ANAEROBIC MEDIA, SAMPLE COLLECTION, AND CULTURE TECHNIQUE

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Bacterial classification by O_2 requirement:-

1. **Obligate aerobes**: require O_2 for growth i.e. O_2 acts as electron acceptor e.g. *Ps aeruginosa.*
2. **Obligate anaerobes**: grow only in complete absence of O_2. O_2 is toxic to bacteria e.g. *Cl. tetani.* Inorganic compound acts as an electron acceptor.
3. **Facultative anaerobes**: can grow under aerobic and anaerobic conditions e.g. *E. coli*.
4. **Microaerophilic**: grow best at low O_2 tension e.g. *H. pylori*.

When to suspect Anaerobic infections:

- **Foul smelling discharge.**
- **Necrotic gangrenous tissue and abscess formation.**
- **Free gas in tissue.**
- **Black discoloration of exudates (*Bacteroides melaninogenicus*).**
- **Sulphur granules in discharge (*Actinomyces spp.*).**
- **Bacteraemia or endocarditis with NO growth on aerobic** blood cultures.

What are risk factors for anaerobic infection?

- **Poor blood supply and tissue necrosis:**

  - Trauma.
  - Foreign body.
  - Malignancy.
  - Surgery.

- **Diabetes mellitus.**
- **Splenectomy.**
- **Immuno-compromised patients.**

Sites with normal anaerobic flora:

- Mouth
- Throat
- Vagina
- Cervix
- Skin folds
- Intestine

Caution: when sampling these sites not suitable for anaerobic culture but can cause anaerobic infections in nearby tissues after trauma.

Suitable specimens for Anaerobic culture?

- These include:-

- Abscesses, Bites,
- Blood, Cerebrospinal fluid (CSF),
- Exudative body fluids,
- Deep wounds,
- Sterile surgical samples
- Dead tissues.
- Tissue samples and biopsies.
- Transtracheal aspirate.
- Endometrial swabs.

Unsuitable specimens:

1. Specimens from sites in which anaerobic bacteria are normal flora (e.g., throat, rectal swabs, urine, bronchial washes, cervico-vaginal mucosal swabs, sputum, saliva).
2. Voided and catheterized urine.
3. Gastric contents (lavage), small bowel contents, feces, colocutaneous fistula and colostomy contents should not be cultured for anaerobic bacteria.

4. Specimens not submitted in anaerobic transport media.
5. Improperly labeled specimen.
6. Follow the rejection policy for unacceptable specimen.

Sampling for anaerobic culture:

1- Aspirate the specimen using a NEEDLE AND SYRINGE is the best and convenient way for sampling following the usual procedure and remove the air from syringe immediately.

2- If no pus or fluid comes on aspiration inject sterile saline subcutaneously and resample.

3- The last and least way is to use deep swab and rapidly to transfer to anaerobic transport media.

Anaerobic Specimen transport

- Must be done immediately to the lab within 1 hr and less than 2 hr.
- Never refrigerate samples for anaerobic culture.
- If delay can not be avoided use the anaerobic transport kit especially for swabs and small volume samples.
- The anaerobic transport system is commercially available and consists of group of vials, tubes and bags that remove O2 and maintain the anaerobic atmosphere for up to 72 H at 20-25 °C.

Fluid transport (aerobic & anaerobic)

Anaerobic transport kit

Blood culture bottles for anaerobic blood culture

Caution

- Avoid shock to the anaerobic bacteria by exposing them to Oxygen or dryness of sample.
- Avoid exposure to cold as anaerobic microorganisms are sensitive to cold.
- Avoid swabs in sampling as Swab fibers contain ambient air and introduce oxygen to the sample.

Anaerobic culture methods:

1. Use of media containing reducing substances (Robertson Cooked Meat broth or Thioglycolate broth).
2. Culture away from O2 (Deep agar tubes).
3. Chemical exclusion of O2 (anaerobic Gas Pak system).
4. Mechanical exclusion of O2 (anaerobic incubator).

1- Use of media containing reducing substances.

A- Robertson Cooked Meat broth

- Composition: 5gm of cooked meat particles + nutrient broth.
- The reducing substances are haemin and glutathione in meat particles
- Uses: for anaerobic cultivation
- Sterilisation: Autoclave at 121°C for 30 min

- Robertson Cooked Meat Broth

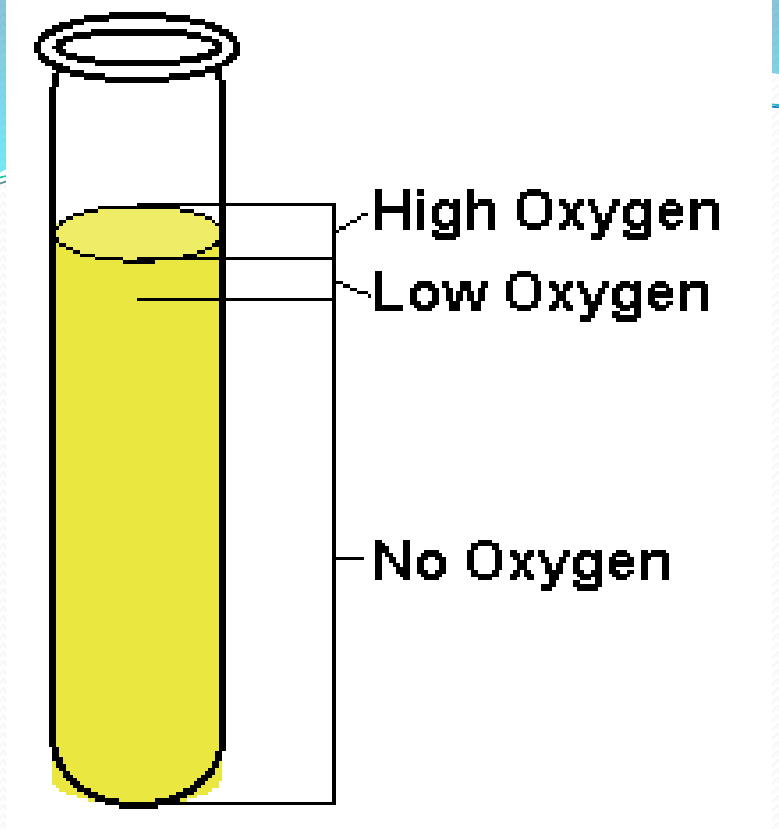
B- Thioglycolate broth:

- Media for anaerobes supplemented with nutrients like hemin and vitamin K, 1% glucose, 0.1% thioglycollate, 0.1% ascorbic acid, 0.05% cysteine or red hot iron filings .
- Sterilize by autoclaving at 121°C for 15 minutes.
- Cool to 25°C and store in a cool dark place preferably below 25°C.
- Before use the medium must be boiled in water bath to expel any dissolved oxygen and then sealed with sterile liquid paraffin.

Thioglycolate broth

Culture away from O2 (Deep agar tubes).

- Simple way to produce anaerobic condition
- The agar surface can be overlaid with oil to maintain the anaerobic condition.
- Sterilization of the media can be carried out in the autoclave at 121 0C for 30 minutes.
- Inoculation is by deep stabbing.



O2 content of culture tube

Growth in deep agar

3- Chemical exclusion of O2 (anaerobic Gas Pak system).

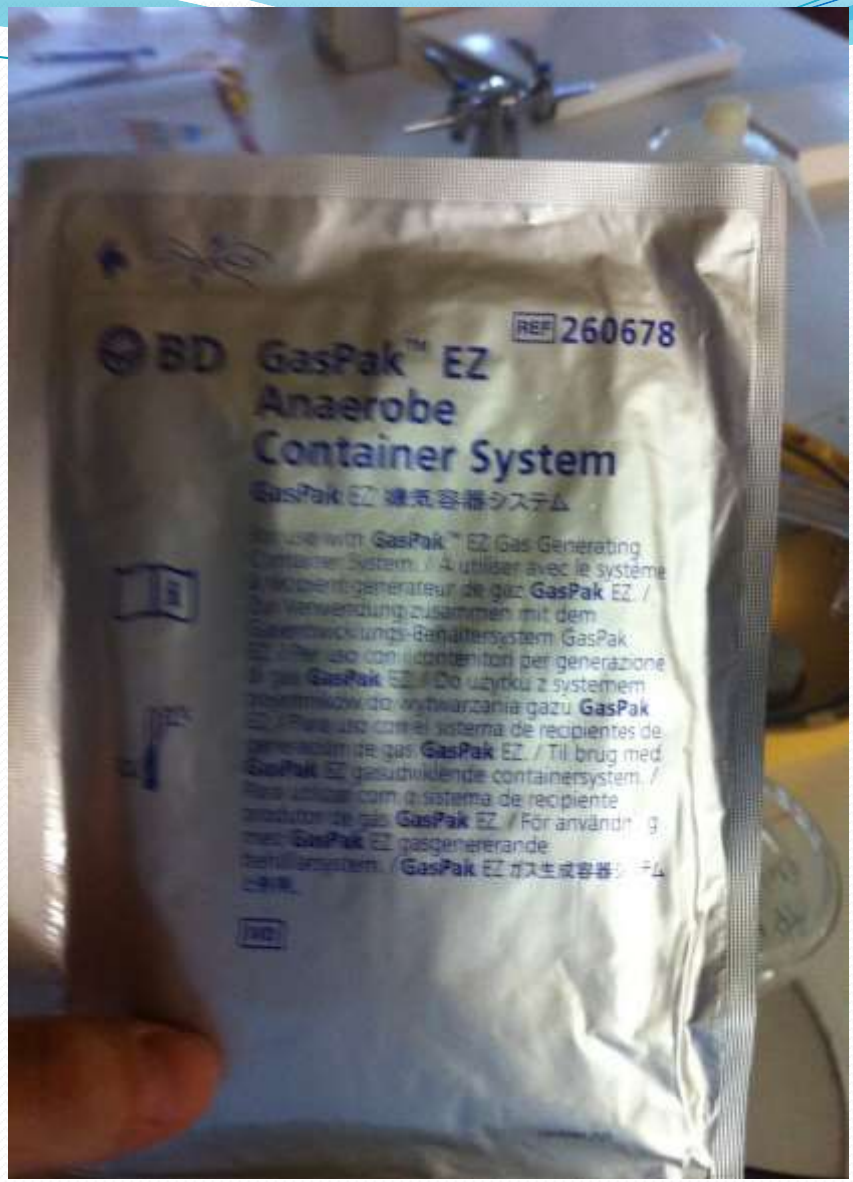
- Uses H2 to convert air O2 to H2O in the presence of a catalyst.
- The reaction formula is ($2H_2 + O_2 \rightarrow 2H_2O$).
- The source of H2 is the Gas Packet commercially supplied.
- The catalyst is palladium contained in the lid of the jar.
- Anaerobic indicator strips included to monitor the anaerobic condition.

GasPak Envelope

Wire mesh containing palladium catalyst


methylene blue
indicator strip
anaerobe
jar
GasPak

- **GasPak needing H2O**



- **GasPak Not needing H2O**


OXOID
ANAEROBIC INDICATOR
CODE NO. BR 55
STORE AT 2 TO 8°C
FOR LABORATORY USE ONLY
Made for Oxoid Ltd.
Basingstoke, Hants., England.

4- Mechanical exclusion of O2 (anaerobic incubator).

Thank You