

Anaerobic Bacteria

Part I

General information

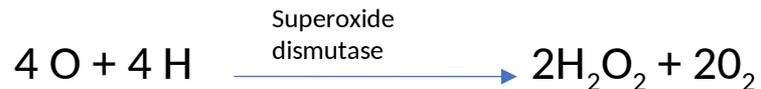
Anaerobic characteristics

Bodily location

General Pathogenesis

What are Anaerobes?

- Bacterium that replicate and thrive in the absence of oxygen
- To recover anaerobes, clinical labs must create special micro- atmospheric conditions
- Molecular O₂ is toxic
 - Oxygen radicals accumulate and damage cell
 - Lack enzyme superoxide dismutase and/or catalase



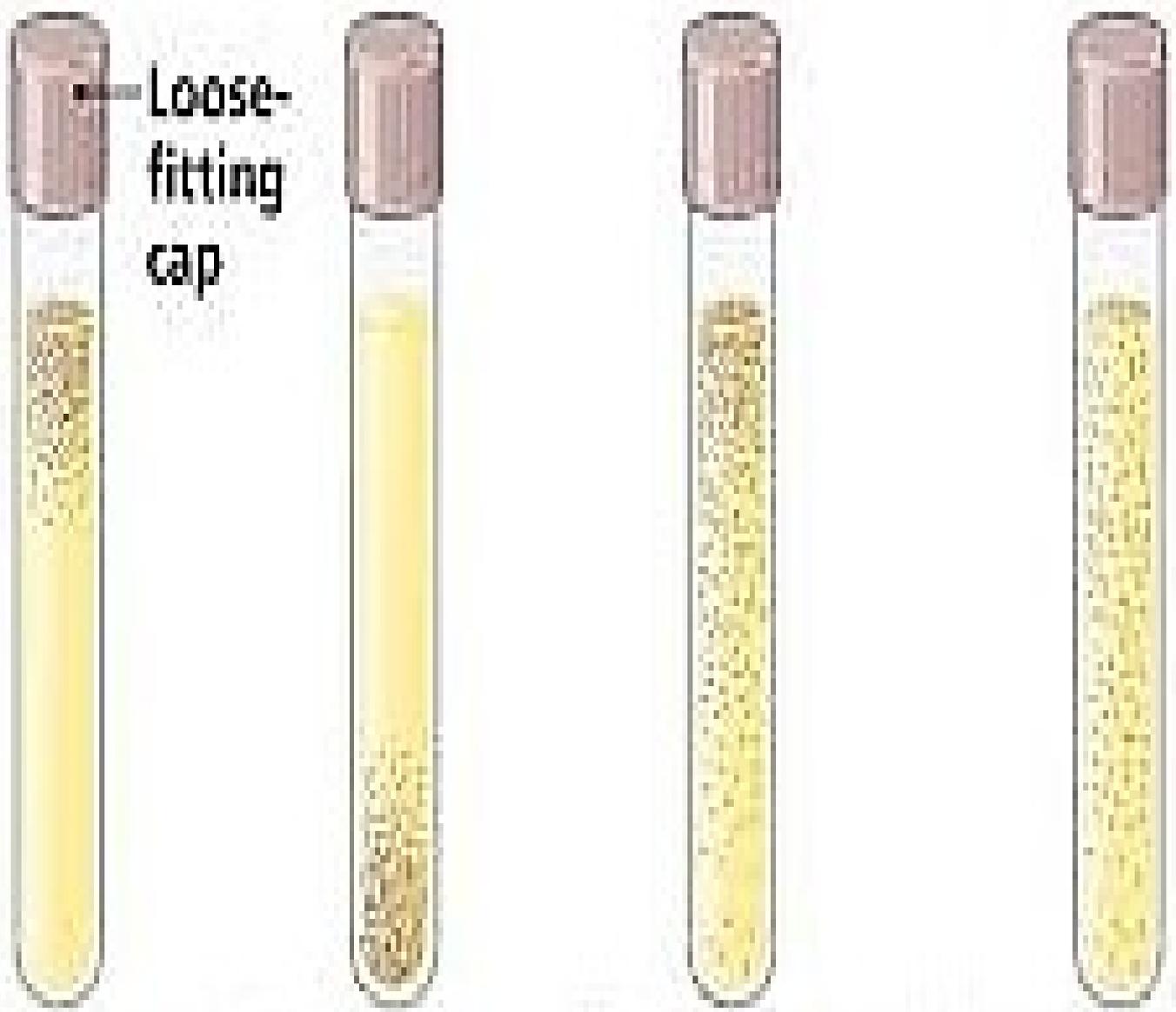
Classifying Bacteria by O₂

- Obligate anaerobes [Example: Clostridium spp]
 - Requires 0% oxygen
- Obligate aerobes
 - Requires 15 - 23% oxygen
- Facultative anaerobes [Example: E. coli]
 - Requires 0 - 23% oxygen
- Capnophilic organisms [Example: Neisseria gonorrhoea]
 - Requires 5 - 10% CO₂
- Micro-aerophilic organisms [Example: Campylobacter spp]
 - Requires 5% oxygen
- Aerotolerant organisms [Example: Actinomyces spp]
 - Requires reduced oxygen % [< 15%]
 - Some survival but limited metabolism

