Laboratory Diagnosis of *Clostridium* spp & Citrate Utilization Test

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Introduction

- Clostridia are strictly anaerobic to aerotolerant spore-forming bacilli found in soil, sewage, marine sediments, animal and plant products, the intestinal tract and in wounds of man and animals.
- *Clostridium* spp. are ubiquitous, gram-positive motile (except *C. perfringens*) bacteria that cause a wide range of diseases, including **tetanus**.
- The toxins produced by the organisms of tetanus and botulism attack nervous pathways and are referred to as **neurotoxins**.
- The organisms associated with gas gangrene attack soft tissues by producing toxins and aggressions and are referred to as **histotoxic**.

Species of clostridium genus

The genus *Clostridium* includes more than 140 species, such as

- *Clostridium tetani*
- *C. difficile*
- *C. botulinum*
- *C. septicum*
- *C. novyi*
- *C. perfringens* (type A, B, C, D, E)

Macroscopically

- Growth of the bacteria in the laboratory on media is difficult and testing for the bacteria has become easier with molecular and serological testing (One exception is *C. perfringens).*
- Clostridia possess no one typical colony morphology.
- They are generally a large colony (>2mm) with irregular edges.
- Some Clostridia form small, convex, non-haemolytic colonies with a smooth edge.
- Other Clostridia produce several different-looking colony types, so the culture appears mixed.

- A few *Clostridium* spp. have distinctive colony characteristics.
- *Clostridium perfringens* usually produces a double zone of beta-haemolysis on blood agar and it is positive for lecithinase on EYA, which differentiates them from other *Clostridium* spp.
- The inner zone shows complete haemolysis, whereas the outer zone may display partial haemolysis.
- *Clostridium difficile* produces a yellow ground-glass colony on CCFA.
- On blood agar, *Clostridium difficile* are usually fluoresce yellow-green, and emit a horse stable odour.

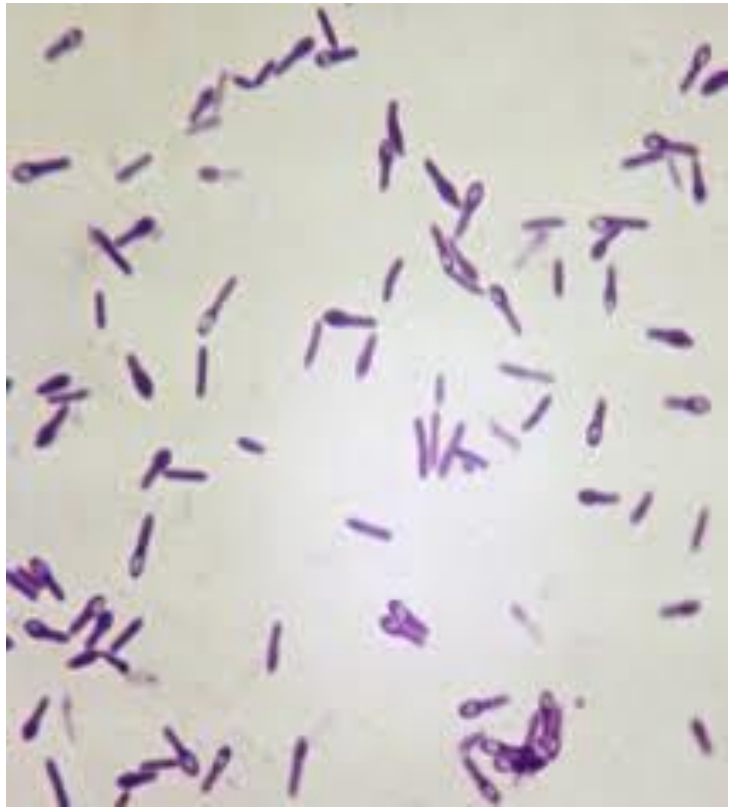
Recommended Media

Reason	Type of medium
Culture	Anaerobic Blood Agar
Selective isolation	Anaerobic Phenylethyl Alcohol (PEA), Cycloserine-Cefoxitin Fructose Agar (CCFA; for *Clostridium difficile*).
Identification	Egg Yolk Agar.
Maintenance	Cooked Meat Media or Thioglycollate with Supplements

Microscopically

- They usually gram-positive.
- They appear as rods bacilli, similar in appearance to *Bacillus* spp.
- Spore forming bacteria.

