Anaerobic bacteria

Anaerobic bacteria

can be divided into:

- strict anaerobes that can not grow in the presence of more than 0.5% oxygen
- moderate anaerobic bacteria that are able of growing between 2 to 8% oxygen.
- Anaerobic bacteria usually do not possess catalase
- some can generate superoxide dismutase which protects them from oxygen.

The clinically important anaerobes

- 1. Gram-negative rods

Bacteroides, Prevotella, Porphyromonas, Fusobacterium,

- 2. Gram-positive cocci

primarily *Peptostreptococcus* spp.);

- 3. Gram-positive spore-forming (*Clostridium* spp.)
- and nonspore-forming bacilli

Actinomyces, Propionibacterium, Eubacterium, Lactobacillus

- 4. Gram-negative cocci (mainly *Veillonella*)

ANAEROBIC BACTERIA-GRAM STAIN

Gram-positive anaerobes

Gram-positive anaerobes include the following:

- *Actinomyces* (head, neck, pelvic infections; aspiration pneumonia)
- *Bifidobacterium* (ear infections, abdominal infections)
- *Clostridium* (gas, gangrene, food poisoning, tetanus, pseudomembranous colitis)
- *Peptostreptococcus* (oral, respiratory, and intra-abdominal infections)
- *Propionibacterium* (shunt infections)

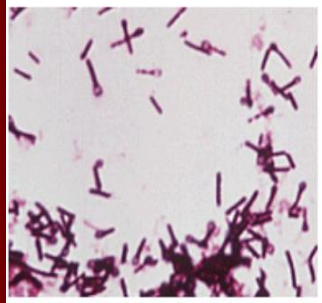


Fig. 56 *Clostridium tetani* - Gram stain.

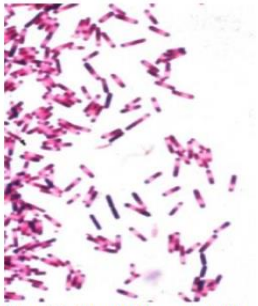


Fig. 67 *Clostridium botulinum* - Gram stain.

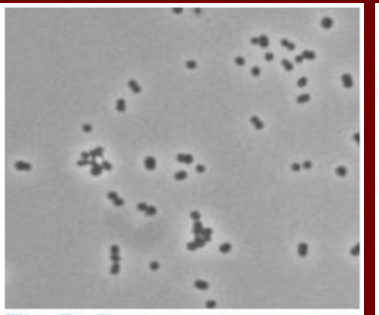


Fig. 78 *Peptostreptococcus* sp.

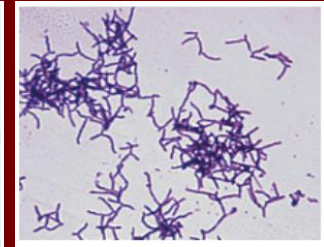


Fig. 90 Actinomycetes - Gram stain.

Gram-negative anaerobes

- *Bacteroides* (the most commonly found anaerobes in cultures; intra-abdominal infections, rectal abscesses, soft tissue infections, liver infection)
- *Fusobacterium* (abscesses, wound infections, pulmonary and intracranial infections)
- *Porphyromonas* (aspiration pneumonia, periodontitis)
- *Prevotella* (intra-abdominal infections, soft tissue infections)

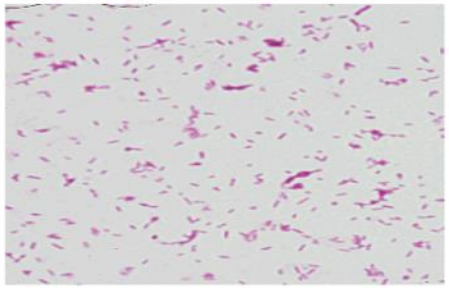


Fig. 91 Bacteroides - Gram stain.

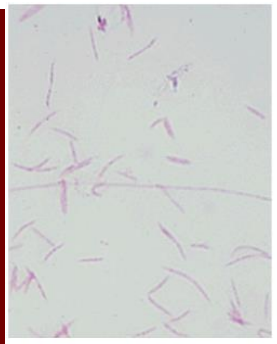


Fig. 102 Fusobacterium - Gram stain.