Oxygen concentration
High

Loose-fitting cap

Low



(a) Obligate aerobes

(b) Obligate anaerobes

(c) Facultative anaerobes

(d) Aerotolerant anaerobes

Where Are Anaerobes Found?

- Found in ecological niches
 - Soil, freshwater and saltwater sediments, endogenous microbiota, or exogenous anaerobes
- Endogenous – inside the human body
 - More common cause of human disease
- Exogenous – outside the human body
 - Usually, spore forming bacilli
 - Soil or water contamination into human tissue

Where Are Anaerobes Found?

- Anaerobes are considered normal flora in some areas of the human body
 - Outnumber aerobes on mucosal surfaces of the:
 - Oral cavity
 - Gastro-intestinal tract
 - Genito-urinary tract
- Normally are beneficial and essential for normal human function/metabolism
 - When endogenous anaerobes migrate to sterile body fluids = infection and mortality

Common/Optimal Anaerobic Environmental Conditions

- Anaerobic environments should consist of
 - 85% Nitrogen
 - 10% Carbon dioxide
 - 5% Hydrogen gas
 - 0% Oxygen

Endogenous Examples

- Bacteremia
- Brain abscess
- Female UTI
- Intra-abdominal abscesses:
 - Liver, peritonitis, perineal, perirectal
- Oral, sinus, dental infections
- Pneumonia + lung infections

Clinical Signs of an Anaerobic infection

- Usually are pus producing
- Infection near mucosal surface
- Infection that persists despite aminoglycoside therapy (tobramycin, gentamicin, amikacin)
- Foul odor
- Large quantities of gas present
- Presence of sulfur granules
- Infection secondary to human or animal bite

Predisposing Factors & Causes of Infection

- Trauma to mucosal membranes or skin
- Vascular stasis [ischemia]
 - Decreased tissue oxygenation and necrosis
- Bacterial anaerobic toxins

Common Causes of Anaerobic Infections

Human or animal bites

Aspiration of oral contents into lungs during emesis

Tooth extraction or dental surgery

GI surgery or traumatic GI punctures

Genito-urinary surgery

Indications that Anaerobes in Microbiology Labs

- Foul odor on opening the anaerobic system
 - May indicate *Clostridium*, *Fusobacterium*, or *Porphyromonas*
- Colonies present on the anaerobic blood agar plates, but not on aerobic check plates
- Good growth of grey colonies on BBE agar (>1 mm in size)
- Colonies on LKV agar that fluoresce brick red under UV light and are brown/black on the anaerobic blood agar plate
- Double zone of beta hemolysis on an anaerobic blood agar plate (indicates *Clostridium perfringens*)



Anatomic Site	Cultured Anaerobically	Not Cultured Anaerobically
Pulmonary	Transtracheal aspirates; thoracentesis fluids; bronchial brushes; lung tissue or aspirates	Tracheal secretions; Expectored sputum; Leukens aspirates; Bronchial Washings; Throat, nasopharyngeal, nose or mouth swabs
Surgical Specimens	Appendix, gallbladder; muscle biopsy from suspected gas gangrene (biopsied from any normally sterile site); Any normally sterile tissue collected by aseptic surgical excision	
Urinary	Suprapubic percutaneous aspirate of bladder urine. Kidney aspirate	Voided urine; catheterized specimens
Wounds Sinus tracts	Aspirate into syringe through catheter introduced as deeply as possible through decontaminated skin orifice: "Sulphur granules."	Swab from external portion of sinus tract or of drainage through external orifice, decubitis, burn eschars

