

How to isolate and maintain anaerobic bacteria

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Clinical indications for anaerobic infections

- Infection of bite wounds
- Infection of sterile sites
- Abscess formation
- Tissue necrosis
- Infection after antibiotic treatment

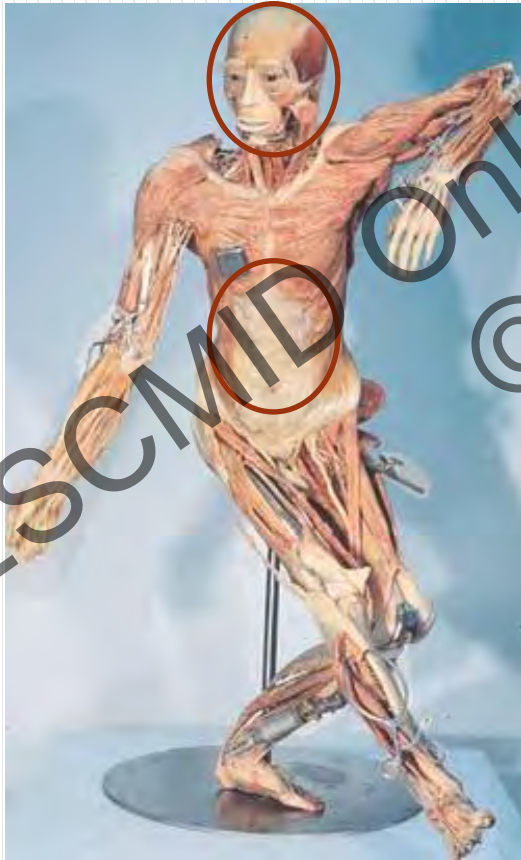


Bite wound



Tissue necrosis

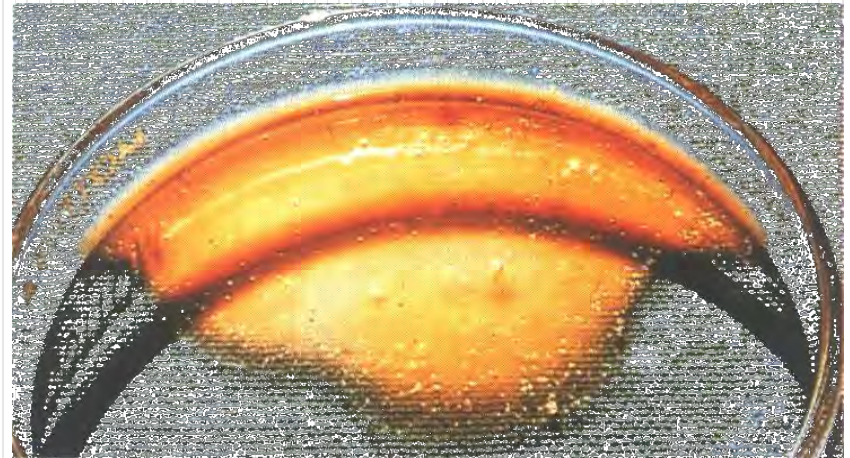
Occurrence anaerobic infections



- Head and neck area
- Abdominal infections

Clinical material suspicious for presence anaerobic bacteria

- Smell
- Gas
- Black discoloration in bloody samples
- Presence of sulfur granules



Conditions for specimen collection and transport

- No contact with the commensal microbiota of the patient
- Store at roomtemperature
- Small volume: transport time < 30 minutes
- Large volume: transport time < 24 hours

Transport devices

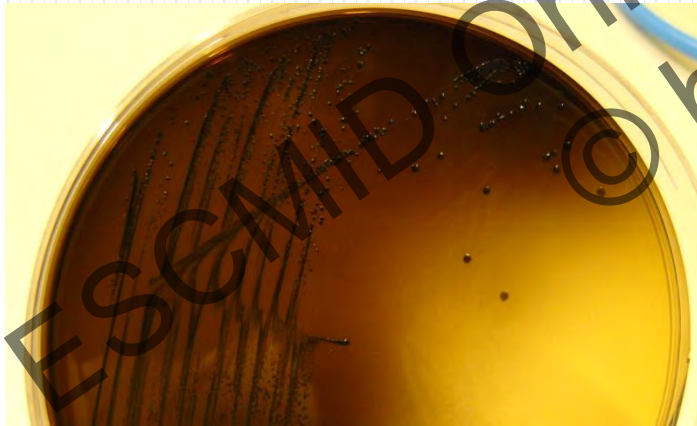
- Container (tissue, pus)
- Syringe (pus)
- Swab with transport medium suitable for anaerobes
- Anaerobic blood culture bottle (fluid, blood)



