Anaerobic culture....

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Our topic today includes:

- Definitions
- Purpose
- Features suggestive of anaerobic infection
- Precautions
- Risk factors
- Description
Definition:

* An anaerobic bacteria culture is a method used to grow anaerobes from a clinical specimen.

* Obligate anaerobes grow only in the absence of oxygen. They are destroyed when exposed to the atmosphere for as briefly as 10 minutes.
There are *microaerophilic anaerobes* that can tolerate 5% oxygen and *aerotolerant ones* that can grow in air e.g. 20% oxygen.

**Few** are *facultative anaerobes* that can grow in all atmospheres.

Anaerobic conditions must be maintained during the collection, transportation, processing, and identification of specimens.
To identify bacteria that grow only in the absence of oxygen and which may cause human infection.

If overlooked or killed by exposure to oxygen, anaerobic infections result in such serious consequences as amputation.

Culture is required to identify anaerobic pathogens and institute effective antibiotic treatment.
Features suggestive of anaerobic infection include:

- **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.**
Precautions

* obtain the sample for culture via **aseptic technique**. Anaerobes are commonly found on mucous membranes and other sites such as the vagina and oral cavity.

* Therefore, specimens likely to be contaminated with these organisms should not be submitted for culture (e.g., a throat or vaginal swab).
Some types of specimens should always be cultured for anaerobes if an infection is suspected. These include abscesses, bites, blood, cerebrospinal fluid and exudative body fluids, deep wounds, and dead tissues.
Risk factors:

- Poor blood supply and tissue necrosis:
  - Trauma.
  - Foreign body.
  - Malignancy.
  - Surgery.
  - Diabetes mellitus.
  - Splenectomy.
  - Immunocompromise.
**Anaerobes** are normally found within certain areas of the body but result in serious infection when they have access to a normally sterile body fluid or deep tissue that is poorly oxygenated.
Some anaerobes normally live in the crevices of the skin, in the nose, mouth, throat, intestine, and vagina. Injury to these tissues (i.e., cuts, puncture wounds, or trauma) especially at or adjacent to the mucous membranes allows anaerobes entry into otherwise sterile areas of the body and is the primary cause of anaerobic infection.
A second source of anaerobic infection occurs from the introduction of spores into a normally sterile site. Spore-producing anaerobes live in the soil and water,
* **Gram-negative anaerobes and some of the infections they produce include the following genera:**

* **Bacteroides** (the most commonly found anaerobes in cultures; intra-abdominal infections, rectal abscesses, soft tissue infections, liver infection)
* **Fusobacterium** (abscesses, wound infections, pulmonary and intracranial infections)

* **Porphyromonas** (aspiration pneumonia, periodontitis)

* **Prevotella** (intra-abdominal infections, soft tissue infections)
* **Gram-positive anaerobes** include the following:
  * Actinomyces (head, neck, pelvic infections; aspiration pneumonia)
  * Bifidobacterium (ear infections, abdominal infections)
  * Clostridium (gas, gangrene, food poisoning, tetanus, pseudomembranous colitis)
  * Peptostreptococcus (oral, respiratory, and intra-abdominal infections)
* **specimens from which anaerobes are likely to be isolated** are:???
Some of the specimens that are not suitable for anaerobic cultures include:

1. Specimens from sites in which anaerobic bacteria are normal flora (e.g., throat, rectal swabs, urine, bronchial washes, cervico-vaginal mucosal swabs, sputum).

2. Voided and catheterized urine.

3. Gastric contents, small bowel contents, feces, colo-cutaneous fistula and colostomy contents should not be cultured for anaerobic bacteria.

5. Improperly labeled specimen.

6. Specimens with a transit time exceeding 2 hours will be rejected.

7. If an unacceptable specimen is received, the physician or patient’s nurse will be notified before discarding.
The **best** specimen for anaerobic culture is an aspirate obtained by **needle and syringe**

1. Disinfect skin surface with 70% alcohol, allow to dry.
2. Aspirate specimen directly into the syringe.
3. Remove air from syringe.
4. Aseptically transfer material into an anaerobic transport vial for fluids.
5. If unable to aspirate;

- If the initial aspiration fails to yield material, sterile 0.9% saline may be injected SC and the aspiration attempt repeated.

- Obtain a swab from deep in the wound, firmly sampling the lesion’s advancing edge. Place swab into anaerobic transport tube.
Tissue samples and biopsies are also very good specimens for anaerobic culture.