Anatomic Site	Cultured Anaerobically	Not Cultured Anaerobically
Abscesses	Needle and syringe aspirate of closed abscesses after decontamination of the surface	Swab from surface of abscess (burn, cyst or ulcer); Swab after incision and drainage
Blood	All blood cultures are cultured for both aerobic and anaerobic organisms. No special order is needed	
Body Fluid	Ascitic fluid, bile, bone marrow, synovial, pericardial, thoracentesis, pleural, and peritoneal fluid, cerebrospinal fluid	
Catheter tips	Tenchkoff cath tips: CMG only	All other catheters
Gastrointestinal		Stool, ileostomy or colostomy effluent, gastric contents
Genital	Uterine specimens obtained by protected brush; placenta, Bartholin's gland, culdocentesis, endometrial, fallopian tube, and septic abortion specimens; IUD	Vaginal, Cervical, or Urethral Swabs

Anaerobic Bacterial Testing

Specimen selection, Collection, Transport, and Processing
Procedures for Identifying Anaerobic Isolates - Pg 510 - 528

Specimen Quality

- Anaerobic infections must be collected directly from the site of infection and not superficial swabbing
- Avoid prolonged oxygen exposure
- Transport to lab ASAP in anaerobic conditions
- Specimen collection and type varies by hospital so follow SOP
- Improperly collected samples may have contamination from other bacteria (makes ID + workup difficult)
 - Bad workups negatively affect patient outcomes and treatment

Specimen Transport and Processing

- Collected properly and transported to lab ASAP
- Anaerobic samples in transport should not be refrigerated
- The amount of time in room temp should be minimalized

Acceptable Samples

- Aspirated body fluids
- Tissue biopsy
- Aspirated pus
- Surgical samples
- Suprapubic aspirates
- Sterile body site fluids
- Eswabs

Unacceptable Samples

- Throat swabs
- NP swabs
- Most sputums
- Mouth swabs
- Feces
- Midstream or catheterized urine
- Exposed wounds

Commonly used Specimens + Samples

- Aspirates
 - Needle or syringe collection
 - Less likely to have contamination from other bacteria
 - Expel air prior in needle prior to collection
 - Stored in **pre-reduced, anaerobically sterilized** transport media [**PRAS**]
 - Example = Cairy Blair medium
 - The lab should vortex the sample, aseptically plate to media, and perform a direct gram stain

Commonly used Specimens + Samples

- Swabs
 - Most swabs are unacceptable
 - Only used when aspiration or tissue biopsy is not possible
 - The lab should vortex the swab and store the swab in thioglycollate broth
 - The liquid media from the swab tube is used for plating and direct gram stain
- Eswabs
 - Swab stored in 1mL of pre-reduced Amies liquid
 - Maintains anaerobic conditions for 48 hours at room temp



Commonly used Specimens + Samples

- Tissue
 - Collected by surgical biopsy or autopsy
 - Stored in anaerobic liquid media like PRAS
 - Keeps the tissue from desiccation
 - Commercially made anaerobic creating bags / pouches may be used
 - Tissue samples often are ground up in the lab via aseptic technique
 - Thioglycolate is added to a tissue grinder and the biopsy is added
 - Add emulsified tissue to plates, liquid media, and direct gram stain based off SOP



Commonly used Specimens + Samples

- Blood
 - Aseptic collection of blood culture bottles are suspected in bacteremia and sepsis cases
 - Rapid transport to the lab is vital
 - Incubated at 35-37 C on instrument
 - Bacterial metabolism trips the chromogenic sensor
 - Plate and gram stain
 - Critical results are reported to physician with date/time and name



Lab Processing of Anaerobic Samples

- Should be received ASAP and plates should be incubated in anaerobic conditions
- Anaerobic bacteria should be assessed:
 - Macroscopically
 - Appropriate specimen
 - Appropriate transport
 - Fluorescence under UV light [366 nm]
 - Tissue sample appearance
 - Microscopically
 - Gram stain morphology
 - GNR staining may be enhanced with 0.1% basic fuchsin or extended safranin staining

Primary Plating Media for Anaerobes

- Anaerobic plates have additional nutritional requirements in each media
 - Vitamin K
 - Hemin
 - Yeast extract
- At minimum, anaerobes should be plated onto non-selective blood agar [CDC ANA or Brucella], Bacteroides Bile Esculine [BBE], and Kanamycin and Vancomycin with Laked Sheep Blood [KVLB]
- Other labs may use selective media Phenylethyl Alcohol [PEA] and Colistin Nalidixic Acid [CNA] and Cooked Meat or Thioglycolate Broth
 - Inhibits facultative anaerobes

