

Gram-Positive Bacilli: Spore-formers

***Bacillus* species**

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Medically Important Gram-Positive Bacilli

Three general groups:

1. Endospore-formers

Bacillus, Clostridium

2. Non-endospore-formers

Listeria

3. Irregular shaped and staining properties

Corynebacterium, Mycobacterium

***Bacillus* species**
(Spore-formers)

There are 48 species included in the genus bacillus with the following general characteristics:

- 1. Gram-positive bacilli**
- 2. Spore former**
- 3. Aerobic and facultative anaerobic**

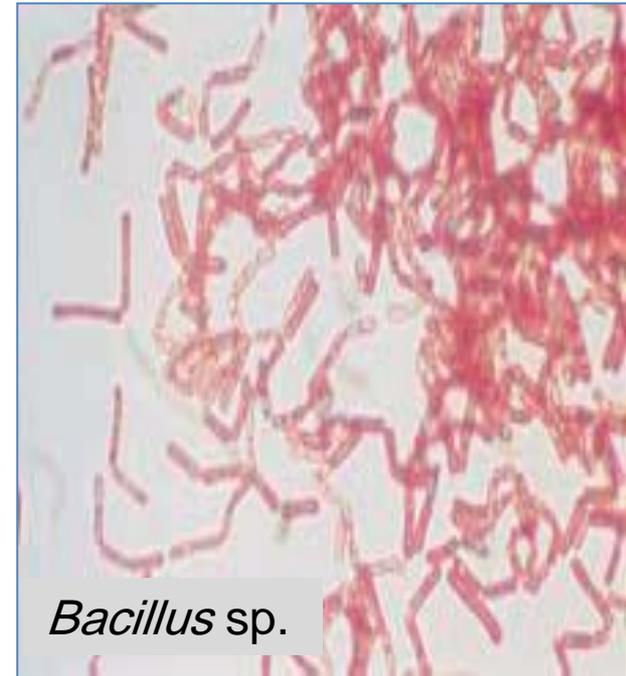
THE MOST IMPORTANT PATHOGENS:

1. Bacillus anthracis

- *Agent of anthrax, a disease in livestock*
- *Humans acquire infection by contamination of wound or ingestion or inhalation of spores*

2. Bacillus cereus

- *Causes food poisoning*



Bacillus sp.

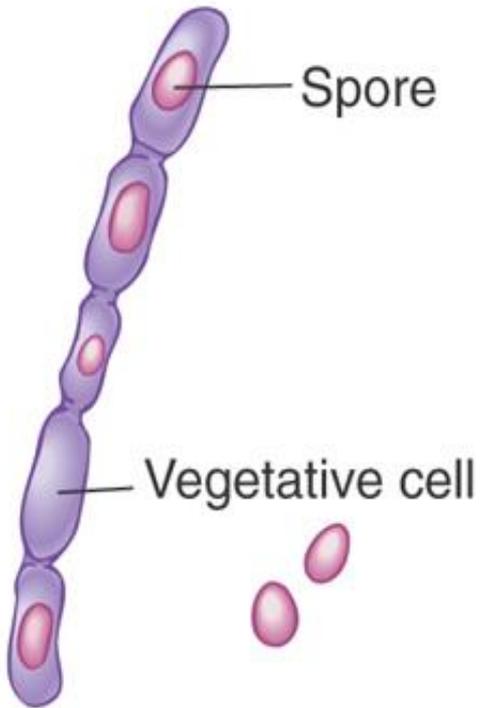
Bacillus anthracis

Morphology:

1. Large gram positive bacilli.
2. Non-motile.
3. Found in pairs or in long chains.
4. Capsule could be demonstrated during growth in infected animals.
5. Spores are formed in culture, dead animal's tissue but not in the blood of infected animals.
6. Spores are oval and centrally located.

Survival in Soil

- Spores remain viable in soil for decades.
- Changing environmental conditions (temp. rain etc.) help in survival and multiplication.



(a)

Cultural Characteristics:

- Blood Agar and Nutrient Agar are commonly used to cultivate the bacilli.
- Plates are incubated aerobically at 37 C.
- On blood agar plates, colonies have irregular borders and are *non-hemolytic*.
- On nutrient agar: They are described as "*Medusa head*" or "*Comet tail*".

