The Biology of Anaerobic Bacteria and Predominant Propagation Practices

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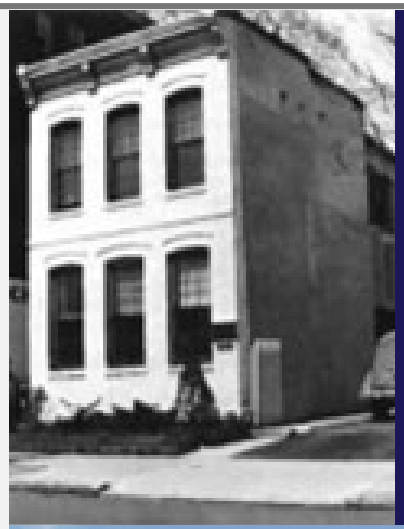
About ATCC

- Founded in 1925, ATCC is a non-profit organization with headquarters in Manassas, VA
- World's premiere biological materials resource and standards development organization
- ATCC collaborates with and supports the scientific community with industry-standard biological products and innovative solutions
- Strong team of 400+ employees; over one third with advanced degrees



Established partner to global researchers and scientists

Outline

1. ATCC's anaerobe collection
2. Classification of anaerobes and specific examples
3. Nutritional and atmospheric considerations
4. Propagation methods

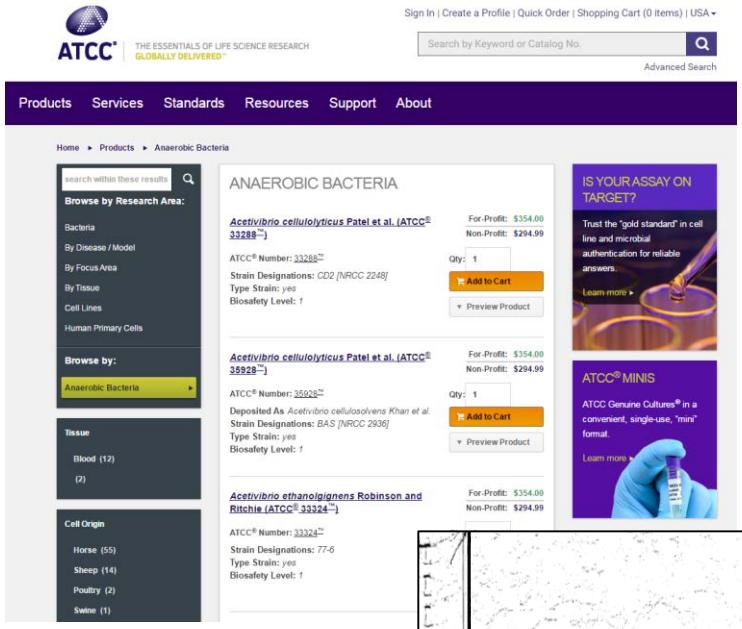
ATCC's anaerobe collection

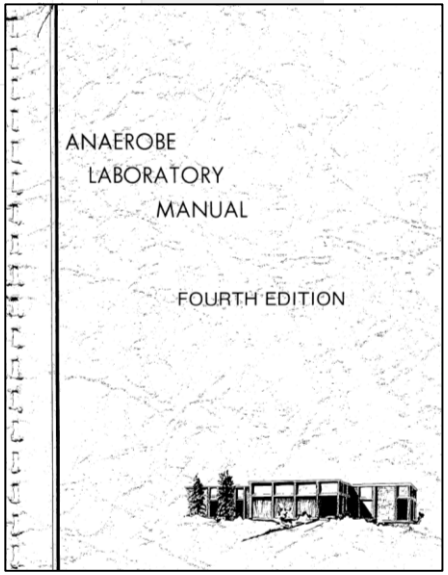
942 active anaerobe holdings

- 465 are type strains

VPI Anaerobe Laboratory

- W.E.C. Moore, Lillian V. Holdeman-Moore, Tracy Wilkins, Elizabeth P. Cato
- Anaerobe Laboratory Manual





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Top 5 anaerobes at ATCC

- *Clostridium sporogenes* (ATCC® 11437™)
- *Clostridium sporogenes* (ATCC® 19404™)
- *Clostridium perfringens* (ATCC® 13124™)
- *Bacteroides fragilis* (ATCC® 25285™)
- *Porphyromonas gingivalis* (ATCC® 33277™)

