Anaerobes

Clues Suggestive of Anaerobic Infections

- There are many clues that can be suggestive of an anaerobic infection such as:
  - Foul odors
  - Black discoloration of specimens or colonies
  - Inability to grow organisms seen in Gram stain
  - Unique morphologies seen on direct smear Gram stain
    - Fusiform GNB
    - Large spore-forming GPB
    - Branching GPB
  - Additional characteristics are listed in **Table 24-2**

Specimen Types

- Anaerobes can be isolated from a number of different specimen types that include:
  - Aspirates
  - Body fluids
  - Lower respiratory
    - BAL and BW only
  - Abscesses
  - Tissues
  - Deep wounds
  - Urine
    - Suprapubic and cystos only
  - Genital sources
    - Intrauterine and vaginal abscesses only

Specimen Processing

- Primary Media includes:
  - CDC-Anaerobe Sheep Blood Agar
  - Laked KV
    - Kanamycin to inhibit growth of Gram-negative facultative anaerobic bacilli
    - Vancomycin to inhibit growth of Gram-positive organisms
    - Contains Vitamin K and Hemin to aid in the growth of *Bacteroides* spp. and *Prevotella sp*.
  - CDC-CNA
    - Colistin and Nalidixic acid: inhibits facultative GNBs

Anaerobic Conditions

- Anaerobic environments can be generated through various systems that include:
  - Gas-impermeable bags, containers or jars
    - Anaerobic environment is achieved through chemical reactions within a gas pack
  - Anaerobic chamber
    - Environment is maintained by gas-tank delivery system and the use of a palladium catalyst
    - Hydrogen from the gas mixture combines with oxygen to form water in the presence of the catalyst

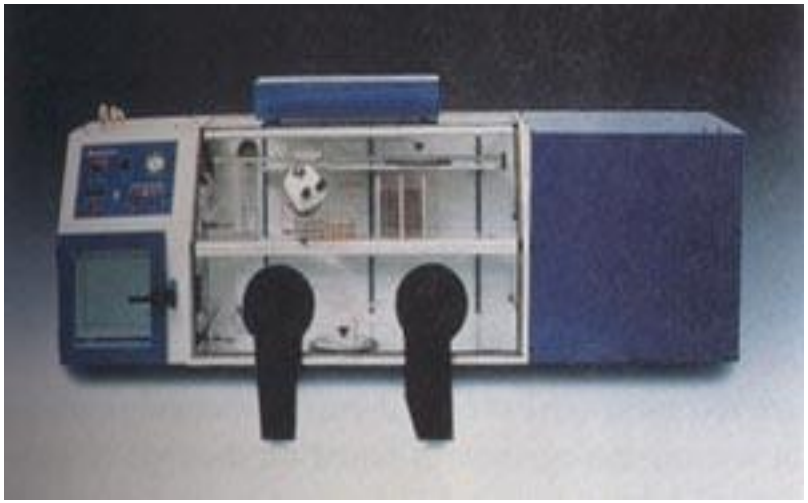
    —Oxygen indicator is required

Anaerobic Culture Environment

- Atmosphere:
  - Nitrogen: 80-90%
  - Hydrogen: 5-10%
  - CO_2: 5-10%

H2 + O2----→H20

palladium catalyst



Anaerobic Systems

- GasPak Jar
- Anaerobic Chamber

Anaerobic Environment Indicator System

- Indicator systems include:
  - Resazurin
    - **PINK** when O2 is present
    - **WHITE/COLORLESS** when O2 is not present
  - Other indicator systems
    - Methylene Blue
      - **BLUE** when O2 is present
      - **WHITE/COLORLESS** when O2 is not present
      - —Up to 2 hours may be required to establish appropriate anaerobic conditions

Important tests used on the ANA bench

- Aero-tolerant test (radials)
- Gram Stain
- Location of spores if present (terminal, sub-terminal, central)
- Potency antimicrobial disks (vankomycin,kanamycin,colistin)
- Egg yolk agar (lecithinase and/or lipase)
- Bacteroides Bile Esculin agar (BBE)
- Double zone hemolysis
- Beta lactamase
- Rapid ANA system (4 hrs ID system)

Gram Stain of Aspirate Material

Table 28-1. Characteristics of anaerobes based on Gram stain morphology

Organism	Gram stain reaction and morphology
Actinomyces spp.	Branching gram-positive bacilli
Clostridium perfringens	Large gram-positive bacilli with blunt ends ("boxcar-shaped"), no spores
Clostridium tetani	Gram-positive bacilli with round or oval terminal spore ("drumstick" or "tennis racket" shaped)
Propionibacterium spp.	Small, thin pleomorphic gram-positive bacilli
Bacteroides, Porphyromonas, or *Prevotella* spp.	Faintly staining gram-negative coccobacilli
Fusobacterium nucleatum	Thin, gram-negative bacilli with tapered ends
Fusobacterium necrophorum or *F. mortiferium*	Extremely pleomorphic, thin gram-negative bacilli with bizarre shapes
Veillonella spp.	Tiny gram-negative cocci with a tendency to stain gram variable

Color Atlas of Medical Microbiology, ASM Press, p203.

Gram Stain Pitfall

- Over decolorization can lead to the false impression of a Gram-Negative organism
- Susceptibility to special potency antimicrobial disks can help preliminarily categorize of the anaerobic pathogen

Potency antimicrobial Disks

- Vancomycin (5 µg)
- Kanamycin (1 mg)
- Colistin (10 µg)

- Gram Positive: Vancomycin S, Colistin R
- Gram Negative: Vancomycin R
  - Exception: *Porphyromonas spp.* – Vancomycin S

Potency Antimicrobial Disks: Presumptive ID

Table 28-3. Presumptive identification of anaerobes based on special-potency antimicrobial disk results

Organism	Result[a] with disk containing:		
	Kanamycin (1,000 μg)	Vancomycin (5 μg)	Colistin (10 μg)
Bacteroides fragilis group	R	R	R
Bacteroides ureolyticus group	S	R	S
Fusobacterium spp.	S	R	S
Porphyromonas spp.	R	S	R
Veillonella spp.	S	R	S
Peptostreptococcus anaerobius	R^S	S	R
Other anaerobic gram-positive cocci	S	S	R
Anaerobic gram-positive bacilli	V	S^b	R

[a] R, resistant; S, susceptible; R^S, resistant, rarely susceptible; V, variable reaction.
[b] Rare *Lactobacillus* spp. and *Clostridium* spp. may be vancomycin resistant.