Clostridium tetani

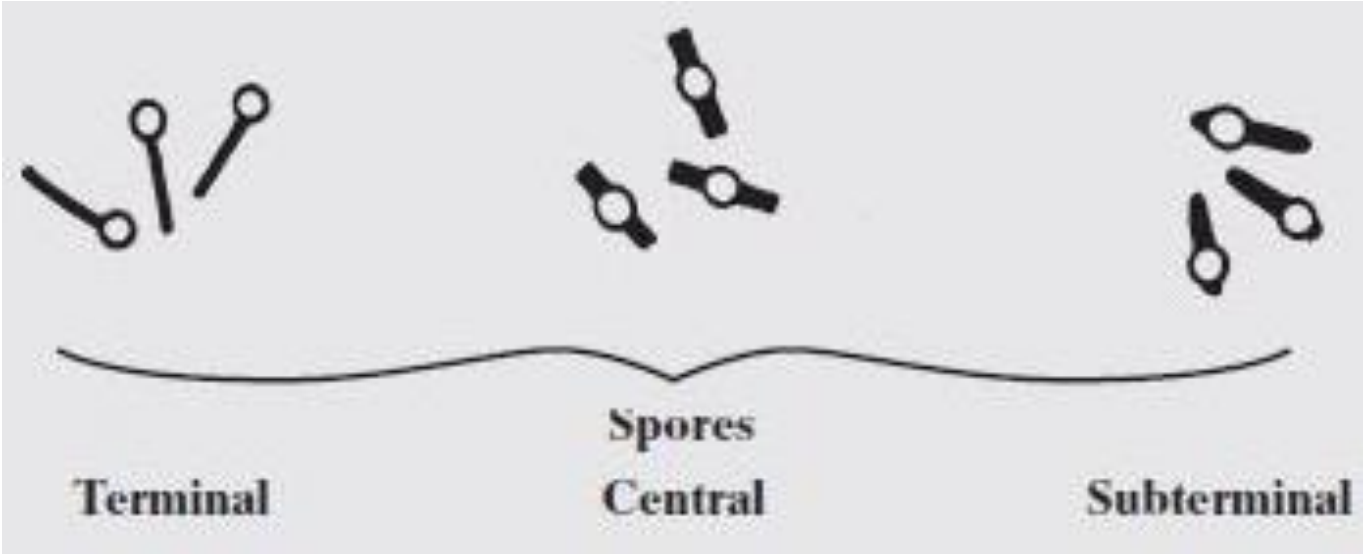


Spores

- The size, shape, and location endospores are particularly useful for identifying *Clostridium*

C. tetani: spherical and terminal (drum sticks)

C. perfringens: sub terminal



Spore location in *Clostridium* bacteria

Biochemical tests of some *Clostridium* spp

Species	Egg – yolk agar		Hydrolysis of gelatin	Digestion of casein	Indole production	Acid production			
	Lecithinase	Lipase				Glucose	Lactose	Sucrose	Maltose
C. tetani	-	-	+	-	v	-	-	-	-
C. botulinum I	-	+	+	+	-	+	-	-	+
C. chauvoei	-	-	+	-	-	+	+	+	+
C. septicum	-	-	+	+	-	+	+	-	+
C. novyi A	+	+	+	-	-	+	-	-	+
C. perfringens	+	-	+	+	-	+	+	+	+

Some disease caused by *Clostridium* spp

Clostridium Species	Diseases	Hosts
C. tetani	Tetanus	horse, ruminant, human
C. botulinum	Botulism	Human and animals
C. chauvoei	Black leg	cattle, sheep, pigs
C. septicum	Malignant edema Necrotic dermatitis	cattle, sheep, pigs, chicks
C. novyi type A	Big- head of rams	sheep
C. novyi type B	Black disease (Necrotic hepatitis)	Cattle, sheep
C. perfringens type A	Gas-gangrene, Food poisoning Enterotoxemic jaundice	Human, lamps

Diagnosis

- Macroscopy
- Microscopy
- Chemical test
- Molecular techniques
  - PCR technique: one of the most important technique that use for Bacterial identification
  - Amplification of specific sequences from a gDNA of the interesting sample.
  - Sequencing of the amplified DNA

Citrate Utilization Test

Citrate Utilization Test

- The **citrate utilization test** determines the ability of bacteria to use **citrate** as a sole carbon source for their energy needs.
- This ability depends on the presence of a **citrate permease** that facilitates transport of citrate into the bacterium.
- Once inside the bacterium, citrate is converted to pyruvic acid and CO2.
- **Simmons citrate agar slants** contain sodium citrate as the carbon source, NH4+ as a nitrogen source, and the pH indicator bromothymol blue.

- This test is done on slants since O_2 is necessary for citrate utilization.
- CO_2 combines with sodium (supplied by sodium citrate) and water to form sodium carbonate (an alkaline product).
- This raises the pH, turns the pH indicator to a blue colour, and represents a **positive citrate test**; absence of a colour change is a **negative citrate test**.
- Citrate-negative cultures will also show no growth in the medium.

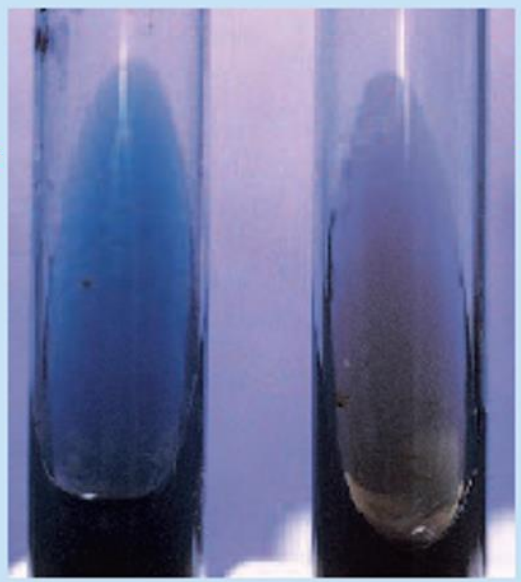
Biochemistry within bacteria

Sodium citrate $\xrightarrow[\text{citrase}]{\text{Citrate permease}}$ Pyruvic acid + oxaloacetic acid + CO_2

Biochemistry within tubes

Excess sodium from sodium citrate + CO_2 + H_2O $\longrightarrow$ Na_2CO_3 (alkaline) pH $\uparrow$

Enterobacter aerogenes → ← *Escherichia coli*



Citrate + **Citrate -**

Procedure

- Inoculate Simmons citrate agar slants with *E. coli* and *E. aerogenes* by stab and streak.
- Incubate these cultures for 24 to 48 hours at 35-37°C.
- Examine the slant cultures for the presence or absence of growth and for any change in colour from green to blue.
- The development of a deep blue colour is a positive test.

Based on your observations, determine and record the results in your lab book.