Specimen Collection and Laboratory Diagnosis:

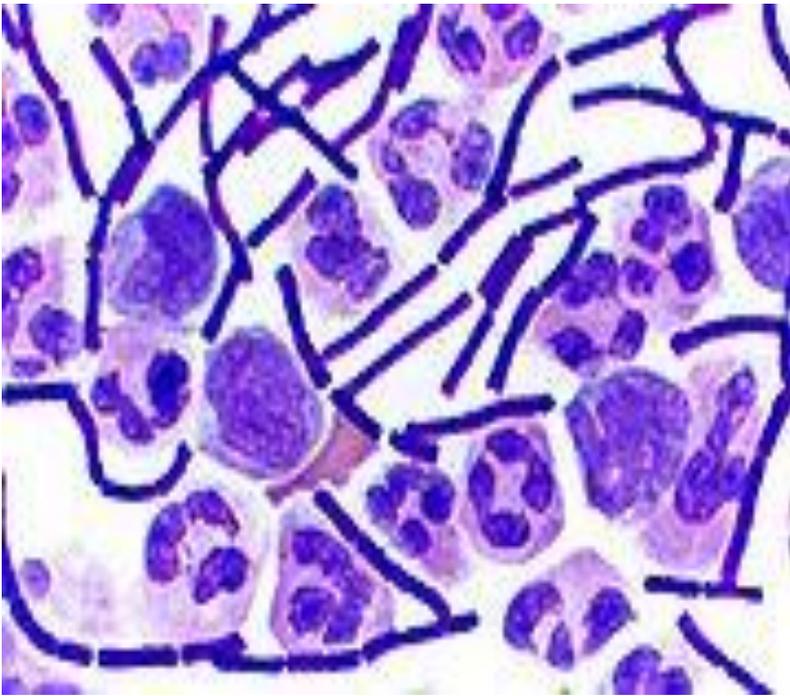
CAUTION: *Laboratory safety is very important when working with any materials suspected of containing Bacillus anthracis.*



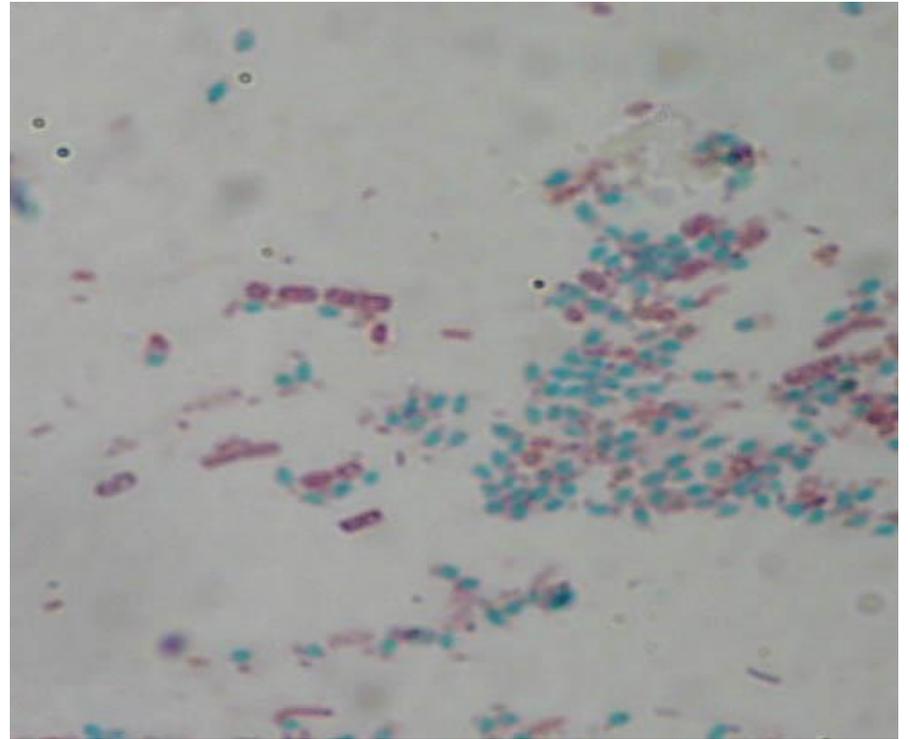
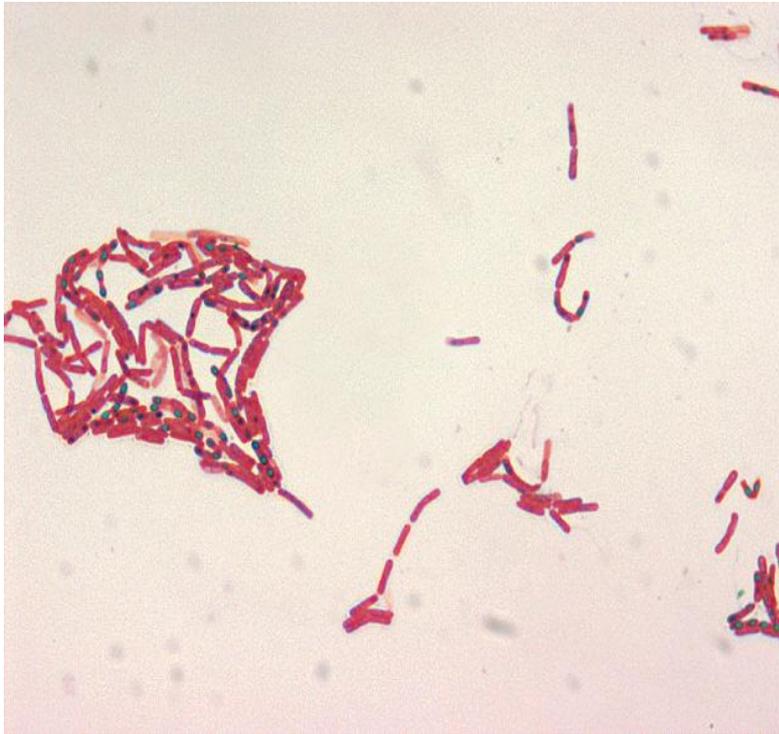
Colonial growth of *Bacillus anthracis*. When lifted by an inoculation loop, colonies, in this case on sheep blood agar, show a tenacity that allows them to be pulled up and stay upright with a texture similar to egg whites.

