

Anaerobic cultivation and diagnosis of anaerobic bacilli

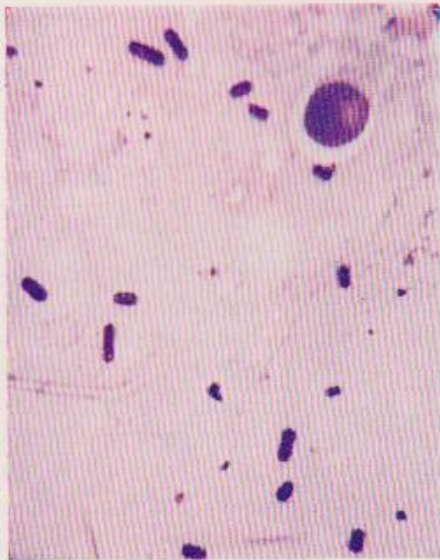
Dr. Shehab A. Lafi

- Clostridia are Gram positive spore forming ,motile except *Clostridium perfringens* grow anaerobically. The Natural habitat is soil, dust , and the intestinal tract of .

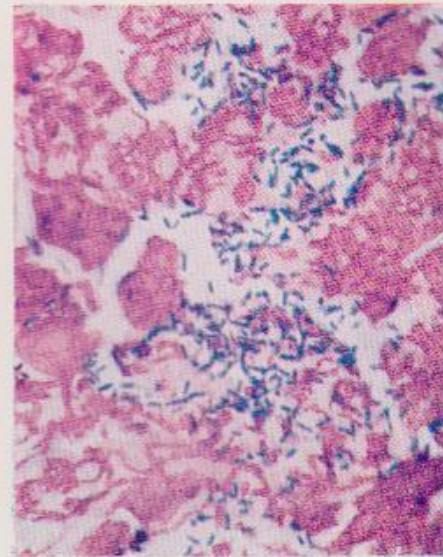
Morphology of anaerobic bacilli :

- This group represents large gram positive bacilli , spores are usually wider than the diameter of the bacillus and they are bulging , some of them are located centrally , subterminal or terminal .

Microscopy. Relatively large bacilli, 5μ by 1μ , with square or rounded ends. Gram-positive, non-motile (all other *Clostridia* are motile), capsulate in animal tissues. Spores are oval, subterminal and non-projecting.



Two Gram-stained preparations showing *Cl. welchii*. On the left is a smear made directly from muscle in a case of gas gangrene; on the right is a section of muscle from the same case. Separation of the muscle fibres, which are also oedematous, by gas production can be noted. $\times 1000$, $\times 500$.

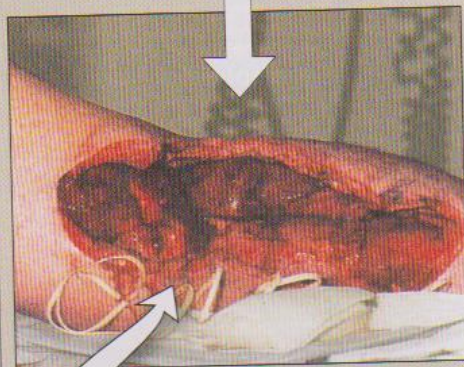


Cultural appearances. Not as strictly anaerobic as *Cl. tetani*; grows very

- The most important species are :
- **Cl. perfringens** causes gas gangrene
- **Morphology gram positive rods with rounded or cut ends, spores are subterminal or central**

- Clinical specimens for *Cl. Perfringens* :
- A- gangrene
- Wound swab
- Wound aspirate
- Tissue biopsy
- Blood
- B- food poisoning:
- Stool

Gas gangrene of 5 cm superficial laceration across the left antecubital fossa (depression in front of the elbow) on presentation 3 days after injury.