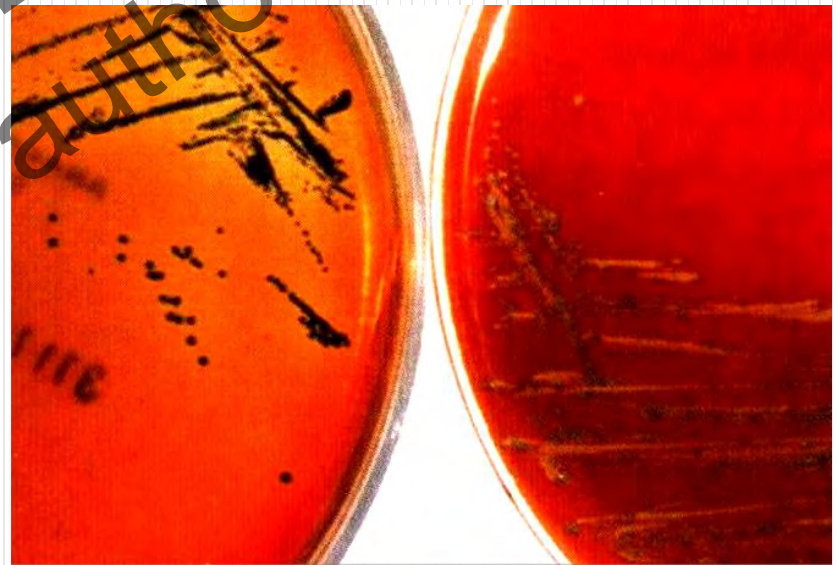
Culture media

Medium	Features
BBA Brucella Blood Agar	Universal medium
PEA PhenylEthyl Alcohol blood agar	Isolation of most anaerobic bacteria, inhibits <i>Enterobacteriaceae</i> and prevents swarming
BBE Bacteroides Bile Esculin agar	Isolation of <i>Bacteroides</i> spp. and <i>Bilophila</i> spp., inhibits all bile sensitive bacteria
BBKV BBA with kanamycin, vancomycin and laked blood	Isolation of gram-negative anaerobic bacteria, promotes the pigmentation of <i>Prevotella</i> and <i>Porphyromonas</i> spp.

Examples of growth



Esculin reaction on BBE



BBKV

BBA

Pigmentation on BBKV and BBA

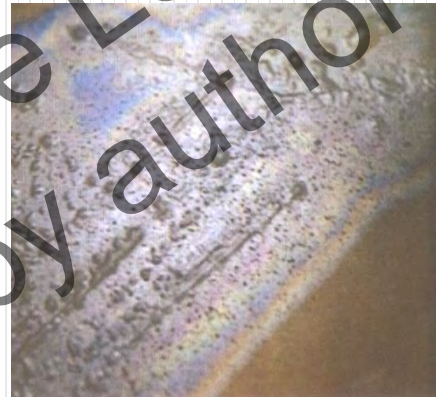
Additional culture media

Medium	Features
CCFA Cycloserine Cefoxitin Fructose Agar	Isolation of <i>Clostridium difficile</i> , inhibition of most anaerobic bacteria
MMBA Metronidazole Mupirocine Blood Agar	Isolation of Actinomyces spp., inhibition of most anaerobic bacteria
EYA EggYolk Agar	Recognition of lipase and/or lipase positive anaerobic bacteria
FB Fastidious Broth	Accumulation of most anaerobic bacteria