Samples are collected depending on the site affected:

1. **Swab samples from cutaneous lesions and blood cultures.**
 2. **Sputum and blood for pulmonary anthrax**
 3. **Gastric aspirate, feces and blood for enteric anthrax.**
- **Gram stained smears:** Made from clinical samples, show large gram positive bacilli in long chains "Bamboo-like appearance".
 - **Giemsa stained smears:** Purple bacilli with red capsule.
 - **Culture**
 - **Animal inoculation test:** Experimental animals are injected intraperitoneally by a suspension of the test organism "Suspected *B. anthracis* culture".
 - The animal dies in 48-96 hours due to respiratory failure.*
 - Large number of typical bacilli can be found in the blood and tissue of spleen of the infected animal.



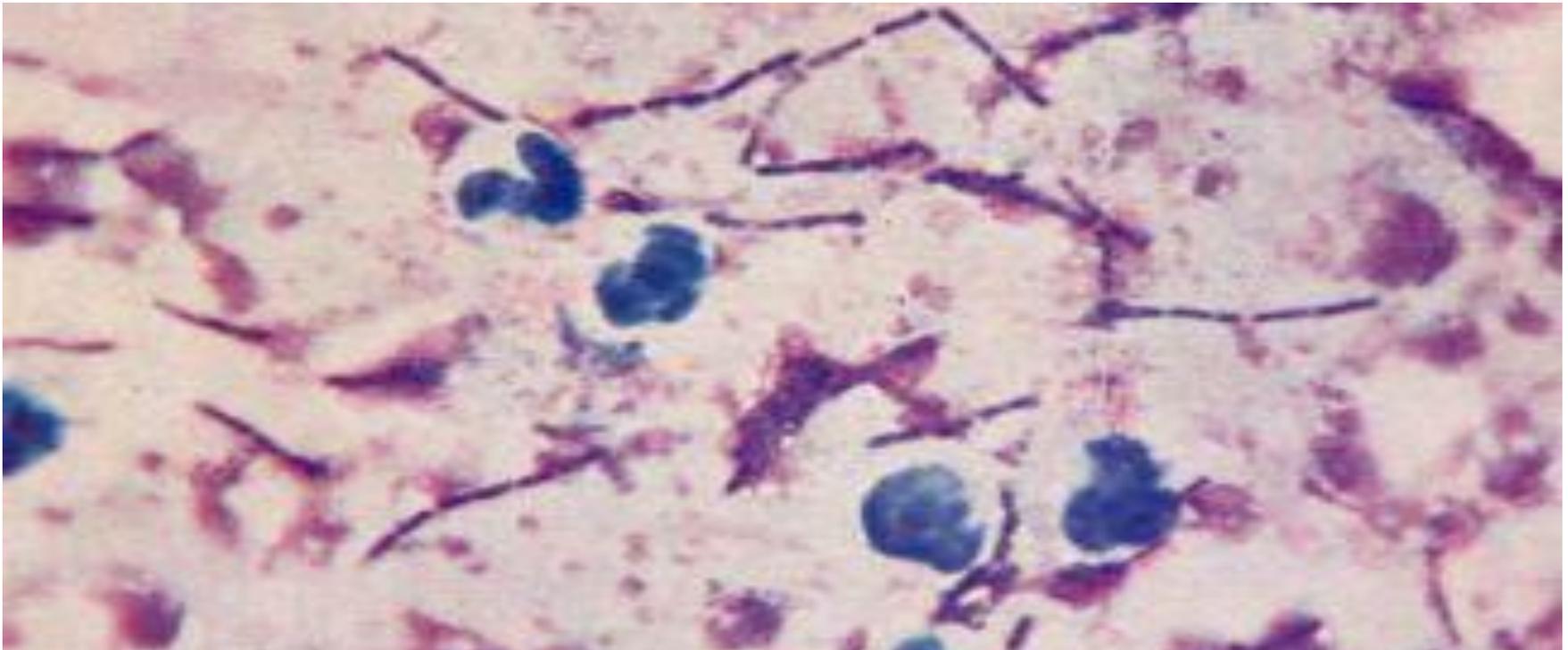
Gram stain of *Bacillus anthracis* showing Gram-positive rods forming long filaments. “Bamboo –like appearance”



showing endospores as green and the vegetative cell as red

McFadyean's reaction

- McFadyean's reaction: is a special staining reaction , demonstrating a pink capsule around a blue cell, after staining with methylene blue, which is used as a presumptive diagnosis for anthrax in blood smear .

