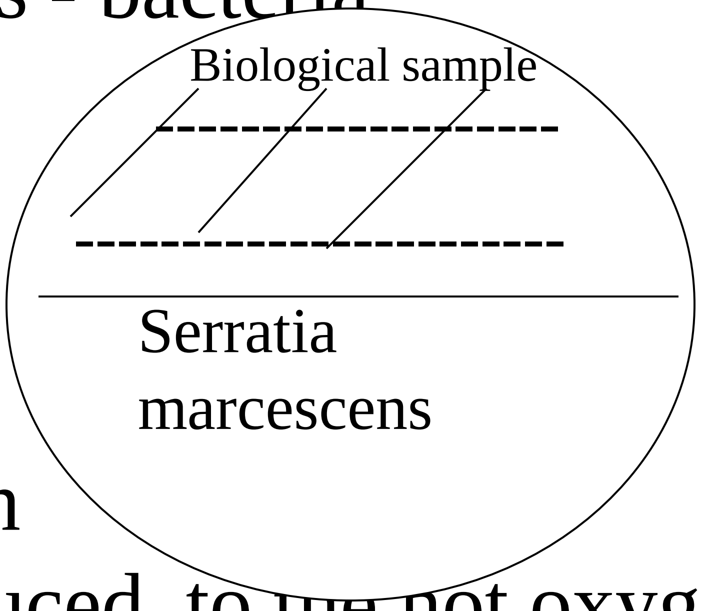

(a)



(b)

# Biological method

- Half of medium in the Petri´s dish is innoculated 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 hot oxygen - free incubator




obrigado
Dank U
Merci
mahalo
Köszi
спасибо
Grazie
Thank you
mauruuru
Takk
Gracias
Dziękuję
Děkuji
danke
Kiitos