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VMC- 602 (BACTERIOLOGY II), UNIT III

ANAEROBIC BACTERIA CULTURE

(PRACTICAL)

Dr. Savita Kumari
Assistant Professor-cum-Jr. Scientist
Department of Veterinary Microbiology
Bihar Veterinary College, BASU, Patna

Anaerobic bacteria

- Unable to grow in an atmosphere containing oxygen
- Use fermentative pathways in which organic compounds serve as final electron acceptors
- killed when exposed to the atmosphere for as briefly as 10 minutes
- Some anaerobes tolerant to small amounts of oxygen
- Facultative anaerobes grow with or without oxygen
- Anaerobic bacterial culture: method used to grow anaerobes from a clinical specimen

Anaerobic bacteria

- Spore forming Gram positive bacilli e.g. *Clostridia* sp.
- Nonsporeforming Gram-positive bacilli e.g. *Actinomyces* sp.
- Nonsporeforming Gram-negative bacilli e.g. *Fusobacterium* sp.
- Anaerobic cocci e.g. *Peptostreptococcus* sp.

Specimen:

- Blood, bile, bone marrow, cerebrospinal fluid, direct lung aspirate, and tissue biopsy from a normally sterile site
- Fluid aspirated from a normally sterile site, such as a joint
- Pus specimens from dental abscess, burn wound, abdominal or pelvic abscess
- Specimens from knife, gunshot, or surgical wounds

Important considerations in cultivation of anaerobic bacteria:

1. Proper collection and transport of the material to be examined
2. Culture of the material as soon as possible after collection
3. Use of freshly prepared and properly reduced media
4. Proper anaerobic conditions

- Sample - from the active site of infection

- Precautions should be taken to exclude surface contaminants and aeration of the sample

- without contaminating the sample with bacteria from the adjacent skin, mucous membrane, or tissue

- Sterile rubber stoppered transport vials and tubes containing an oxygen free CO_2 atmosphere are available commercially
- Abscesses or fluids - aspirated with a needle and sterile syringe, injected directly into the transport bottles; care must be taken to exclude any air
- Tissue samples- placed into a degassed bag and sealed, or into a gassed out screw top vial that may contain oxygen-free pre-reduced culture medium and tightly capped
- Material can be placed in a medium containing a reducing agent such as cysteine or thioglycollate at room temperature for a period not exceeding 2 hours
- Swabs not preferred, material on swabs should never be allowed to dry out
- Sputum, rectal swab, nasal or throat swab, urethral swab, and voided urine samples not suitable

- Samples should not be refrigerated since chilling is detrimental to some anaerobes, and oxygen absorption is greater at lower temperatures

- Blood specimens:

✓ 5-10 ml of blood should be inoculated into 50-100 ml of liquid media and the blood cultures incubated up to 14 days

✓ Blood cultures should be subcultured to plating media

- Broth media containing 0.025% sodium polyanethol sulfonate (liquoid) and an anaerobic or partial CO2 atmosphere are commercially available

- Tryptic soy broth, trypticase soy broth, thioglycollate medium and pre-reduced brain heart infusion broth designed for anaerobic blood culture equally satisfactory

- Plating media for primary isolation should be prepared on the day it is used, or freshly prepared media should be placed under anaerobic conditions for a period no longer than 2 weeks

- Plating media can be stored in an anaerobe jar, glove box, or in an air-tight cabinet containing an oxygen free CO_2 atmosphere

- Liquid media containing reducing agents should be stored in the dark at room temperature in tightly capped tubes for not longer than 2 weeks

- In order to gain some insight into the quantity and type of organisms in the specimen, examine a gram stained smear (except blood)

Culture media

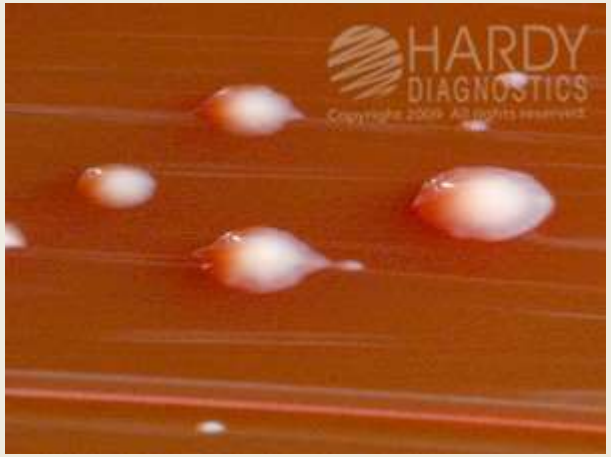
- Enriched Thioglycollate media
- Robertson cooked meat (RCM) broth
- Blood agar with vitamin K-hemin solution
- Blood agar plates with vitamin K-hemin and antibiotics

e.g. Kanamycin-vancomycin blood agar

- Phenylethyl alcohol blood agar
- A ‘Fastidious Anaerobe agar’ (Lab M) is available commercially with various antibiotic supplements, depending on the anaerobe that is being sought
- Liquid media: useful adjuncts to agar media if the initial sample contains very small numbers of the required anaerobe and also for growing and maintaining pure cultures
- The inoculated tubes or bottles are incubated anaerobically, with loose caps, at 35–37 °C