Image of *Bacteroides fragilis* courtesy of the CDC

Image of *Clostridium perfringens* courtesy of the CDC

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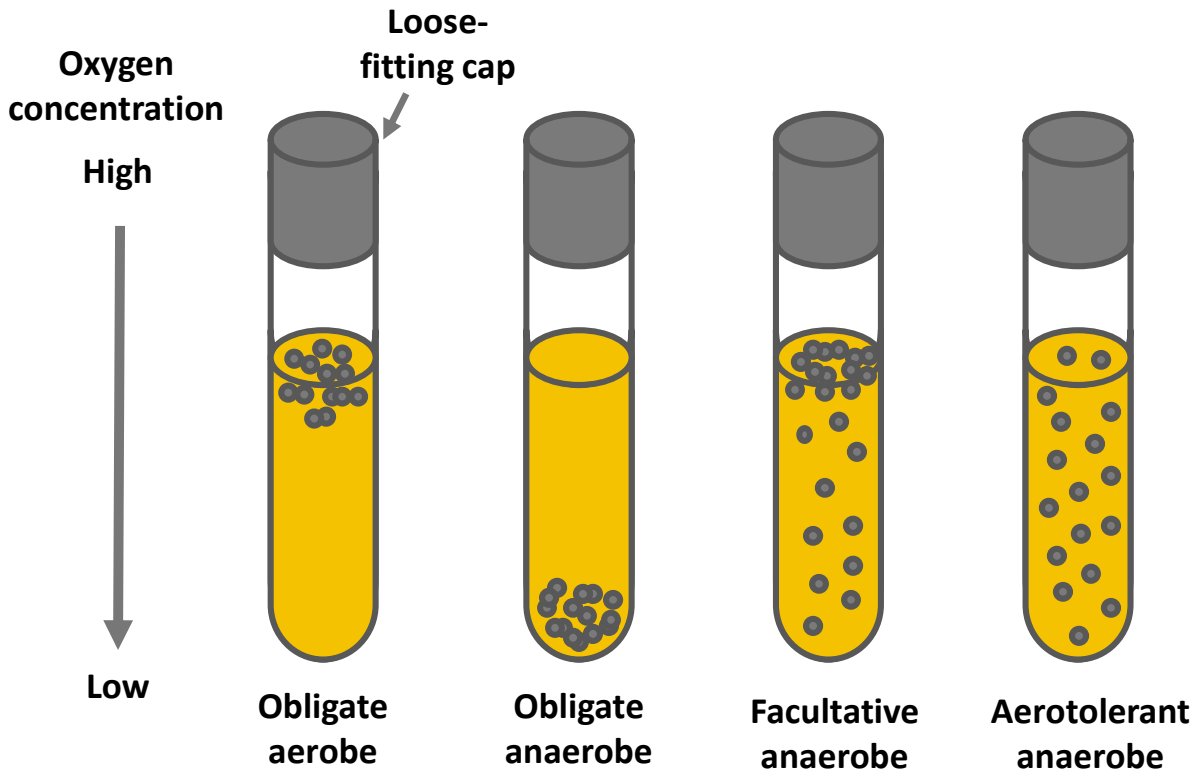
Classification of anaerobes

- Facultative
- Aerotolerant
- Strict

- Environmental
- Methanogens



Facultative anaerobes

- Can grow with or without the presence of oxygen
- Can metabolize energy aerobically (respiration) or anaerobically (fermentation)
- Ex: *Propionibacterium acnes* (ATCC® 6919™)
  - Isolated from facial acne
  - Has variable aerotolerance
  - Nutritional requirements:
    - All strains require Vitamin B5
    - Thiamine, biotin, and nicotinamide are stimulatory