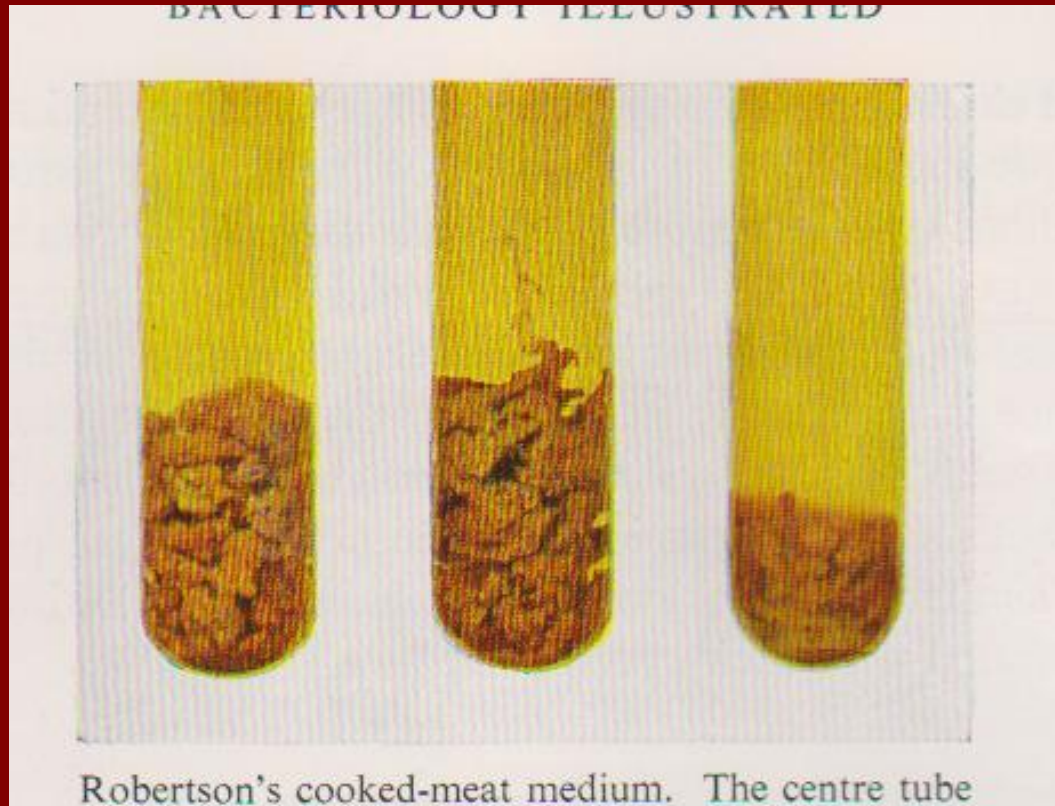
Following surgical debridement.

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Figure 14.4
Gas gangrene of arm.

- Cultivation :
- Cultivation of specimen on cooked meat broth or thiol broth for 24 hours anaerobically at 37 c.

Cultivatin in cooked meat broth



- Subculture on blood agar to study the hemolytic activity and colony morphology of the test bacterium .

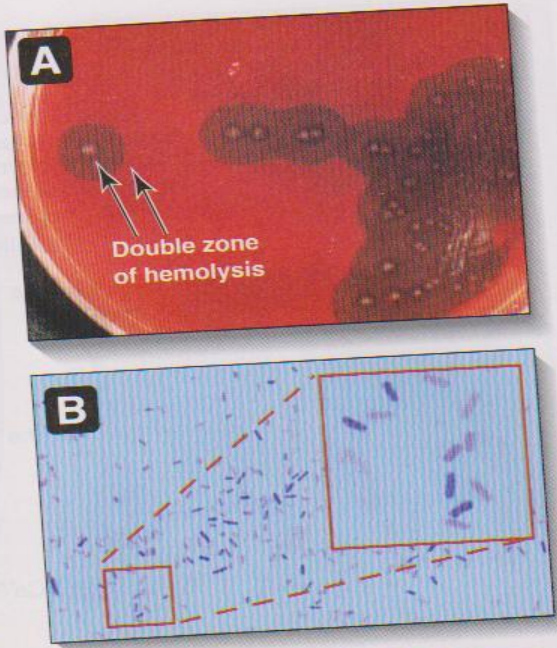


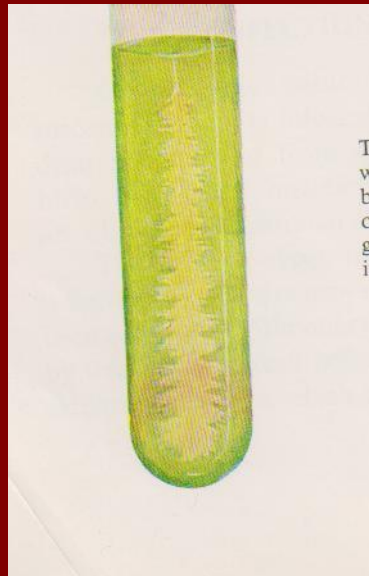
Figure 14.5
Clostridium perfringens. A. Colonies on blood agar showing double zone of hemolysis. B. Photomicrograph of Gram stain.