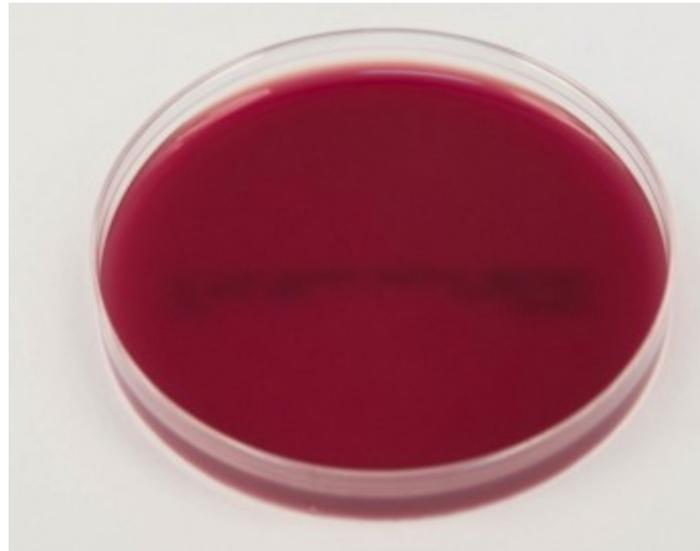
Solid and Liquid Media for Anaerobes

- Anaerobic CDC BAP [CDC ANA]
- Bacteroides Bile Esculin [BBE]
- Brucella Blood Agar [BRU]
- Kanamycin, Vancomycin, Laked Blood [KVLB]
- Phenylethyl Alcohol agar [PEA]
- Colistin Nalidixic Acid Agar [CNA]
- Thioglycolate or chopped meat broth
- (all contain vitamin K, yeast extract, and Hemin)



CDC ANA plate

- Supports growth of obligate and facultative anaerobes
- Best for anaerobic GPC
- Useful for hemolysis detection



BBE Plate

- Selective agar containing gentamicin
 - Inhibits most aerobes
- Contains 20% bile
 - Inhibits most anaerobes
- Contains esculin
 - Identifies *B. fragilis* group
 - Turns agar black
- Supports growth of bile tolerant *Bacteroides* spp
 - some *Fusobacterium mortiferum*, *Kleb. Pneumo*, *Enterococci*, and yeast may grow



Brucella Agar

- Supports growth of obligate and facultative anaerobes
- Best for GNR



KVLB Agar

- Supports growth of *Bacteroides* and *Prevotella* spp., yeasts, and kanamycin resistant GNR
 - Facultative anaerobes may grow
- Selective media
 - Kanamycin inhibits most facultative GNR
 - Vancomycin inhibits most GPO
 - Laked blood facilitates *Prevotella* pigmentation (black / brown)



PEA agar

- Supports growth of obligate anaerobes
 - Mostly gram positive organisms
- Selective media to suppress Enterobacteriaceae



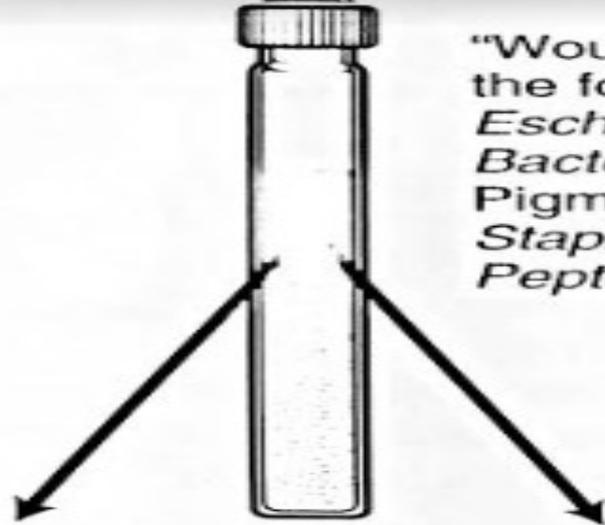
CCFA

- Cycloserine cefoxitin fructose agar
- Selective and differential media that supports the growth of *Clostridium difficile*
 - Yellow “ground glass” colony morphology
 - Has a “horse-stable” odor
- Does not test for toxin/virulent strains so many labs
- Fluoresce chartreuse under UV light



Broth Media

- Chopped meat agar
 - Media that contains solid meat particles and is excellent for initiating growth from a small inoculum
 - Viability can be sustained over long periods
 - Used to recover small amounts of anaerobes that do not grow on the plates



"Wound" aspirate containing the following:
Escherichia coli
Bacteroides fragilis
Pigmented species of *Prevotella*
Staphylococcus aureus
Peptostreptococcus anaerobius