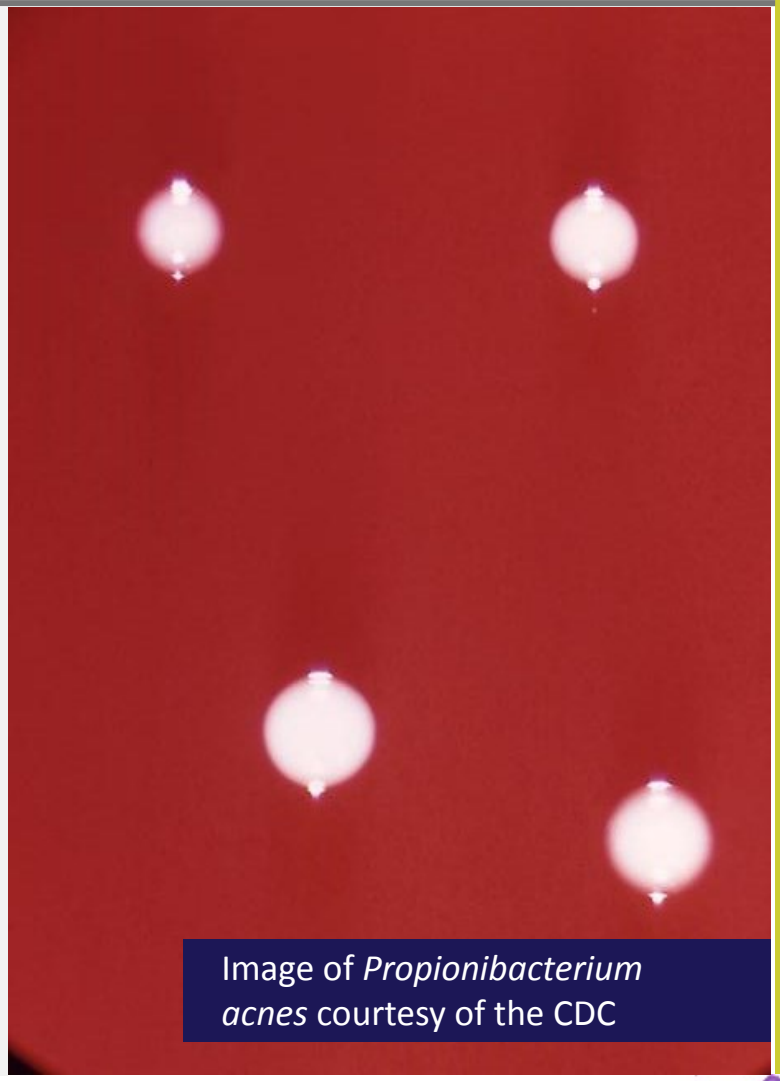
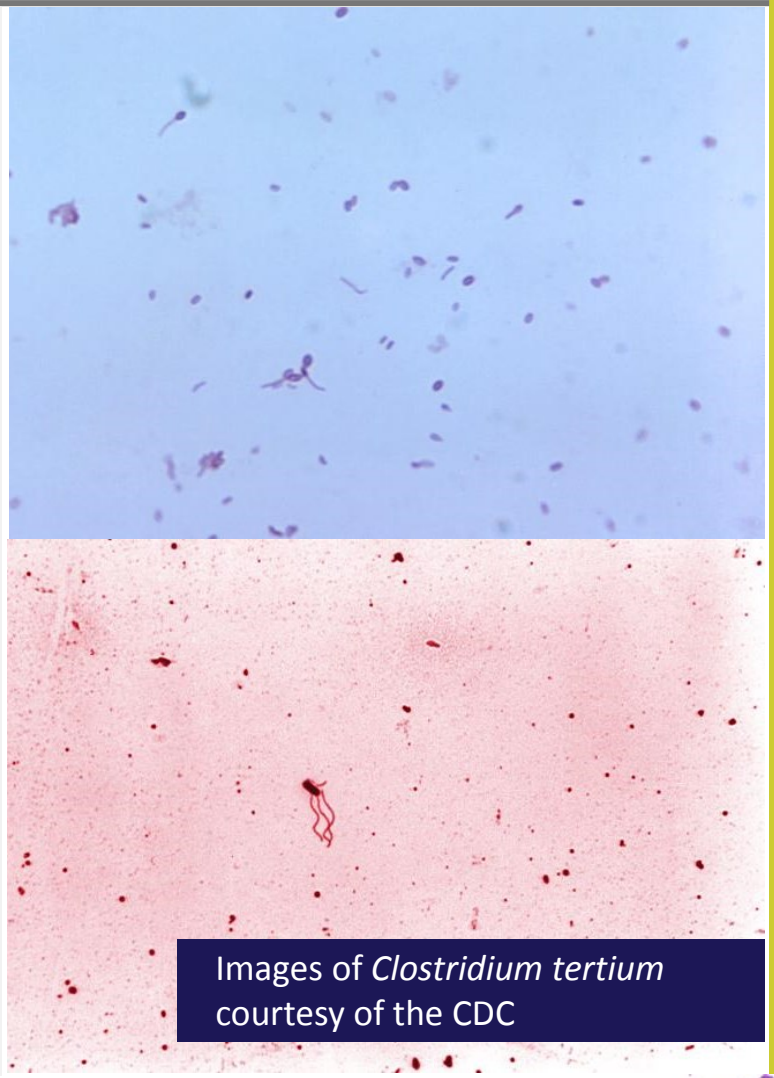