Color Atlas of Medical Microbiology, ASM Press, p204.

Lecithinase (Naegler RX)

- Performed on an egg-yolk agar
- Tests the ability to break down lecithine into diglycerides
- Positive for lecithinase: Produces opaque white clearings
- Performed on suspected Clostridium spp.

Lecithinase (Naegler RX)

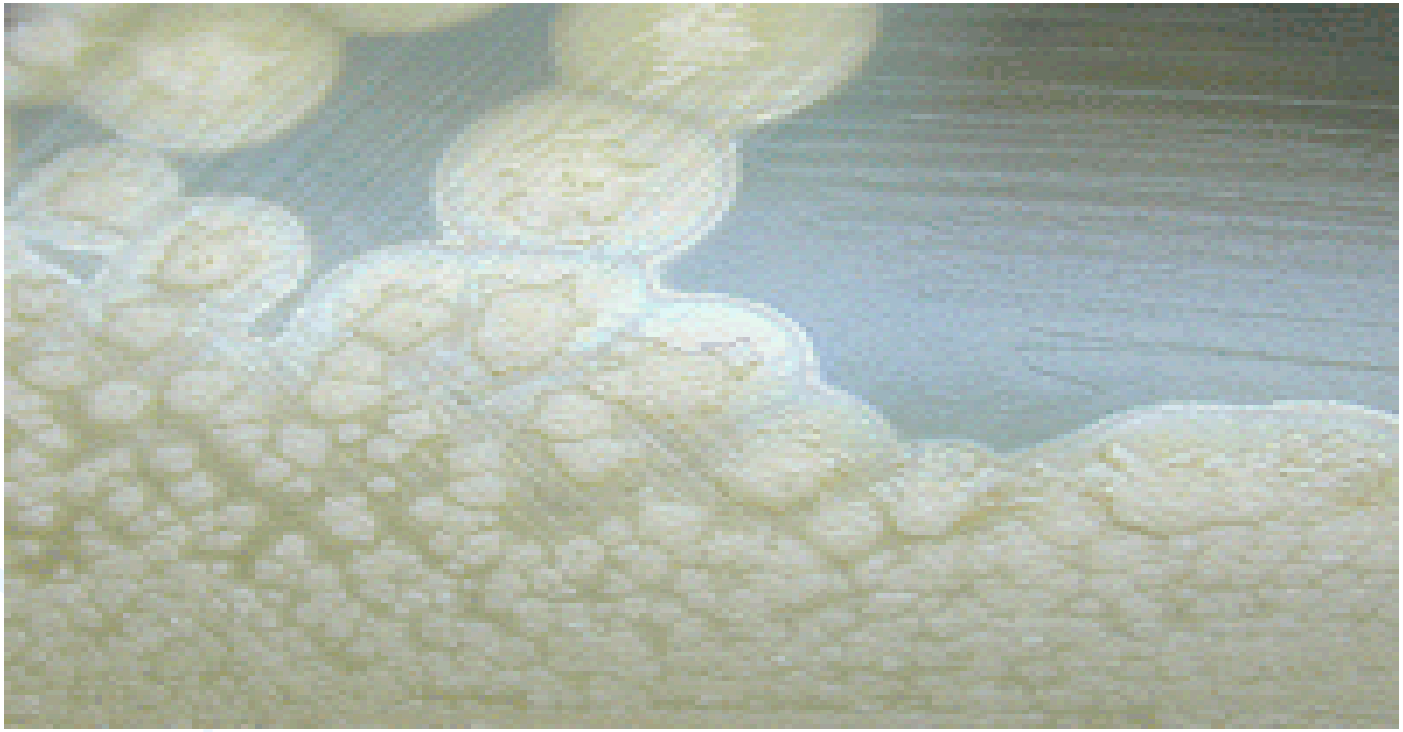
Negative

Positive

Lipase

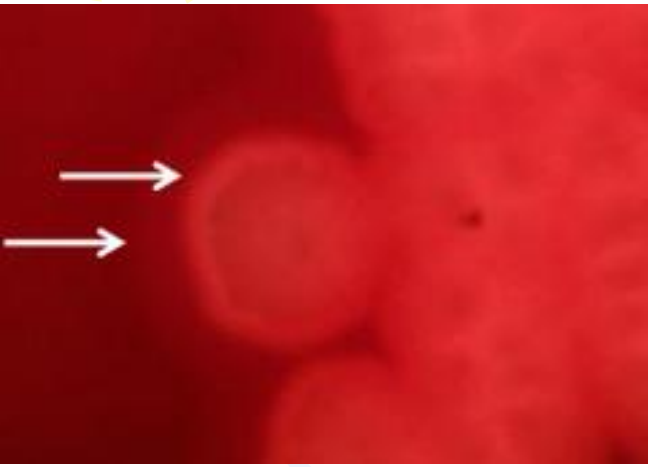
- Performed on an egg-yolk agar
- Tests the ability to break down fat with the enzyme lipase
- Positive: Production of lipase causes an iridescent oil-on-water effect (oil slick)
- Differentiates Fusobacterium spp.

Lipase (positive)



BBE,Double zone hemolysis, Rapid ANA

BBE (Bacteroides spp.)



Double Zone Hemolysis

(Clostridium perfringens)

Rapid ANA

(4 hour ID)

Anaerobic, Spore-Forming Gram Positive Bacilli

Clostridium spp.