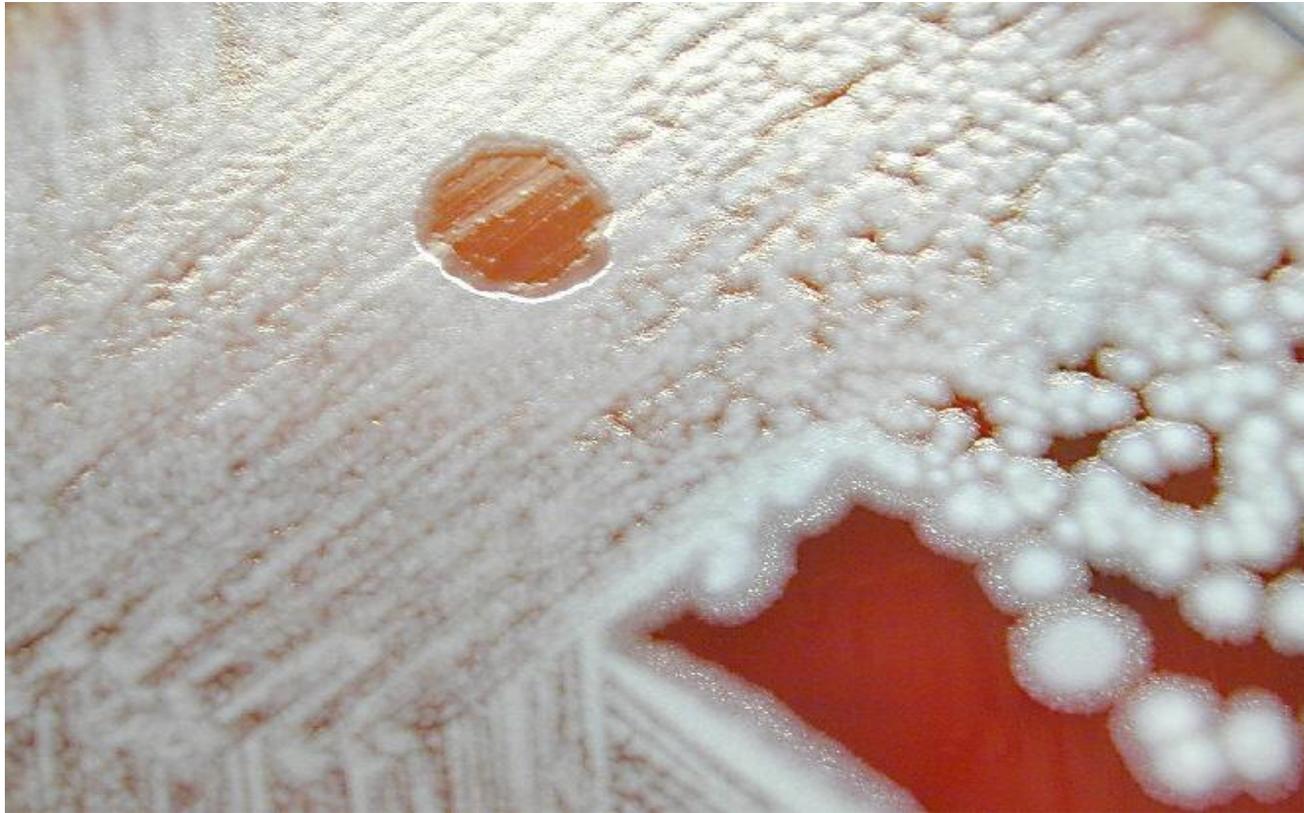


McFadyean's reaction showing short chains of *Bacillus anthracis* cells lying among amorphous, disintegrated capsular material. White blood cells can also be seen.

Biochemical Identification:

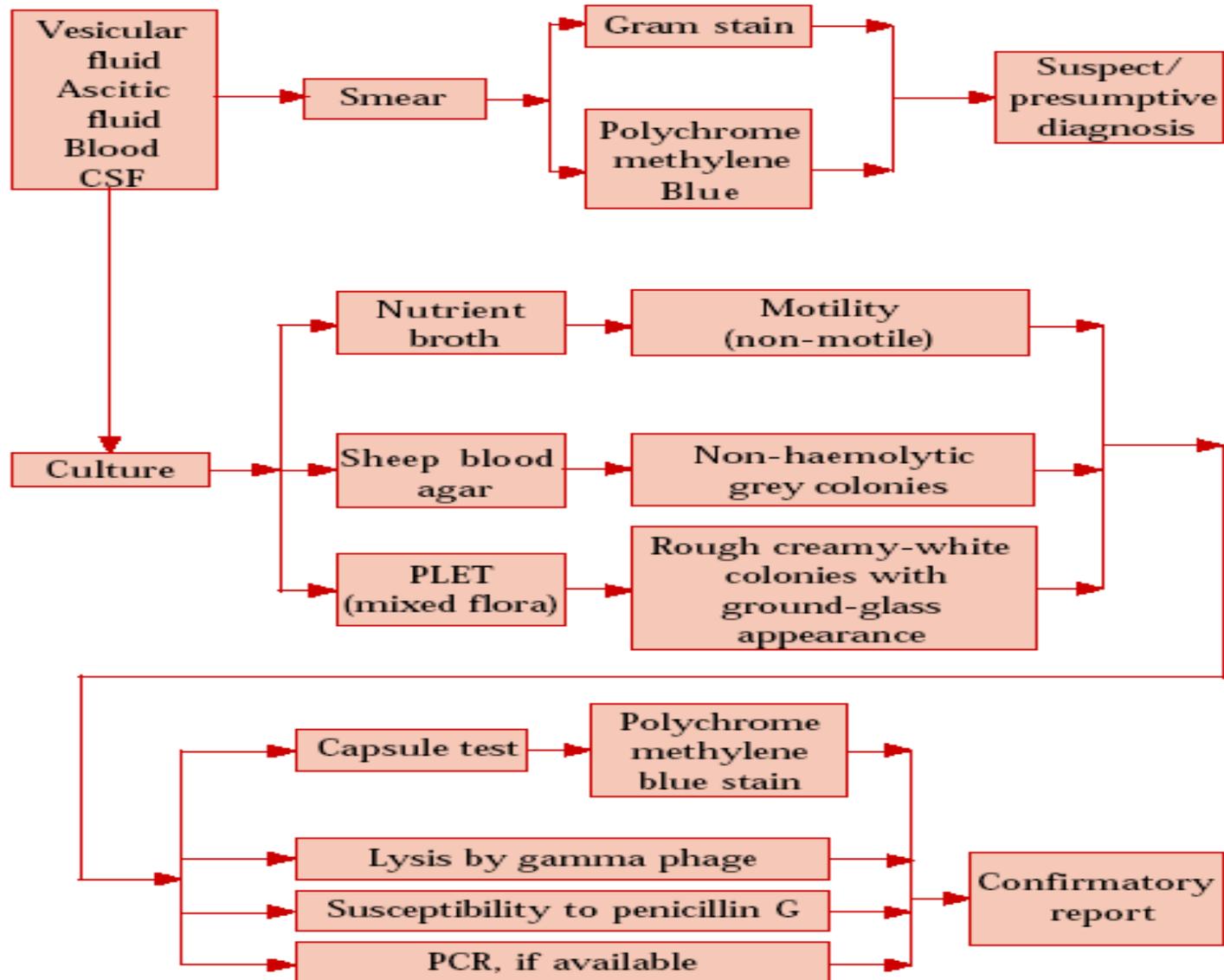
- Sensitivity to penicillin : (Sensitive) .
- String-of-pearls test:(positive) ; As *Bacillus anthracis* is susceptible to penicillin, so when *B. anthracis* is grown on surface of a solid medium containing 0.05-0.50 units of penicillin/ml in 3-6 hrs the cells swell up. The cells become large, spherical and occur in chains on the surface of agar, resembling a string of pearls.
- Lysis by gamma phages: (Positive) This test accurately differentiate *B.anthraxis* from other bacillus species.

Gamma Phage has the ability to lyse *B. anthracis* grown aerobically on blood or other nutrient agar and rarely lyses any other *Bacillus* species.



Lysis of *Bacillus anthracis* by the lytic phage *gamma*.
The plaque (clear area) in the region of confluent growth is where the gamma phage was applied. The plaque results from the phage's .

Suggested procedure for isolation and identification of *B. anthracis* and confirmation of diagnosis



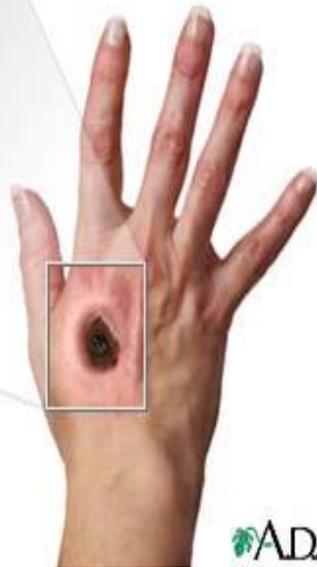
PATHOLOGY

There are different clinical forms of anthrax:

1. **CUTANEOUS ANTHRAX:** 95-98% of anthrax cases are of this type. Infection occur through wounds, burns, which may progress to toxemia and septicemia. The site of entry often produces a painless blister referred to as *Malignant pustule*.
2. **ENTERIC "INTESTINAL" ANTHRAX:** Caused by the ingestion of infected meat.
This form of the disease is severe and fatal.
3. **PULMONARY ANTHRAX:** Caused by the inhalation of large number of *B. anthracis* spores. It is usually fatal. This clinical form is commonly known as "*wool sorter disease*".