Image of *Propionibacterium acnes* courtesy of the CDC

Aerotolerant anaerobes

- Not inhibited by O_2, but does not use O_2 to generate ATP
- Uses fermentation to generate ATP

- Ex: *Clostridium tertium* (ATCC® 14573™)
  - Originally isolated in 1917 by Captain Henry from war wounds
  - Type strain
  - Will grow slightly on an aerobic blood plate



Images of *Clostridium tertium* courtesy of the CDC

Strict anaerobes

- Grows only in the absence of O_2 and may be inhibited or killed by O_2
- Generates ATP through anaerobic respiration or fermentation
- Ex: *Fusobacterium nucleatum* subsp. *nucleatum* (ATCC® 25586™)
  - Isolated from a Cervico-facial lesion
  - Obligate anaerobic, Gram-negative rods with pointed ends
  - Most strains produce H_2S
  - All *Fusobacterium* species produce butyric acid
  - Require a rich medium for growth
- Ex: *Clostridium sporogenes* (ATCC® 19404™)
  - Isolated from gas gangrene
  - Genetically similar to *C. botulinum*

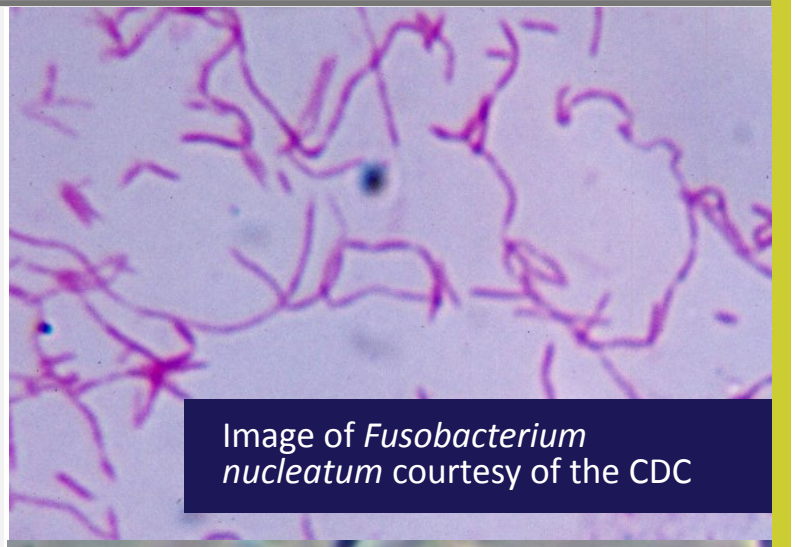
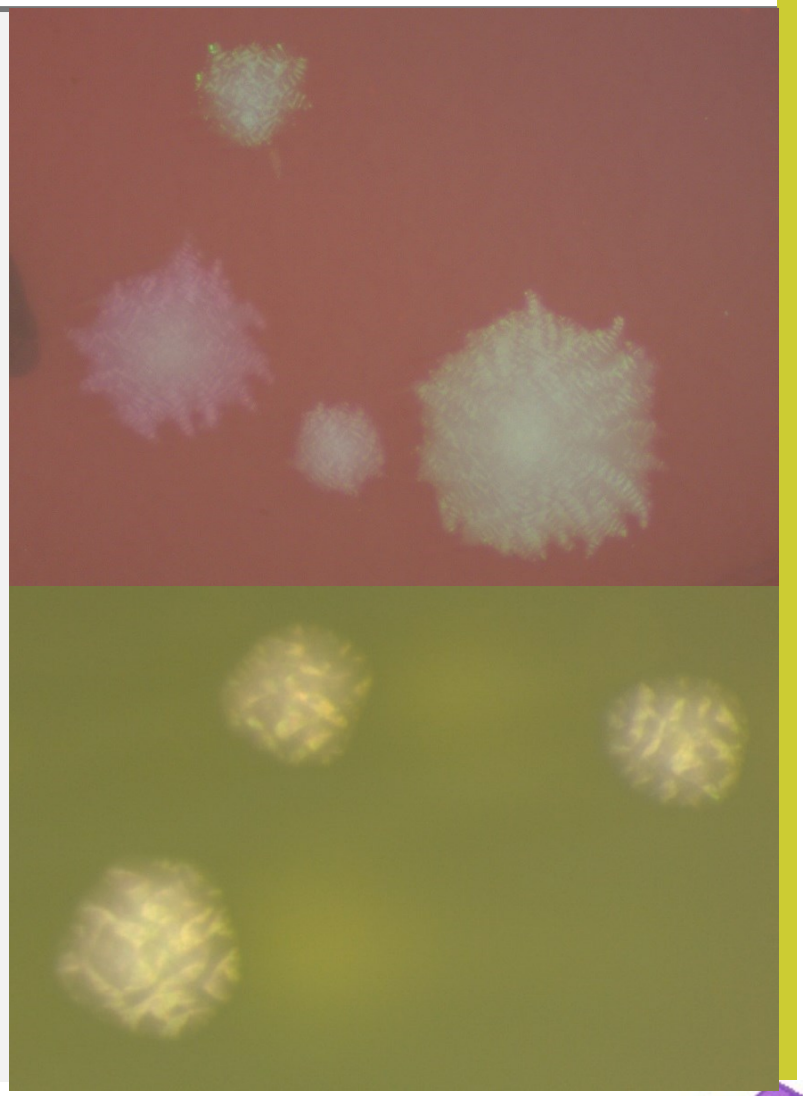