1. Punch biopsies, bone, or any other tissue specimen may be collected surgically.
2. Place specimen in a sterile container without formalin.
When a swab must be used to collect a specimen, use a prepared anaerobic culturette system and take special care to sample the active site of infection.

1. Clean the site of surface debris and disinfect as above.

2. Carefully avoid surrounding surface areas that may be inhabited by normal anaerobic flora.
Proper collection and transport of the specimens.

Immediate delivery of the specimen is crucial.

If delay is anticipated, an anaerobic transport system should be utilized especially for swabs and small volume specimens.
There are commercially available anaerobic transport media in **vials and tubes** with anaerobic atmosphere and **bag systems** that act by removing molecular oxygen.
Swabs can be placed in tubes with reduced transport media and anaerobic atmosphere.
* Specimens should be placed in **leak-proof container** with tight fitting caps.

* Proper labeling with **date and time** of collection should accompany all specimens submitted for culture.

* Put samples in **room temperature** while waiting for delivery to the laboratory.

* Samples are **not refrigerated**.
**SPECIMEN TRANSPORT:**

* Very small volume specimens (<1 ml) should be transported in the syringe. Delivery to the lab. should be within 10 minutes.

* Small volume specimens (1-2 ml) should be transferred to the anaerobic culturette immediately after collection at the bedside. Transport time can be up to 2-3 hrs.
* Large volume specimens (>2 ml) should be transferred to a sterile screw cap container at the bedside, as well as tissue or bone samples. Transport should be as soon as possible, but can be delayed up to 2 hrs.

* Transport all specimens at room temperature and avoid extremes of heat or cold
**Anaerobic transport system instructions:**

* Stored in dark. At room temperature, or refrigerated.
* Freshly prepared.
* Discard if media turns pink/purple.
* Discard after expiration date.

* If routine culture for aerobes is also desired, it is best to collect a separate regular culturette.
Part of the initial processing is a **direct specimen smear**, which will determine quality of specimens and provide the physician with rapid information for diagnosis and initial treatment.
Anaerobic culture methods

1. Producing a vacuum
2. Oxygen displacement
3. Oxygen absorption
4. Reducing agents
5. Anaerobic chambers—(have catalyst, desiccant, H₂, CO₂, N₂ + indicator; airtight gloves)
Displacement of Oxygen

* McIntosh and Filde’s anaerobic jar.
* Metal jar with metal lid—airtight.
* Inlet and outlet to lid
* Lid has electrical terminal
* Outlet tube—vacuum pump to take out air
* Inlet—Hydrogen passed in—normal atmospheric pressure
* Catalyst: Hydrogen + Oxygen = Water
* Reduced methylene blue is indicator. Colorless is anaerobic and blue when exposed to oxygen
A. Pyrogallol (Buchner)

Alkaline Pyrogallol absorbs oxygen

Tube with NaOH and pygallic acid in airtight jars: anaerobic conditions.
* Chromium and Sulfuric acid
* Chromium + Sulfuric acid = anaerobiosis

* React in presence of Oxygen to produce chromous sulfate
Gas Pak

- Pellets of sodium borohydride, cobolt chloride, citric acid and sodium bicarbonate
- Generate hydrogen and Carbon dioxide in presence of water $\rightarrow$ $\text{H}_2 + \text{O}_2 = \text{H}_2\text{O}$
- Inocculated plates in airtight Jar
- Gas-Pak + Water added; lid closed
A is an facultative anaerobe
B is an aerobe (microaerophilic)
C is an anaerobe
Biological Methods

- Incubate aerobes + anaerobes.
- 2 blood agar plates
  1. *Pseudomonas aeruginosa* (aerobic bacteria)
  2. Anaerobic bacteria

Place one over other and seal.

*Method slow and ineffective.*
Oxygen reduced by
- Glucose
- Thioglycollate
- Cooked meat pieces
- Cystein
- Ascorbic acid
Reducing Agents

**Media**

- Tioglycollate broth (nutrient broth and 1% thioglycollate)

- Cooked meat broth/Robertson’s cooked meat medium (nutrient broth, fat-free minced cooked meat of ox heart)
Antibiotic sensitivity:

* Not done routinely

* Antibiotics used:
  * Penicillin (P).
  * Clindamycin (CL).
  * Cefoxitin (FOX).
  * Metronidazole (MTZ) (+ penicillin).
  * Carbapenem (IPM).
  * Chloramphenicol (C).
THANK YOU