Examples of growth



C. difficile on CCFA



Lipase and lecithinase reaction on EYA



Isolation of *C. difficile*

Toxin test on faecal sample

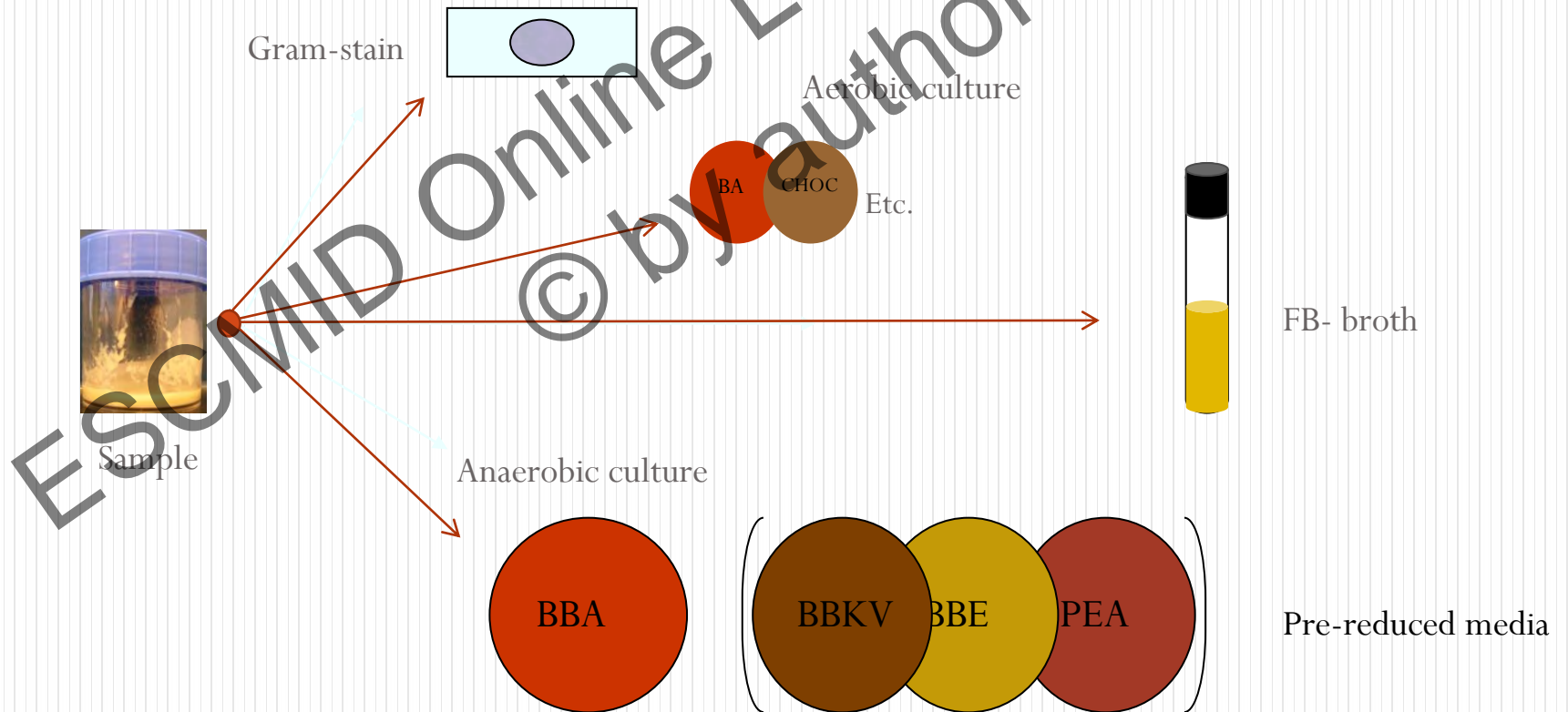
Positive:

- alcohol shock on faecal samples

- culture on CCFA (incubation, 2-4 days)

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Processing of clinical material

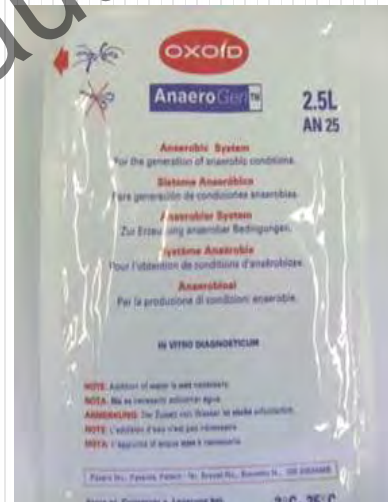


Creating an anaerobic environment

Anaerobic jar



(a)