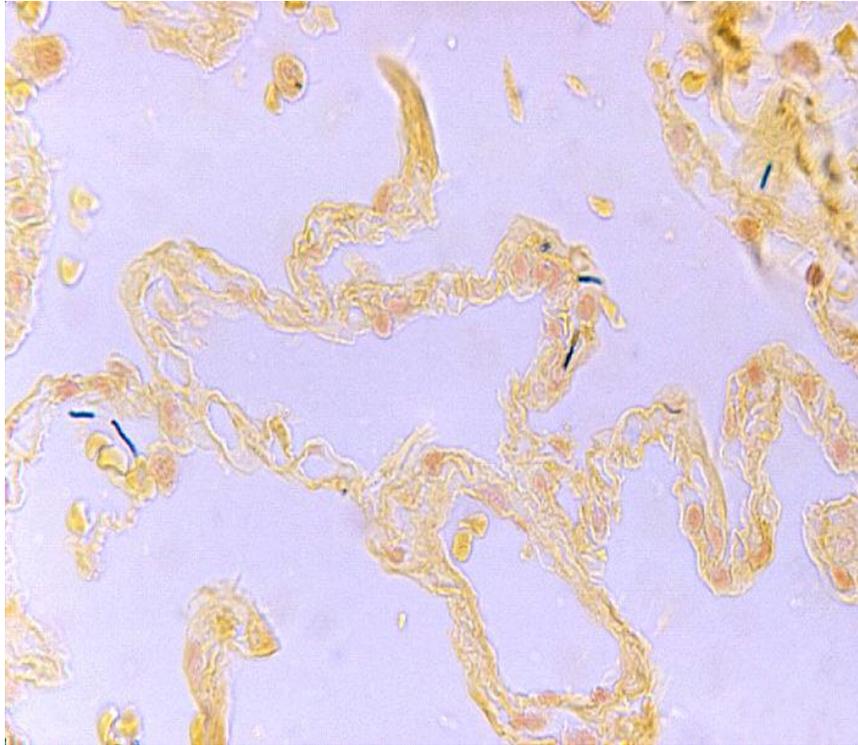


Cutaneous anthrax skin infection