- Obligate anaerobic GPB, form spores
- Ubiquitous in nature (soil)
- Frequent inhabitants of the GI tract
- Frequently isolated species in humans:
  - **C. perfringens*
  - **C. septicum*
  - *C. innocuum*
  - *C. ramosum*
  - **C. difficile*

Clostridia spp.

- Wound infections
  - Surgical site
  - Trauma
- Gas Gangrene (*C. perfringens*)
- Abscess (abdominal, lung, organ, brain, oral)
- Septicemia
- Toxin mediated diseases:
  - *C. difficile*: antibiotic associated diarrhea
  - *C. botulinum*: botulism; flaccid paralysis
  - *C. tetani*: tetanus; muscle spasm; no relaxation
  - *C. perfringens*: food poisoning

Figure 28-16 Disk pattern of *Clostridium*. Although some of the clostridia stain gram negative, the disk pattern is consistent with a gram-positive organism and confirms the correct Gram reaction of the isolate. As shown here, clostridia are vancomycin and kanamycin susceptible and colistin resistant.

Vancomycin – S

Kanamycin – S

Colistin - R

Color Atlas of Medical Microbiology, ASM Press, p210.

Spore Location

- Spore location is one of the identifying characteristics used for *Clostridium sp.*
- Location of spores can include:
  - Central
  - Sub-terminal
  - Terminal

C. botulinum

- **Botulinum toxin - most powerful EXOTOXIN known**
  - Neurotoxin prevents acetylcholine release from the nerve endings producing acute descending, flaccid paralysis
    - Bilateral involvement of Cranial Nerves (face, head, throat), followed by thorax, diaphragm, then extremities
  - Death by respiratory failure
  - Seven toxin types

- **Food disease= Ingestion botulism**
  - Improperly preserved canned foods will not k
  - Spores germinate and secrete botulinum toxin
  - Ingestion of the preformed toxin

C. botulinum

- Neonates ingest bacterial spores
- Typical food source is raw honey
- Germinates in neonatal gut
- Same syndrome as intoxication botulism

- Adults are not susceptible to this type of infection
  - Spores will not germinate in adult gut

Clostridium botulinum

- Key characteristics include:
  - Lipase: positive
  - Lecithinase: negative
  - Sub-terminal spore location

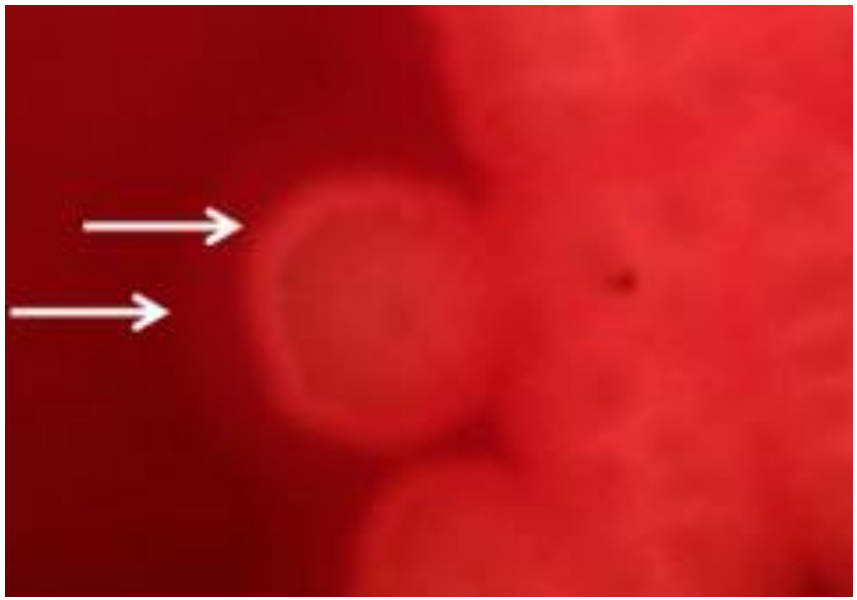
- *Diagnosed primarily by:
  - Serological testing
  - Clinical symptoms

Clostridium perfringens

- Associated with gas-gangrene/food poisoning
- Gram positive “boxcar” shaped bacilli
- Key characteristics include:
  - Lecithinase: Positive
  - Lipase: Negative
  - Central or sub-terminal spore location(rarely seen)
  - Double zone of Beta-hemolysis
  - Reverse CAMP test positive

