ANAEROBIC MEDIA, SAMPLE COLLECTION, AND CULTURE TECHNIQUE

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Bacterial classification by O₂ requirement:-

- **1. Obligate aerobes**: require O_2 for growth i.e. O_2 acts as electron acceptor e.g. *Ps aeruginosa*.
- Obligate anaerobes: grow only in complete absence of O₂. O₂ 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 O₂ 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.

- 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:

- 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

- <u>Composition</u>: 5gm of cooked meat particles + nutrient broth.
- The reducing substances are haemin and glutathione in meat particles
- <u>Uses:</u> for anaerobic cultivation
- <u>Sterilisation</u>: 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 oC for 30 minutes.
- Inoculation is by deep stabbing.



Deep Agar www.pharma-board.com

*O*² *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 $(2H_2 + 0.2 \rightarrow 2H_{20})$.
- 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.

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GasPak Not needing H₂O

GasPak needing H2O



4- Mechanical exclusion of O2 (anaerobic incubator).