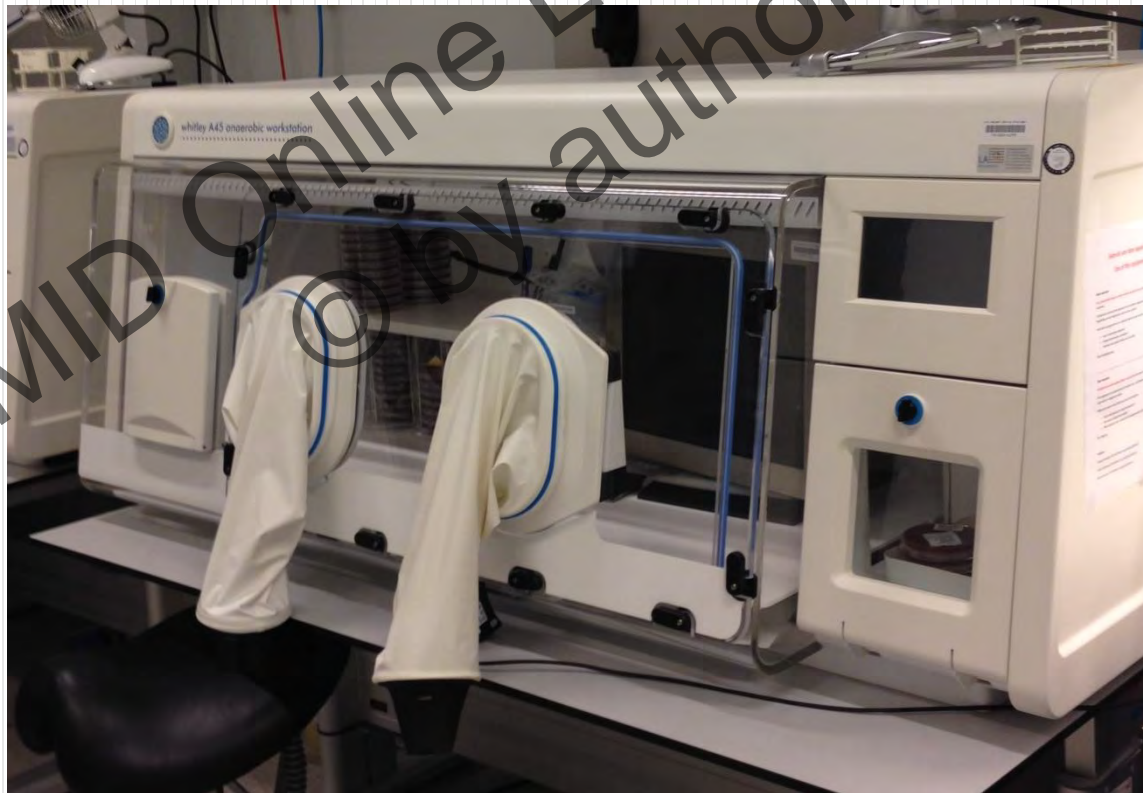
Creating an anaerobic environment

Anaerobic jar/anoxomat®



Creating an anaerobic environment

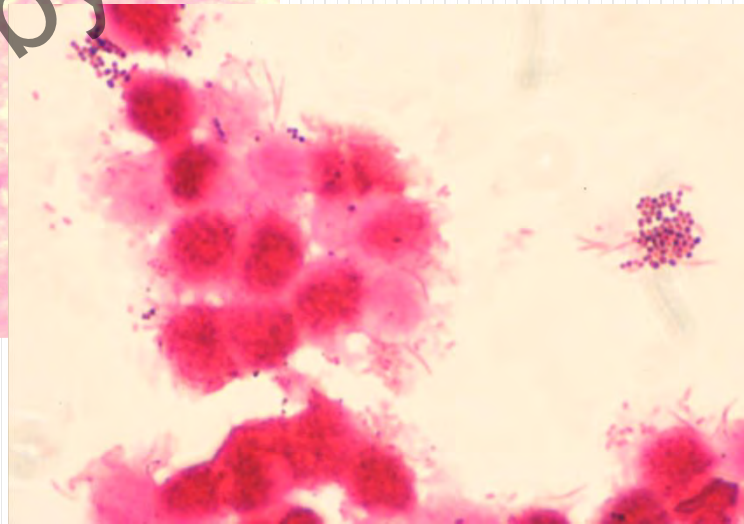
Anaerobic chamber



Anaerobic jar vs chamber

Anaerobic chamber	Anaerobic jar
Constant anaerobic environment	Incidental exposure to oxygen
Examination cultures possible at any moment	Examination cultures after 48 h of incubation
Modification of work method	Normal work method
Sufficient space need in laboratory	No space needed
No odors	Odors due to VFA

Anaerobic bacteria in direct sample



When is a clinical sample suspicious of harboring anaerobic bacteria

- Bacteria present in direct gram-stain but no aerobic growth
- Growth in anaerobic zone of FB broth
- Characteristic colonies on anaerobic media
- Growth of pigmented colonies
- Characteristic smell

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