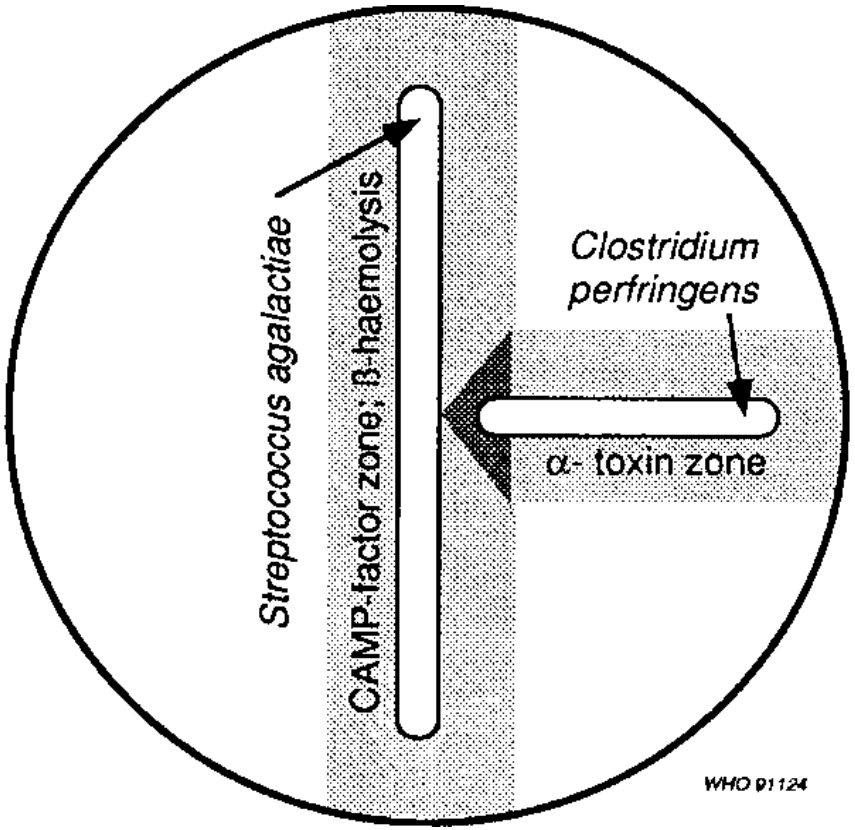
Double Beta-Hemolysis

- *C. perfringens* produces a characteristic double zone of Beta-hemolysis



Reverse CAMP

- *C. perfringens* produces a positive reverse CAMP test.
  - Group B is streaked as a line down the center on the plate
  - C. perfringens is streaked in a line perpendicular
  - Arrow head clearing is positive



Clostridium difficile

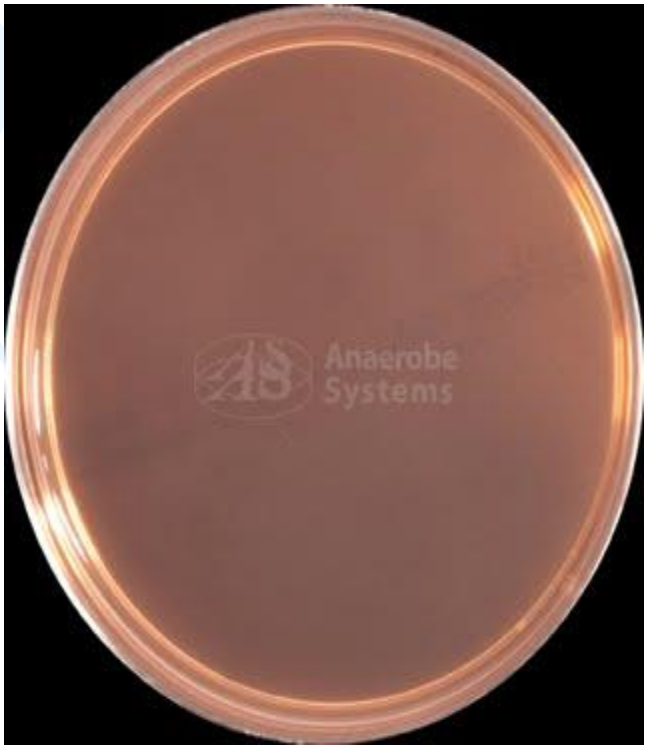
- Implicated in:
  - Antibiotic-associated diarrhea
  - Pseudomembranous colitis
- Key characteristics include:
  - Lecithinase negative
  - Sub-terminal spore location

- **Must be a toxin producer to cause disease**

C. difficile on CCFA

- Cycloserine-cefoxitin-fructose agar (CCFA) is be used to isolate C.difficile

Uninoculated

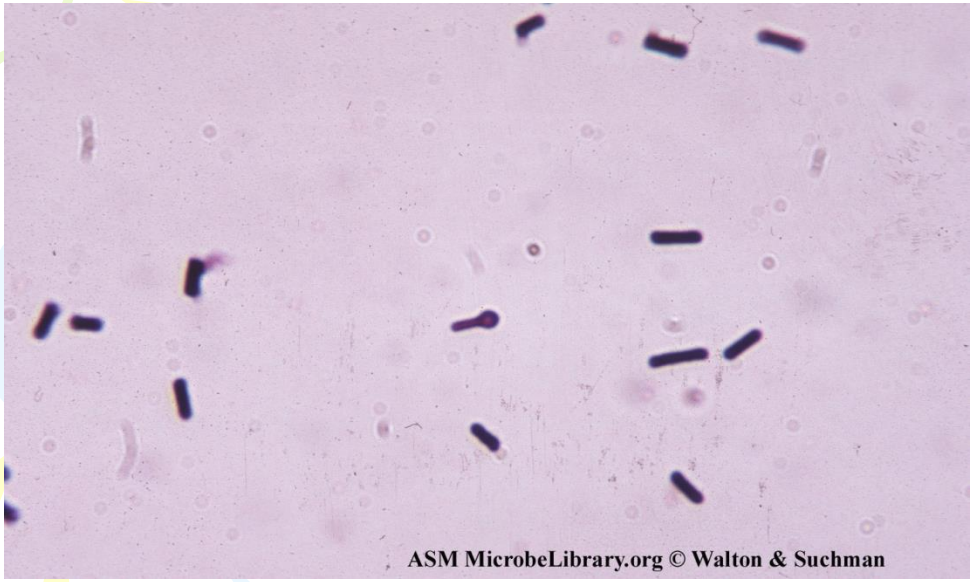


Growth

Clostridium tetani

- Associated with lockjaw and tetanospasims
- Requires a neurological identification
- Key characteristics include:
  - Lecithinase: Negative
  - Lipase: Negative
  - Terminal spore location
    - **Tennis racket shaped cells**
  - Swarming colonies
  - Indole positive(other Clostridia are neg)
  - *Diagnosed primarily by:
  - —Serological testing
  - —Clinical symptoms