Phenylethyl alcohol blood agar

LKV (Laked Sheep Blood Kanamycin Vancomycin) Agar



Robertson cooked meat (RCM) broth:

- Nutrient broth and pieces of fat-free minced cooked meat of ox heart
- Unsaturated fatty acids and even glutathione and cysteine present in the meat extract utilize oxygen for auto-oxidation
- The medium before inoculation usually boiled at 80°C in a water bath to make the medium free of oxygen
- Growth of anaerobes- saccharolytic or proteolytic activities as meat is turned red or black, respectively

Thioglycolate broth

- Multipurpose, enriched, differential medium used primarily to determine the oxygen requirements of microorganisms
- Sodium thioglycolate in the medium consumes oxygen and permits the growth of obligate anaerobes
- Strictly anaerobic bacteria (i.e., those that cannot grow in the presence of oxygen) grow at the bottom of the broth

Methods for anaerobic culture

- Three main methods
- Anaerobic jars with a catalyst, an anaerobic indicator and an atmosphere free of oxygen

Anaerobic jars with vents:

These can be evacuated (to 20–24 inches of mercury), flushed twice with commercial grade nitrogen gas (N_2) and then filled with an anaerobic gas mixture (10 % hydrogen (H_2), 5 % carbon dioxide (CO_2) and 85 % nitrogen (N_2))

- This mixture can be ordered in cylinders from a commercial gas supplier

Anaerobic jar without vents:

- These are used with commercially available envelopes that deliver an H_2–CO_2 atmosphere

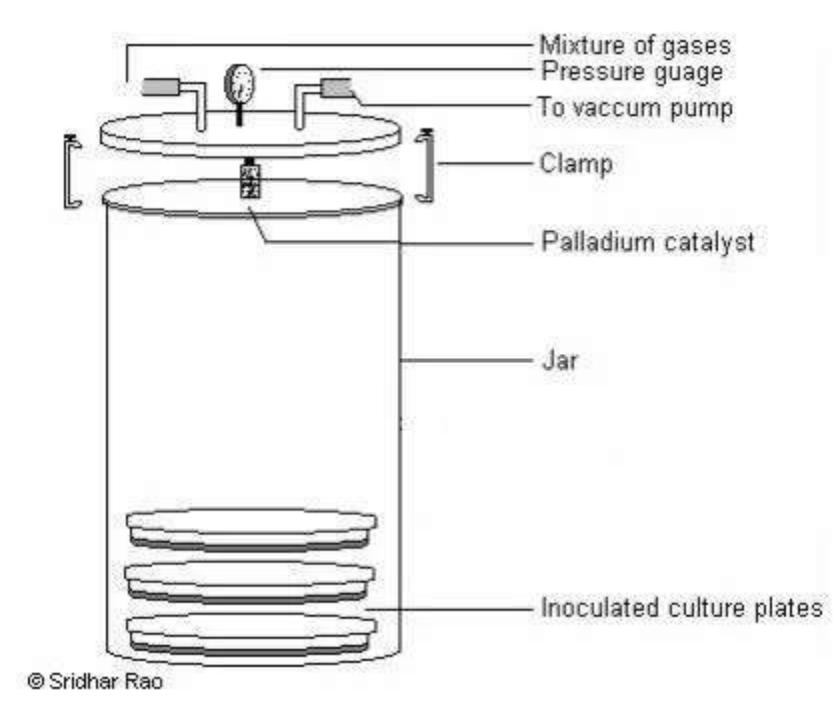
McIntosh–Fildes anaerobic jar:

- Glass or metal jar with a metal lid, clamped air tight with the help of a screw
- The lid has one inlet tube and another outlet tube
- The outlet tube is connected to a vacuum pump by which the air is evacuated out of the jar
- The inlet tube is connected to a source of hydrogen supply
- The lid has two electric terminals also that can be connected to an electric supply
- The underside of the lid contains a catalyst (e.g., alumina pellets coated with palladium) that catalyses the combination of hydrogen with residual oxygen present in the air
- This method ensures complete anaerobiosis