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Motility test on semisolid agar

Cl. perfringens is non motile



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Negative

Naglers reaction

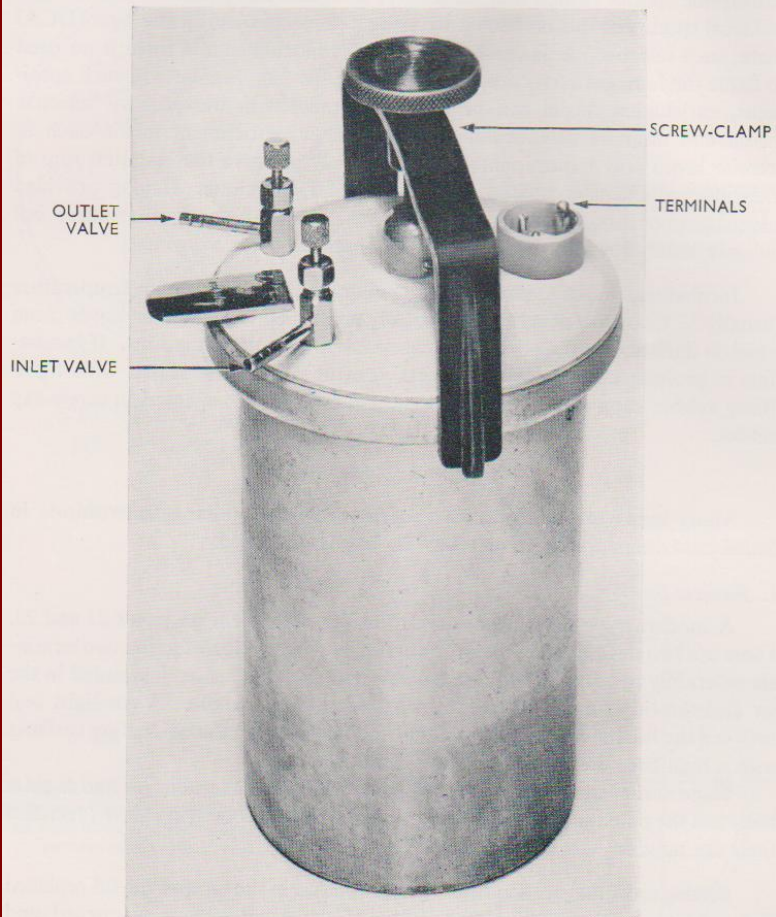


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Positive

Methods of anaerobiasis:

- 1-Anaerobic Jar, agar or culture tubes are placed in empty tightly closed jars evacuated from air then carbon dioxide and nitrogen are pumped into the jar.
- 2-Gas pack kit, Co₂ gas generating kit through certain chemical reaction between chemicals can be used instead of the above mentioned method, this kit is employed with suitable gas pack jar
- .3- cultivation on culture media and incubation in CO₂ incubator .



Anaerobic jar with lid *in situ*.



The simplicity of the 'GasPak' system of generating hydrogen and carbon dioxide is shown here: the foil envelope is opened by peeling back the corner to a printed line and 10 ml. of water is injected. The envelope is immediately placed upright in the anaerobic jar, the lid replaced and screwed down and the jar is then ready for incubation.

Clostridium tetani

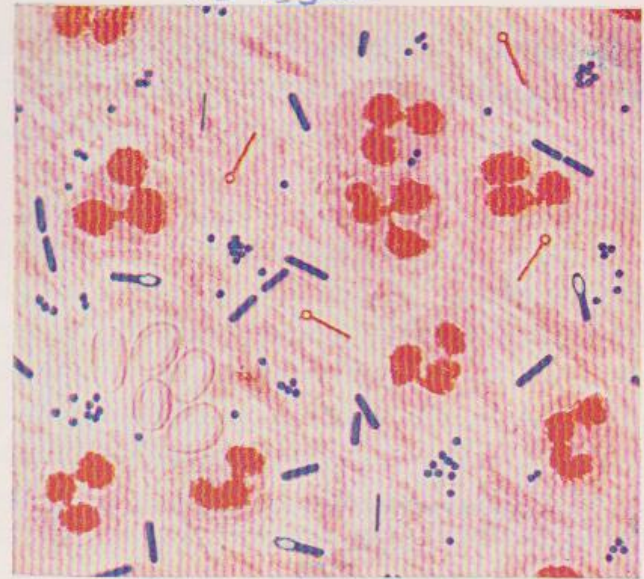
- It is Gram positive spore forming, spores are located terminally giving the organism the drum stick appearance and motile distributed in soil and feces of animals.