C. tetani



www.textbookofmicrobiology.net

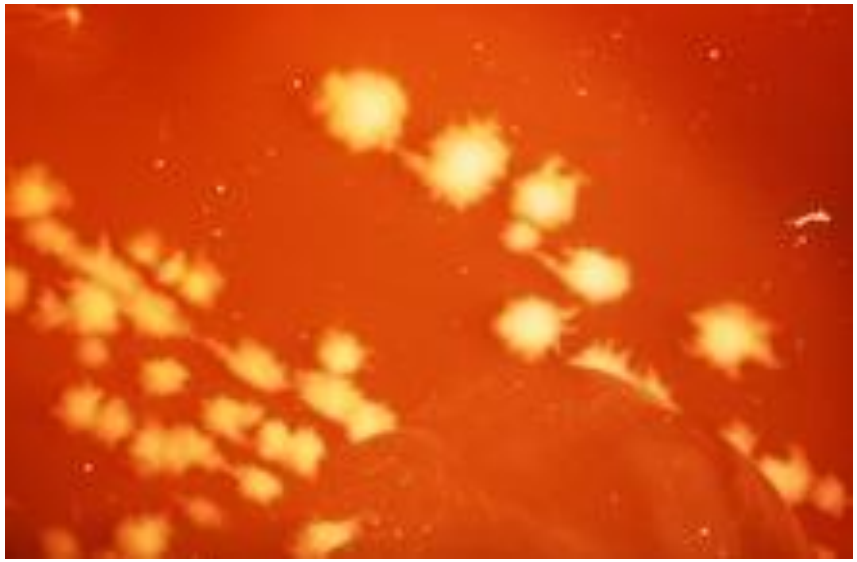
GPB with round/oval terminal spore “tennis racket, drumstick”

Clostridium septicum

- Associated with malignancy when isolated outside the GI tract
- Key characteristics include:
  - Lecithinase: Negative
  - Lipase: Negative
  - Sub-terminal spore location
  - Swarming colonies
    - **Produces "Medusa head" colonies**
  - Indole negative

"Medusa Head" colonies

- *C. septicum* produces finger-like projections from the edge of colonies
- Identification on Rapid ANA system



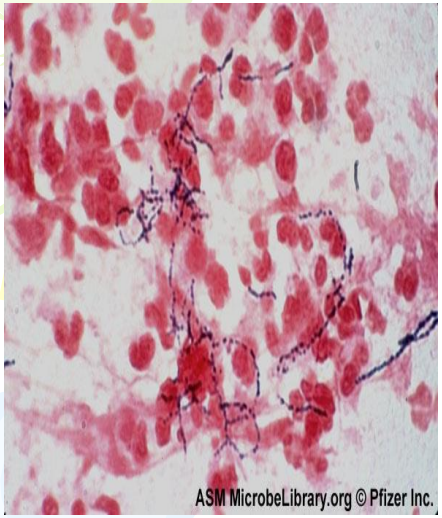
Anaerobic, Non-spore Forming Gram Positive Bacilli

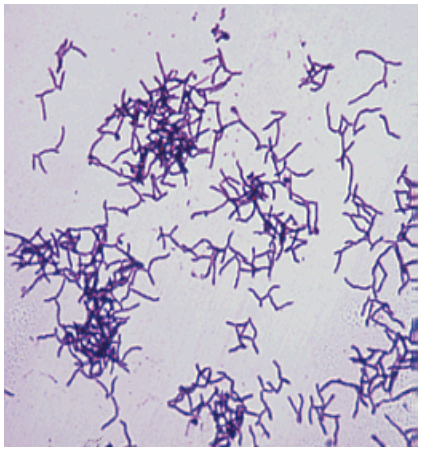
Sensitive to:Vancomycin,Kanamycin
Resistant to: Colistin

Actinomyces sp.

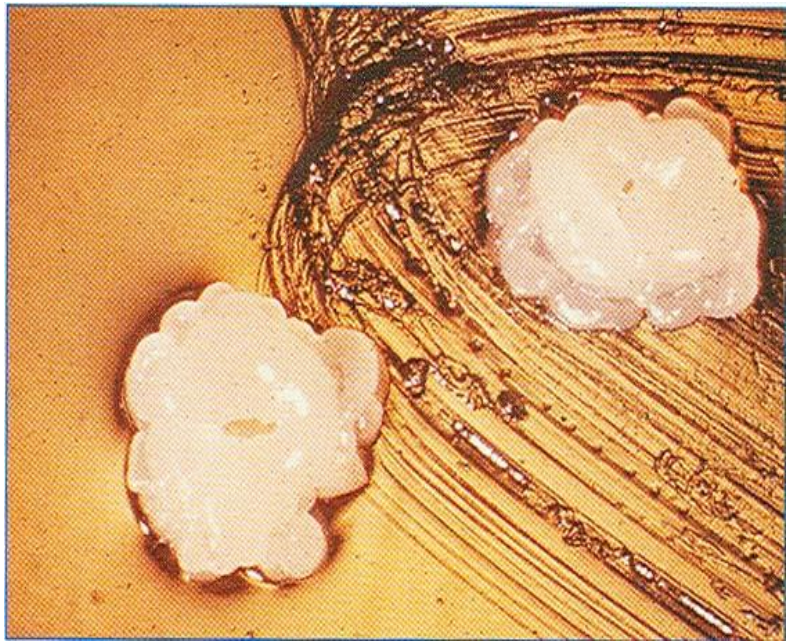
- Branching Gram positive bacilli
- Characteristic white and raised "Molar Tooth" colonies
- Catalase: Negative
- Indole: Negative
- Associated with lumpy jaw

Actinomyces spp.





Branching, GPB, may have beaded appearance, partial acid-fast negative, whereas *Nocardia spp.* are partial acid-fast positive



A. israelli. "molar tooth" colonies produced on BHI agar.

Koneman's Color Atlas and Textbook of Diagnostic Microbiology, 6th Ed. Color plate 16-2, F