Growth on plates incubated in ambient air or CO₂ incubator



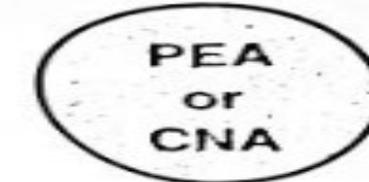
E. coli
S. aureus



E. coli
S. aureus



E. coli



S. aureus

Growth on plates incubated anaerobically



All five organisms



B. fragilis
Pigmented species of *Prevotella*
S. aureus
P. anaerobius



B. fragilis



B. fragilis
Pigmented species of *Prevotella*

Anaerobic Incubation and Chambers

- Anaerobic incubated plates are stored at 35 – 37C using:
 - Anaerobic chambers, anaerobic jars, anaerobic bags/pouches
 - Varies by space and financial limitations of the hospital

Anaerobic Containers in the Lab

- Typically contains:
 - A catalyst
 - Palladium coated pellets – removes O₂
 - A desiccant
 - Silica gel – removes condensation
 - Anaerobic gas with approximate partial pressures of:
 - 5% H₂, 7.5% CO₂, 85.5% N₂
 - An indicator
 - Methylene blue or Resazurin

Anaerobic QC and Troubleshooting

- CAP requires daily verification of anaerobic conditions as QC
- QC is assessed via redox reactions with either methylene blue or resazurin
- In anaerobic conditions
 - Methylene blue is reduced and colorless
 - Resazurin is reduced and colorless
 - Valid to proceed with testing
- In aerobic conditions
 - Methylene blue is oxidized and turns blue
 - Resazurin is oxidized and turns pink
 - QC failure and need to assess anaerobic condition troubleshooting

Anaerobic Chambers

- Sealed glove or gloveless chamber for anaerobic growth conditions
- Allows scientists to work with and view colony morphology without exposing plates to oxygen
- Most expensive but best way to isolate anaerobes



Anaerobic Jars

- Used in smaller microbiology labs
- Anaerobic jar systems use an envelope gas generator
 - Older generation systems require H₂O
 - New generation systems do not
- Gas pack generators contain a QC indicator to determine if anaerobic conditions are achieved
- Disadvantage:
 - The jars must be opened to look at plates
 - Too much oxygen exposure may kill anaerobic bacteria



Anaerobic Bags

- The most cost-effective means of oxygen removal systems for anaerobic conditions
- Plates must be removed from the bag and viewed
- An indicator and pouch are added to each container to create anaerobic conditions
 - Should be white
 - Blue indicates anaerobic conditions were not achieved



Gas jar



Glove box
with gloves



Gloveless
box



Anaerobic Workup and ID

- Some labs may require full identification while others want rapid, presumptive ID; follow SOP
- If anaerobic closed chambers are used, plates can be examined after 24-hour incubation
- If anaerobic jars or pouches are used, plates are examined after 48-hour incubation
- Anaerobic cultures are held for 5 – 7 days to allow for slow growing anaerobes

Anaerobic Workup and ID: Aerotolerance Testing

- Determines whether a bacteria is truly an obligate anaerobe
- After looking at anaerobic cultures post 48-hour incubation
 - Any organisms are compared to aerobic culture workups
 - Gram stained
 - Plated to BAP or CHOC in both CO₂, aerobic, and anaerobic conditions for up to 48 hours

Type	Obligate Aerobe	Capnophile	Facultative Anaerobe	Obligate Anaerobe
Aerobic PLTs	+	-	+	-
CO ₂ PLTs	+	+	+	-
Anaerobic PLTs	-	- * *	+	+

** H. Influenza may grow on anaerobic plates but can be differentiated by growth on CHOC CO₂ plates. H. Influenza will not grow on BAP in aerobic conditions

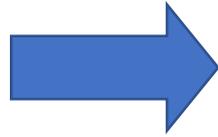
Rapid Biochemical Testing

- Presumptive identification of bacteria is based of anaerobic aerotolerance testing and gram staining
 - Fluorescence under UV light
 - Catalase
 - Spot Indole
 - Urease
 - Motility
 - Antimicrobial disks
 - Kanamycin, vancomycin, colistin disks
 - Sodium Polyanethol Sulfonate [SPS] Disks

More Rapid Biochemical Testing

- Egg Yolk Agar - selective for *Clostridium perfringens*
 - Contains egg yolk and the suspension allows for the detection of lecithinase and lipase activity.
- Lecithinase:
 - A positive lecithinase test is noted by the appearance of a white, opaque, diffuse zone that extends into the medium surrounding the colonies.
 - A negative lecithinase test is indicated by the absence of a white, opaque zone extending from the edge of the colony.
- Nitrate Disk
- Lipase:
 - A positive lipase test is noted by the appearance of an iridescent sheen (oil on water)
 - A negative lipase test is indicated by the absence of an iridescent sheen.

Lipase positive



Lecithinase positive