Image of *Fusobacterium nucleatum* courtesy of the CDC

Image of *Clostridium sporogenes* courtesy of Allison Faust

Environmental anaerobes

- Includes extremophiles
- Selective media are often specific to genus or even species
- May require a gas mixture free of CO_2
- Ex: *Thermoanaerobacterium thermosaccharolyticum* (ATCC® 7956™)
  - Requires a fermentable carbohydrate to grow
  - Type strain
  - Obligate anaerobe
  - Grows at 45°C - 60°C



Methanogens

- Oregon Collection of Methanogens (OCM)
- Very strictly anaerobic with methane always being the product of catabolic metabolism
- Ex: *Methanobrevibacter wolinii* (ATCC® BAA-1170™)
  - Isolated from sheep feces
  - Atmospheric requirements:
    - Media has a redox potential below -110 mV
    - Large amount of headspace and pressure
  - Nutritional requirements:
    - Nitrogen source (ammonia or N_2)
    - One or more B vitamins
    - Requires acetate and/or trypticase or yeast extract
    - Growth inhibited by bile salts

Media

- PRAS commercial media is superior
  - Boiled free of molecular oxygen
  - Autoclaved, dispensed, and packaged anaerobically
  - Light-proof packaging
- Indicator - Rezazurin
  - Monitors redox potential of media
  - Non-toxic to bacteria and effective at low concentrations
  - Becomes colorless at a redox potential below -110 mV, remains pink above -51 mV

Common agar media

- Bases for blood agar media:
  - Brucella
  - Columbia-based blood agar
  - Tryptic Soy-based blood agar
  - Brain heart infusion w/ 0.5% yeast extract
- Supplements to enhance growth:
  - 5% sheep, horse, or rabbit blood
  - Vitamin K1 (1 µg/mL)
  - Hemin (5 µg/mL)
- Selective agars are commercially available
  - Bacteroides Bile Esculin agar (BBE)