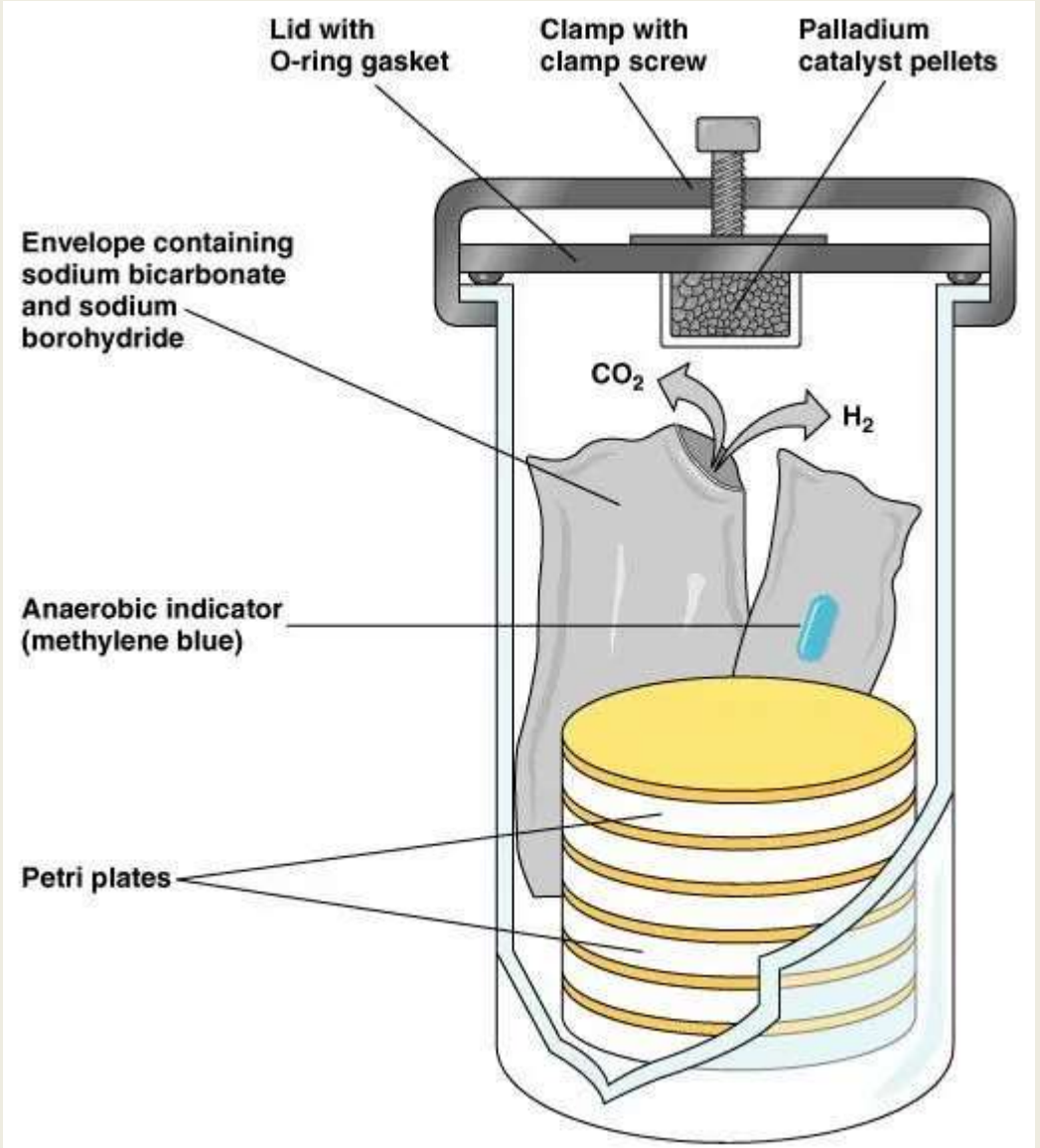
- The culture media are inoculated with the specimens suspected to contain anaerobic bacteria
- The inoculated media are then kept inside the jar, and the lid is closed air tight
- The anaerobiosis in the jar is carried out by first evacuating the air from the jar through outlet tube with the help of a vacuum pump
- The outlet tube is closed, then the sealed jar containing the culture plates is replaced with hydrogen gas passed through inlet tube till reduced atmospheric pressure is brought to normal atmospheric pressure, which is monitored on the vacuum gauge as zero
- The electrical terminals are then switched on to heat

McIntosh and Fildes' jar

- works on principle of evacuation and replacement
- air inside chamber evacuated and replaced with mixture of gase



GasPak Anaerobic System



https://i2.wp.com/microbeonline.com/wp-content/uploads/2010/07/Anaerobic-Jar.jpg

Anaerobic bags or pouches

- Commercially available products
- suitable for culturing small numbers of samples (e.g. Bio-BagTM Type A, GasPakTM Pouch, Becton-Dickinson)
- Plates are placed in the bags and an oxygen-removal system is activated
- These bags can be used for transport of specimens also

https://www.bd.com/assets/images/our-products/microbiology-solutions/gaspak-ez-pouch-system_RC_DS_ES_0616-0003.png

Anaerobic glove box or Anaerobic chambers :

- Most bacteriologic techniques involved in the isolation and identification of anaerobic bacteria can be performed under anaerobic conditions without exposing the microorganisms to air
- Usually large plastic chambers kept constantly under an anaerobic atmosphere
- May contain temperature control devices and other equipment for culturing anaerobes
- Large clear-vinyl chamber with attached gloves, containing a mixture of 80% nitrogen, 10% hydrogen, and 10% carbon dioxide
- A lock at one end of the chamber is fitted with two hatches, one leading to outside and the other to the inside of the chamber

- Specimens are placed in the lock, the outside hatch is closed, and the air in the lock is evacuated and replaced with the gas mixture

- The inside hatch is then opened to introduce the specimen into the chamber

- Manipulations inside are conducted with the operator's hands and arms in gloves that are an integral part of the tent wall

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