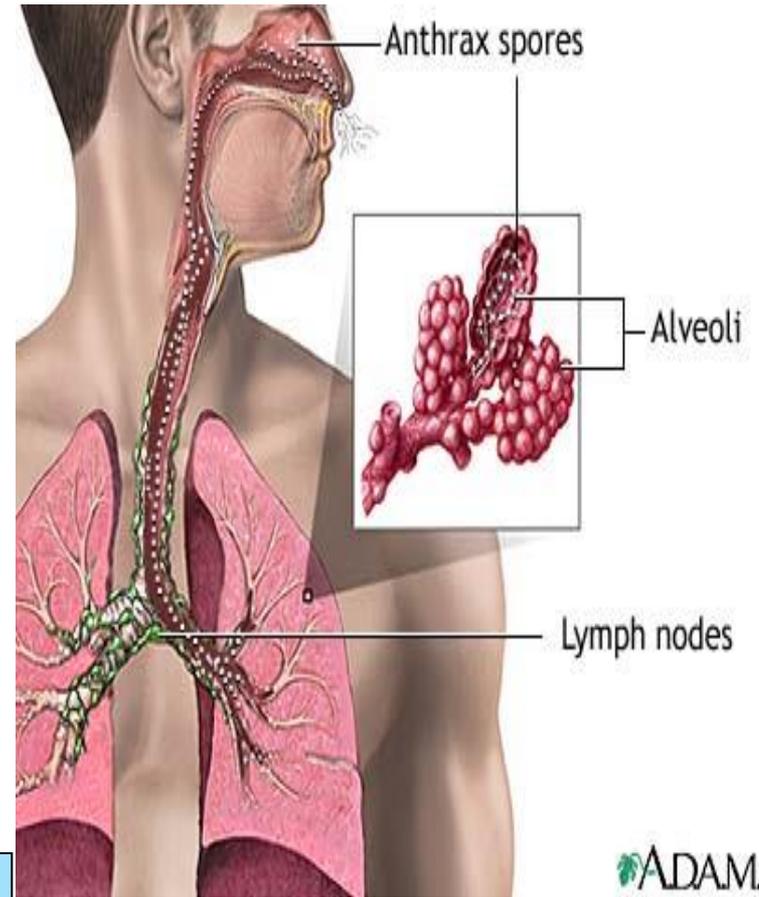


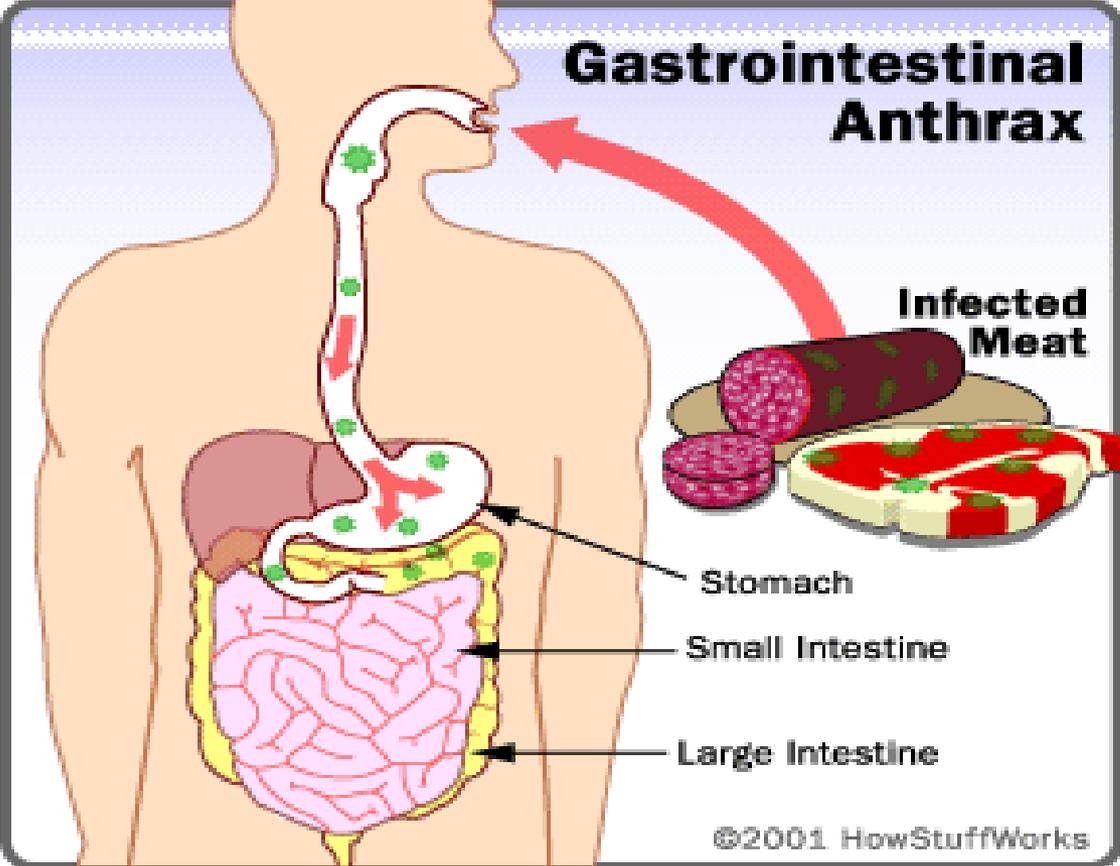
ADAM.