Common broth media

- Chopped meat
- Reinforced clostridial
- Peptone yeast extract broth with glucose (PYG)
- Supplemented tryptic soy
  - ATCC Medium 2722
    - Additions of Yeast extract, hemin, and vitamin K1
- Broth media vessels
  - Hungate tubes
  - Balch tubes

Inhibitors

- Inhibitors that affect the quality of media
  - Oxygen
  - Light
  - Moisture/dehydration
- Inhibitors that affect cell growth
  - Bile
  - Reducing agents
  - Oxygen/incorrect gas mixture
  - Failure to add supplements

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Reducing agents

- Reduces the redox potential of growth media
  - The reducing agent is oxidized by the oxygen in the media and therefore the media is reduced of the oxygen
- Ex: Coenzyme, cysteine, sodium sulfide
  - Select the reducing agent that is already used in the media formulation
  - Typically use 2 mL reducing agent per 100 mL media
  - Incubation at 37°C may speed up the process
  - Allow reducing agent to react for at least 1 hour, preferably overnight

Oxygen toxicity

Defenses

- Hemoglobin
- Superoxide dismutase
- Catalase
- Peroxidase
- Vitamin C
- Vitamin E
- Uric Acid

Damage

- Oxidation of membrane lipids
- Inactivation of enzymes
- Genetic damage

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Why is oxygen toxic?

O_2 + *Energy* → O_2

Ground State → Singlet State

O_2 + e^- → O_2^-

Singlet State → Superoxide Anion

Why is oxygen toxic?

$O_{2}^{-} + e^{-} + 2H^{+} \rightarrow H_2O_2$

Superoxide Anion → Hydrogen peroxide

$H_2O_2 + e^{-} + H^{+} \rightarrow H_2O + OH^{-}$

Hydrogen peroxide → Hydroperoxy radical

$OH^{-} + e^{-} + H^{+} \rightarrow H_2O$

Hydroperoxy radical → Water

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Gas mixtures

- 100% Nitrogen
- 97% N_2 - 3% H_2
- 80% N_2 - 10% H_2- 10% CO_2
- 80% N_2 - 20% CO_2
- 80% H_2 - 20% CO_2
- 100% Methane

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Propagation methods: The roll tube

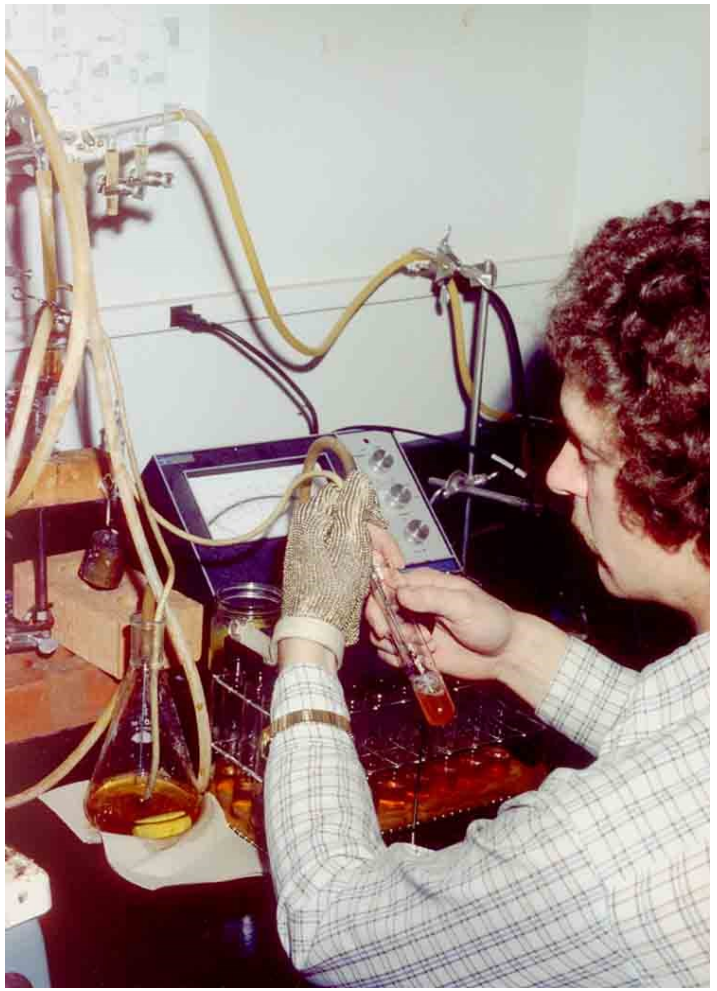
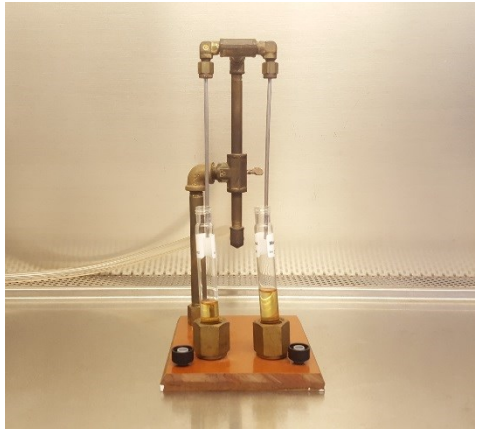