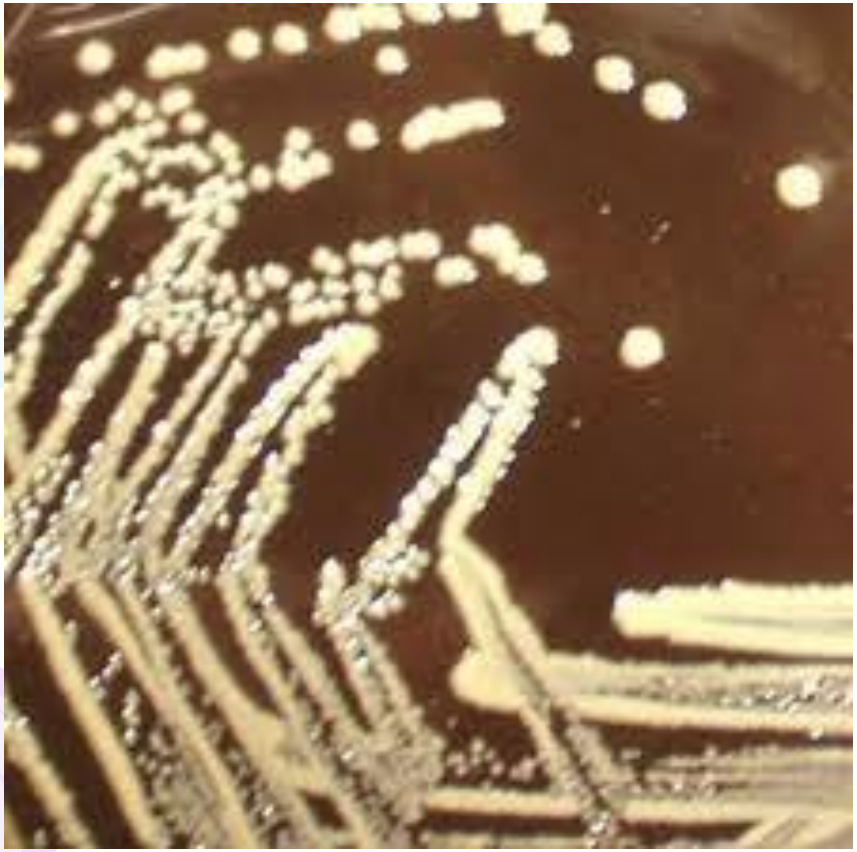
Propionibacterium sp.

- Pleomorphic Gram positive bacilli
- Catalase positive
- Normal flora in oral cavity, gastrointestinal and urogenital tract and on skin
- Associated with acne, endocarditis, bacteremia, meningitis
- P. acnes: Catalase: Positive

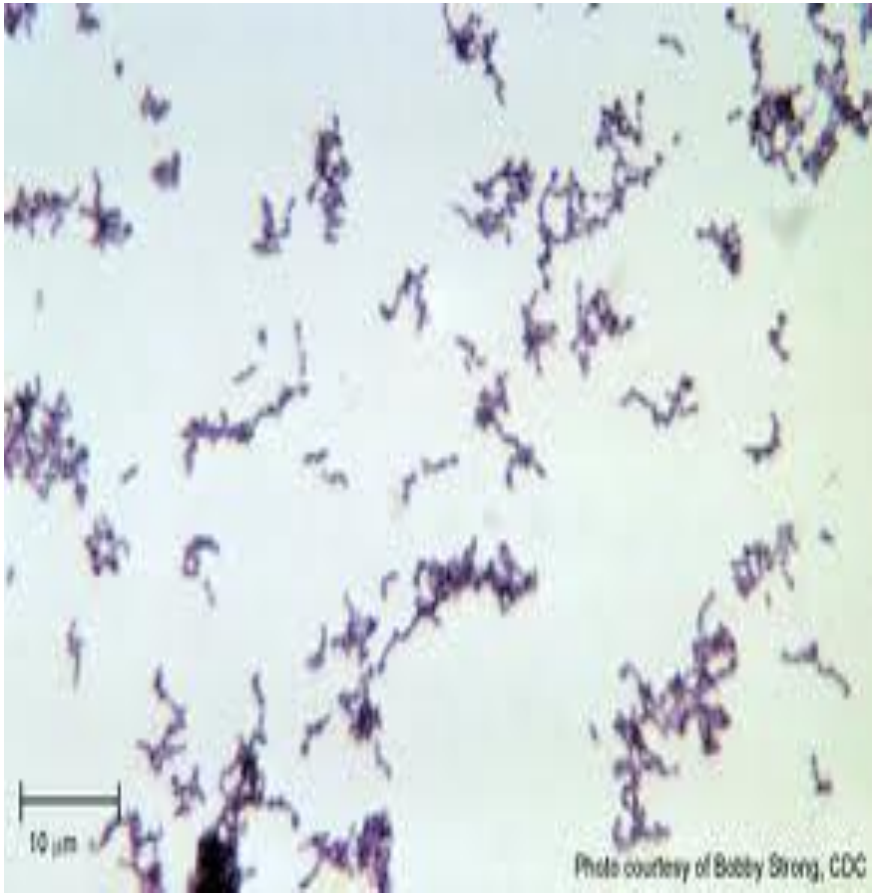
  Indole: Positive

Propionibacterium sp.