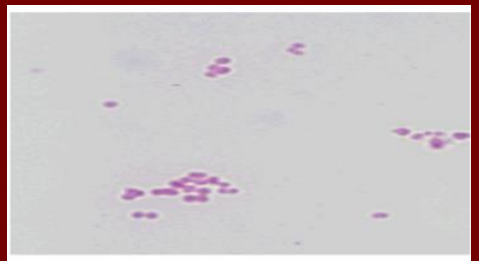


Fig. 113 Veillonella - Gram stain.

Anaerobic bacteria culture

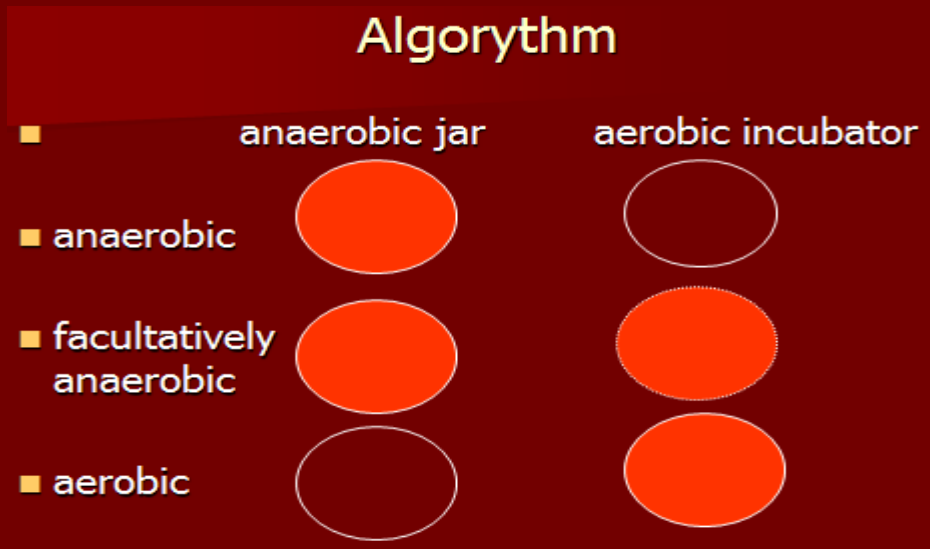
- **Non selective media used in anaerobic bacteriology:**
- 1. Anaerobic blood agar: It is a nonselective medium for isolation of anaerobes and facultative anaerobes.
- 2. Egg-yolk agar (EYA): Nonselective for determination of lecithinase and lipase production by clostridia and fusobacteria.
- 3. Cooked meat broth: Nonselective for cultivation of anaerobic organisms; with addition of glucose, can be used for gas-liquid chromatography.
- 4. Peptone-yeast extract glucose broth (PYG): Nonselective for cultivation of anaerobic bacteria for gas-liquid chromatography.

- **Selective and differential media used in anaerobic bacteriology:**

- 1. Bacterioides bile esculin agar (BBE): It is selective and differential for *Bacteriodes fragilis* group and good for presumptive identification.
- 2. Laked Kanamycin-vancomycin blood agar (LKV): It is selective for isolation of *Prevotella* and *Bacteriodes* spp.
- 3. Anaerobic phenylethyl alcohol agar (PEA): Selective for inhibition of gram negative rods and swarming by some clostridia.
- 4. Cycloserine cefoxitin fructose agar (CCFA): selective for clostridium difficile.
- 5. Thioglycollate broth: Non selective for cultivation of anaerobes; as well as facultative anaerobes and aerobes.