Image of anaerobic roll tubes courtesy of CEBTech Services. Please note that proper personal protective equipment is recommended.

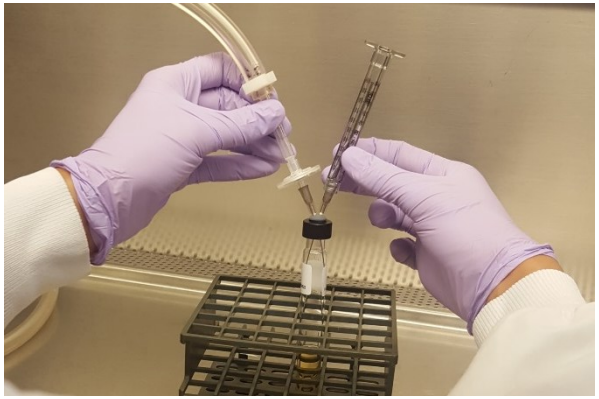
Propagation methods: The roll tube

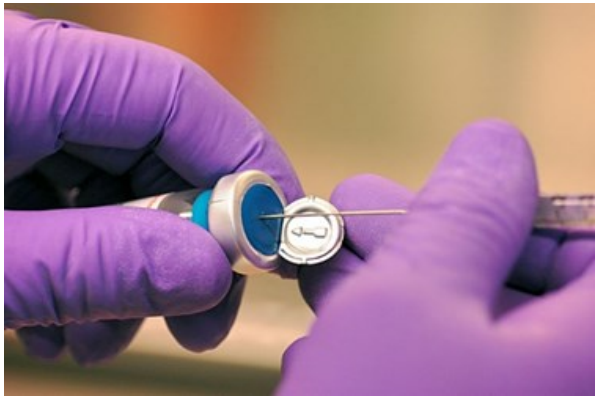
Image of *Clostridium perfringens* n a roll tube courtesy of LeBeau, MicrobeWorld