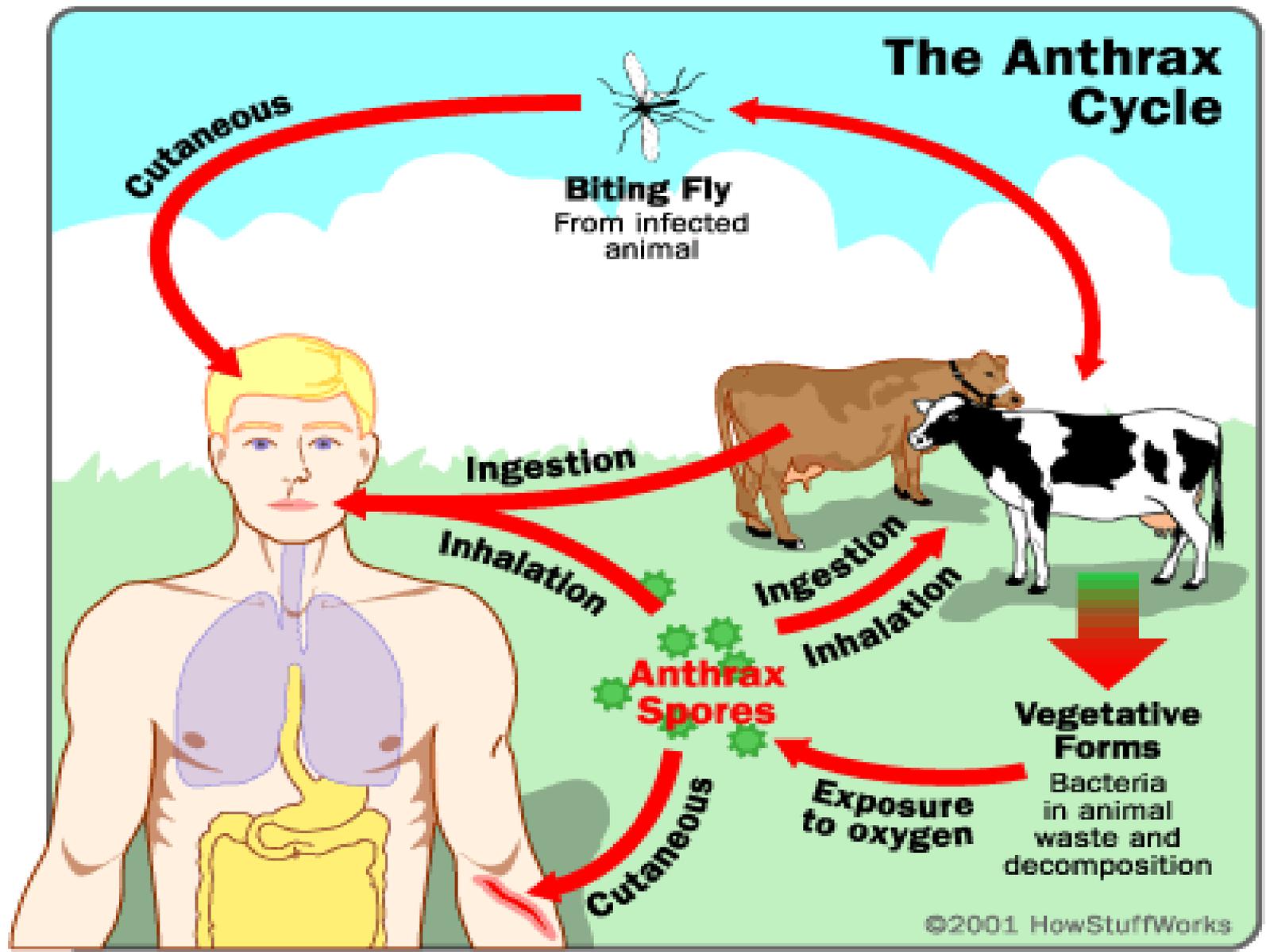


Rod of B anthrax in lung Tissue





The Anthrax Cycle

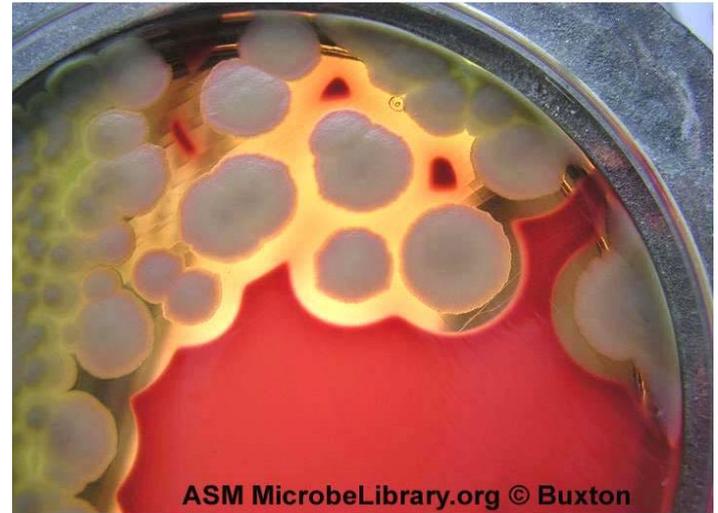


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Bacillus cereus



- Gram-positive spore forming bacilli
- *Bacillus cereus* is a normal inhabitant of the soil, but it can be regularly isolated from foods such as grains and spices
- Produce β -hemolysis on blood agar



Pathogenesis & clinical features

- Spores are found on most raw foods like rice
- Spores are heat-resistant & survive rapid frying
- Produce enterotoxin – ingested → food poisoning.
- Short IP – 4-6 hours – similar to Staphylococcal food poisoning (vomiting & diarrhoea)

B. cereus with Endospores



Characteristic	<i>B. anthracis</i>	<i>B. cereus</i>
hemolysis on sheep blood agar	-	+
glutamyl-polypeptide capsule	+	-
lysis by gamma phage	+	-
motility	-	+
string-of-pearls test	+	-



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Pathogenesis

To produce disease, the spore of the anthrax bacteria enters an opening in the body, either through a break in the skin, through ingestion into the GI tract, or through inhalation into the lungs. The spores are ingested at the site of entry by macrophages, in which they germinate into replicating bacteria and begin to produce toxins.

When spores are inhaled, they are transported to the regional tracheobronchial lymph nodes, where germination occurs. The toxins cause edema, hemorrhage and tissue necrosis at the site. The bacteria enter the bloodstream and seed other organs.

In inhalational cases, death occurs from a combination of respiratory failure with hemorrhagic mediastinitis, pleural effusions, overwhelming bacteremia, toxemia and often, meningitis.