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الغنجرية
GAS GANGRENE
تحتكربن كجاز

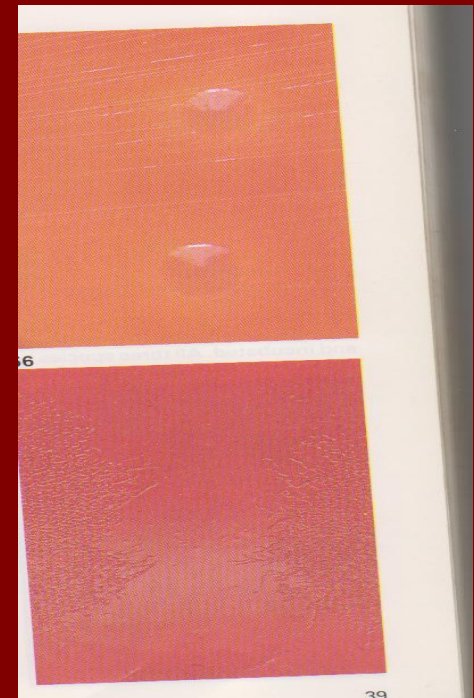


This Gram-stained film of material from a wound shows pus cells (degenerate polymorphonuclear leucocytes), a few necrotic muscle fibres and clumps of Gram-positive cocci which on culture proved to be coagulase positive staphylococci.

Two species of *Clostridia* were also isolated, *Cl. tetani* which in the film is represented by the slender Gram-

Growth characters

Cl. tetani grow on blood agar and show • filamentous with meshwork colony, fresh colony show alpha heamolysis which shifts later into beta heamolysis.



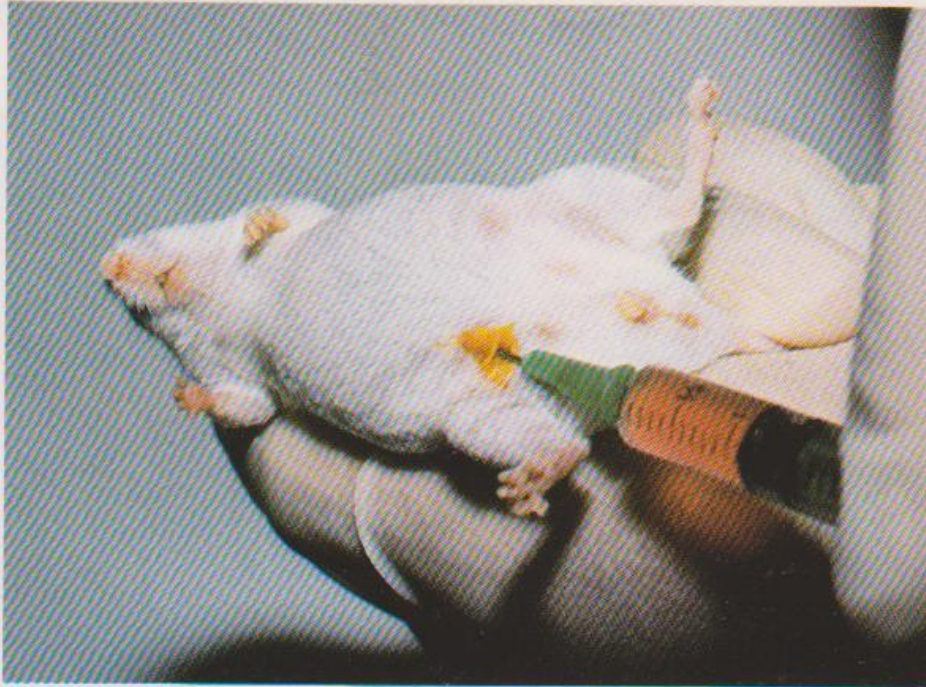
Lab diagnosis

- Anaerobic cultivation for the pathogen diagnosis followed by toxicity test in lab animals (Mice or Guinea pigs) to confirm toxin release by the isolate organism .

Intramuscular injection. Intramuscular injection is done in the thigh. Anaesthesia is unnecessary. Fix the mouse as shown, separating the skin of the back between thumb and fingers. Always disinfect the skin with iodine to prevent abscesses.



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206 Intraperitoneal injection. Fix the mouse by grabbing the skin of the neck and the back in one hand. Disinfect the skin of the injection site. Hold the mouse with the head somewhat downward to move the intestines. The needle is pushed in slowly to prevent perforations.

- API SYSTEM (Analytical Profile Index)is available for anaerobic bacteria diagnosis
- Particularly Clostridium types.