CDC Blood agar

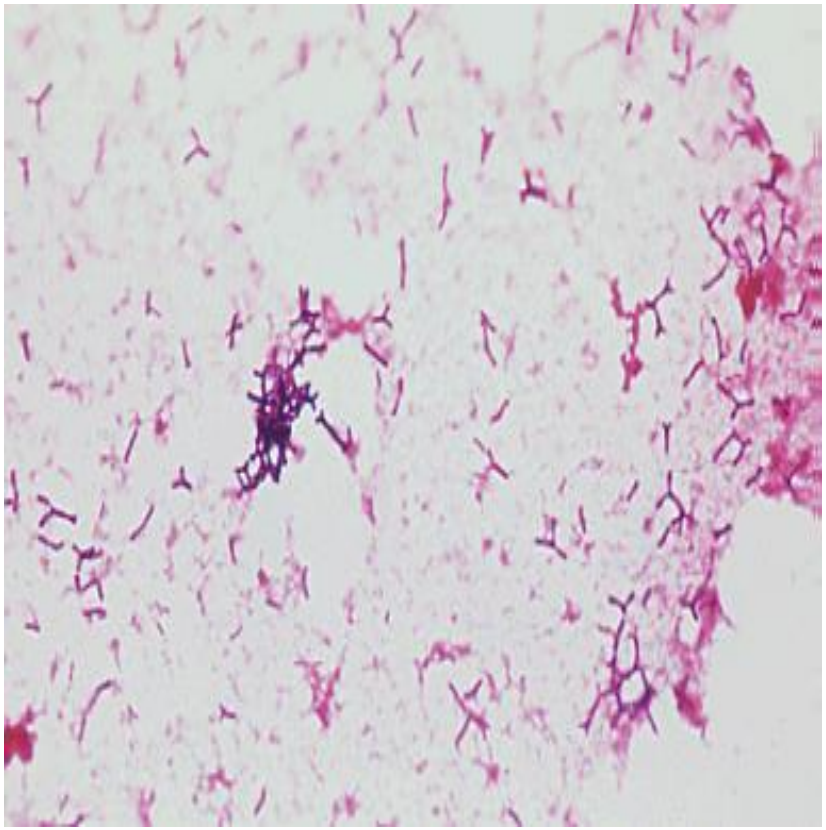


Gram stain



Bifidobacterium sp.

- Dog bone or fork shaped gram positive bacilli
- Catalase negative
- Indole negative
- Normal intestinal and oral flora
- Rarely causes disease

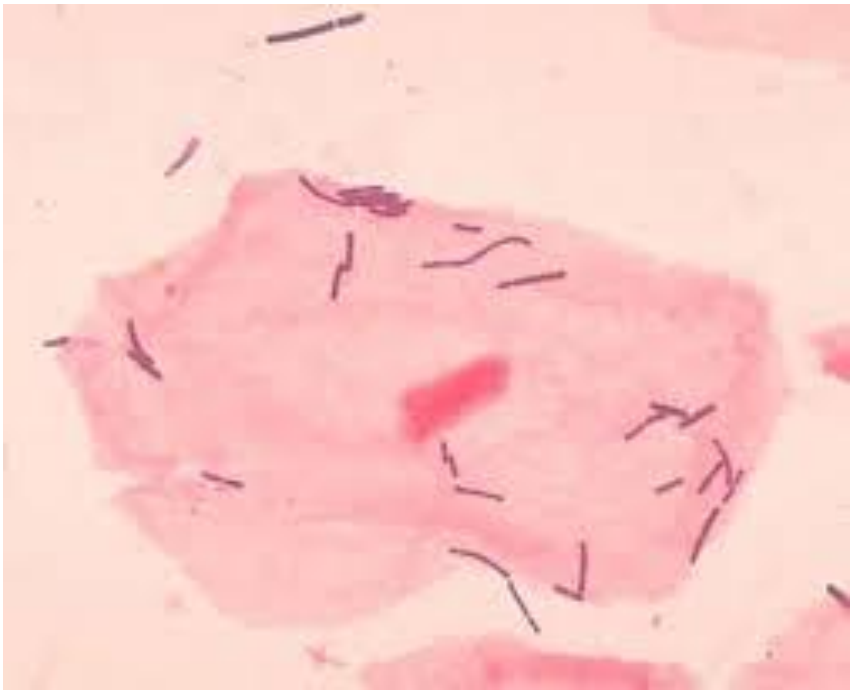


Eubacterium species

- Pleomorphic GPB, may be branching
- Normal intestinal and oral flora
- Catalase : Negative
- Nitrate: Positive
- Not considered a pathogen
- E. lentum is a rare human pathogen

Lactobacillus sp.

- Long, thin gram positive bacilli
- Catalase negative
- Indole negative
- Normal flora in mouth, intestines and vagina



Anaerobic, Gram Negative Bacilli

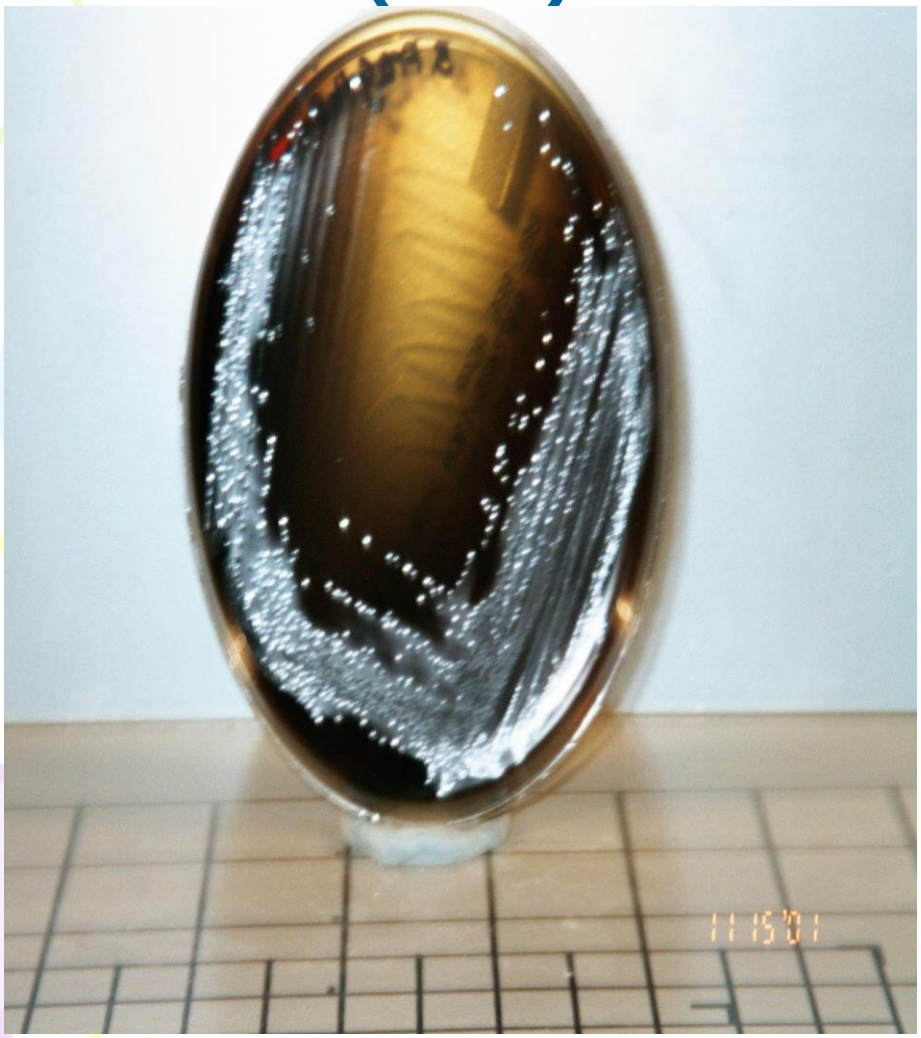
all are normal flora of human mucous membranes