Propagation methods: The cannula system



Propagation methods: Syringe, needle, and oxygen-free gas





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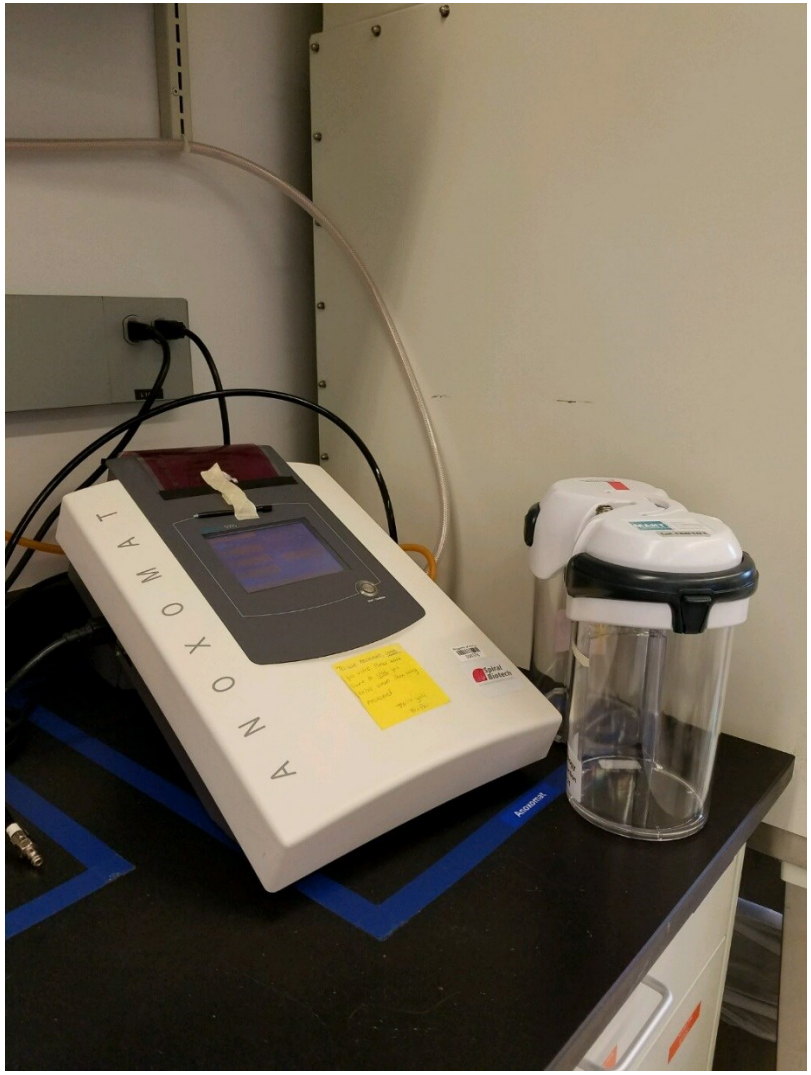
Propagation methods: Jars and catalysts

Propagation methods: Jars and catalysts

Propagation methods: Anoxomat™



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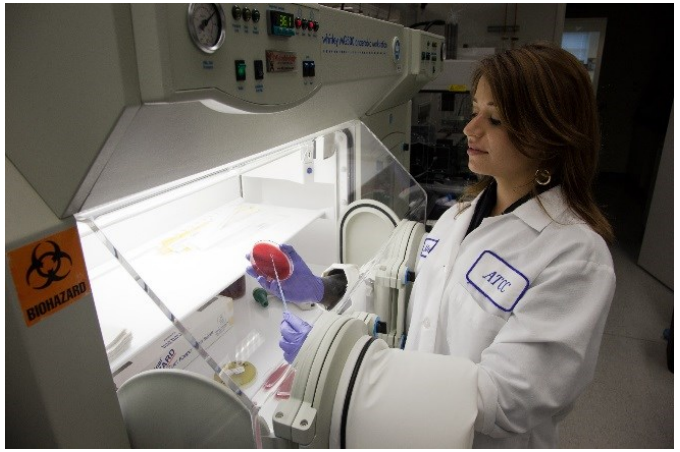
Propagation methods: Anaerobe chambers



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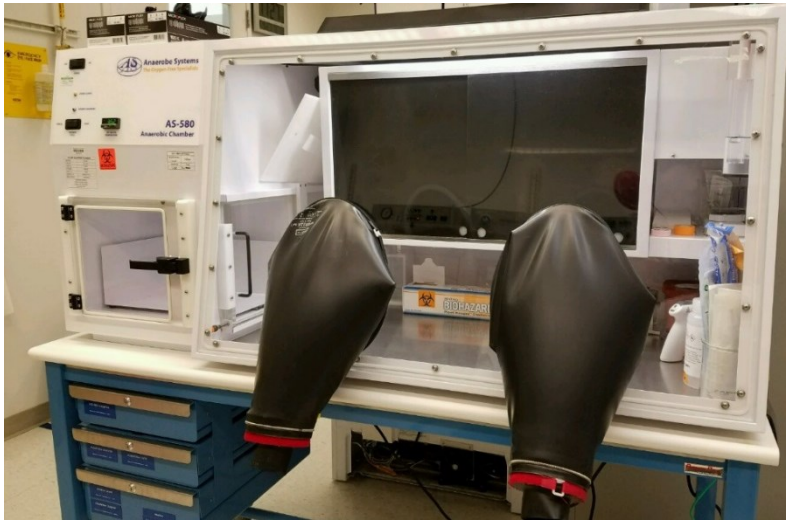

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