Bacteroides fragilis Group

- Gram negative bacilli
- Can be found in wounds, tissues and blood cultures
- All species:
  - Resistant to Vancomycin, Kanamycin and Colistin
    - Grows on Laked KV plate
  - Catalase positive
  - Stimulated to grow in 20% bile
    - Bacteroides Bile Esculin (BBE) plate
      - Up to 20% bile concentration
      - Hydrolysis of esculin turns plate black
  - CDC-ANA BAP
    - Grey, speckled colonies

Bacteroides Group

Bacteroides Bile esculin (BBE)



Gram stain

Bacteroides fragilis Group

	Esculin	Salicin	Trehalose	Indole
B. fragilis	+	-	-	-
B. distanosis	+	+	+	-
B. thetaiotamicron	+	-	+	+
B. vulgatus	-	-	-	-

Pigmented Gram Negative Bacilli (dark brown to black colonies)

- Prevotella sp.
  - No growth on BBE plate
  - Growth on KV plate
    - Resistant to Vancomycin and Kanamycin
    - Variable to Colistin
    - Requires vitamin K and hemim
  - ***Indole negative**
  - Red fluorescence under UV light
- Porphyromonas sp.
  - No growth on BBE plate
  - No Growth on KV plate
    - Susceptible to Vancomycin
    - Resistant to Kanamycin and Colistin
  - ***Indole positive**
  - Red fluorescence under UV light

Fusobacterium sp.

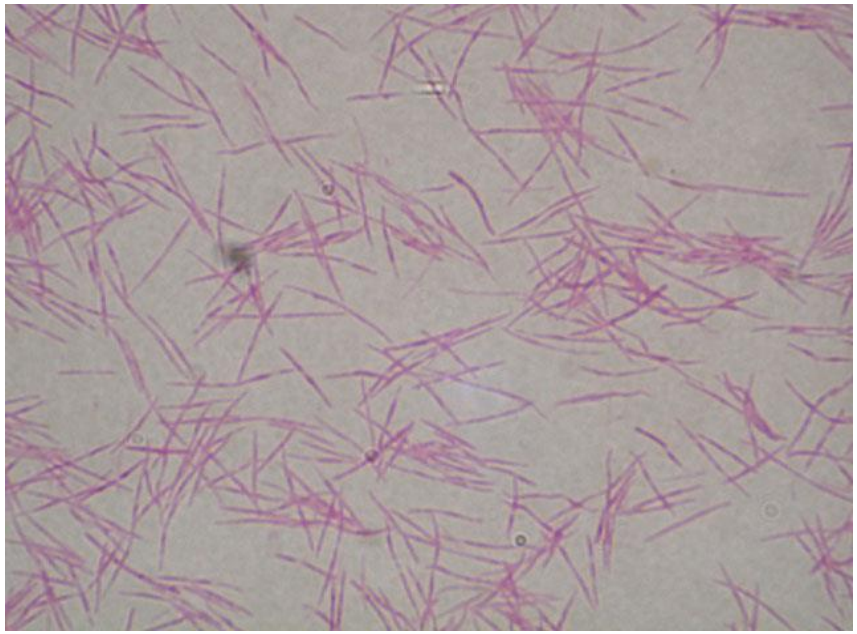
- Key features include:
  - Resistance to Vancomycin
  - Susceptible to Kanamycin and Colistin
  - Catalase negative
  - Indole positive
  - Not stimulated to grow in 20% bile (no growth on BBE)
  - Colonies produce a chartreuse fluorescence under long-wave UV light

Fusobacterium sp.

- *F. nucleatum*
  - Long, slender, fusiform gram negative bacilli
  - ***Lipase negative**
- *F. necrophorum*
  - Pleomorphic gram negative bacilli or coccobacilli
  - ***Lipase positive**
- Key features can be found in **Table 24-8**

Fusobacterium sp.

- *F. nucleatum* produces a characteristic fusiform shape on gram stain



Anaerobic, Gram Positive Cocci

Peptostreptococcus sp.

- Susceptible to Vancomycin
- Resistant to Kanamycin and Colistin
- Catalase negative
- Most common:

➢ P. anaerobius: Indole pos,
SPS sensitive

➢ P. asaccharolyticus: Indole neg,
SPS resistant

Anaerobic, Gram Negative Cocci

Veillonella sp.

- Non-pathogenic normal flora
- Resistant to Vancomycin and susceptible to Kanamycin and Colistin
- Red fluorescence under UV light

Question 1

All of the following specimen types are acceptable specimens for processing anaerobic organisms except:

A. Aspirates
B. Clean catch urine
C. Body fluids
D. Lower respiratory

Question 2

Describe some of the clues that would indicate the possibility of an anaerobic infection?

Question 3

A 36 year old man arrived at the emergency room after noticing a black discoloration in his toe 5 days prior to his visit. Since that time, the discoloration has spread to his lower limb. The attending physician noted severe tissue necrosis and a build-up of gas under the skin and sent a specimen to the lab for examination.

1. Do you think this patient possibly has an anaerobic infection? Why?
2. List some of the laboratory tests that might be ordered for this patient?
3. Describe how this organism might appear on culture media and on a Gram stain?
4. What organism do you think is causing this patients symptoms?