Anaerobic conditions

- Candle jar - microaerophilic, facultative anaerobic
- Anaerobic jar with anaerobic atmosphere generator (foil envelopes): release hydrogen and carbon dioxide -after addition of water.
- Biological method - cultivation with Serratia marcescens



CLOSTRIDIA

- *C. tetani* – tetanus
- *C. botulinum* – botulism

- C. ***difficile***
  - **PSEUDOMEMBRANOUS COLITIS**
  - antibiotic-associated diarrhea (AAD)
  - colitis
- overgrowth of *Clostridium difficile* in the colon
- antimicrobial chemotherapy, prolonged use of antibiotics, elderly , immunocompromised
- **Toxin A** - enterotoxin - fluid accumulation in the bowel
- **Toxin B** - extremely lethal - cytopathic toxin

- ***C. perfringens***
  - ***GAS GANGRENE***
  - tissue degrading enzymes - lecithinase [alpha toxin]
  - proteolytic and saccharolytic enzymes

Clostridium tetani

- Gram-positive, rod-shaped
- produces endospores (swollen sporangium giving it a distinctive drumstick appearance)
- Produces toxin- tetanospasmin
- strictly anaerobic

Tetanospasmin

- initially binds to peripheral nerve terminals
- It is transported within the axon and across synaptic junctions until it reaches the central nervous system
- rapidly fixed to gangliosides at the presynaptic inhibitory motor nerve endings
- The effect of the toxin - to block the release of inhibitory neurotransmitters (glycine and gamma-amino butyric acid) - it produces the generalized muscular spasms characteristic of tetanus.

Tetanus - treatment

- Hospitalization to manage complications of the infection
- Opening and cleaning of the wound, or sometimes surgical removal of the entire wounded area
- Antibiotics
- Tetanus immune globulin (antibodies against tetanus that help neutralize the tetanus toxin)

Clostridium botulinum

- Spores in the soil, fresh water sediment, animal feces
- serious paralytic illness

Botulism

- bacterial toxin- acting in the intestine and causing neuromuscular poisoning (resulting from Clostridium botulinum toxin).
- Food-borne botulism - eating foods that contain the botulism toxin, home-canned foods
- Wound botulism - toxin produced from a wound infected with *Clostridium botulinum*.
- Infant botulism -consuming the spores of the botulinum bacteria, which then grow in the intestines and release toxin

Botulism

- can be treated with an antitoxin that blocks the action of neurotoxin circulating in the blood.
- antibiotics

Spirochetes

Treponema pallidum

Spirochaetes

- double-membrane bacteria
- long, helically coiled (corkscrew-shaped)
- asexual transverse binary fission
- microaerophilic or anaerobic
- extremely sensitive to oxygen toxicity

Disease-causing members	Disease
Leptospira species	leptospirosis
Borrelia burgdorferi B. garinii B. afzelii	Lyme disease
Borrelia recurrentis *Treponema* species	relapsing fever treponematoses
Brachyspira pilosicoli *Brachyspira aalborgi*	intestinal spirochaetosis

TREPONEMA PALLIDUM

Subspecies	Disease
T. pallidum subsp *pallidum*	Venereal syphilis
T. pallidum subsp *pertenue*	Yaws
T. pallidum subsp *endemicum*	Endemic syphilis (Bejel)
T. carateum	Pinta

Syphilis

PRIMARY:

- Chancre -first cutaneous lesion , 18 to 21 days after infection, round indurated papule, eroded surface, serous fluid
- Usually painless, untreated, the chancre heals spontaneously in 1 to 4 months (heals without scarring)
- Inguinal adenopathy 1-2 weeks after chancre
- Extragenital chancre: may be larger, frequently on lips, rarely tongue, tonsil, breast, finger, and anus
- **infectious**

SECONDARY:

- occurs approximately four to ten weeks after the primary infection
- Skin manifestations in 80%
- Early on face, shoulders, flanks, palms and soles, anal or genital areas
- Symmetric, generalized, superficial, macular transient; later papular, pustular , reddish-pink, non-itchy rash on the trunk and extremities, including the palms and soles
- **infectious**

LATENT ⟶ TERTIARY:

- may occur approximately 3 to 15 years after the initial infection, and may be divided into three different forms:
  - gummatous syphilis
  - late neurosyphilis
  - cardiovascular syphilis
- **People with tertiary syphilis are not infectious**
- A gumma is a nonspecific lesion, similar to a granuloma, that occurs in late syphilis.
- most commonly found in the skin, skeletal system, and mucocutaneous tissues, but may occur anywhere.
- single or multiple

Laboratory Testing

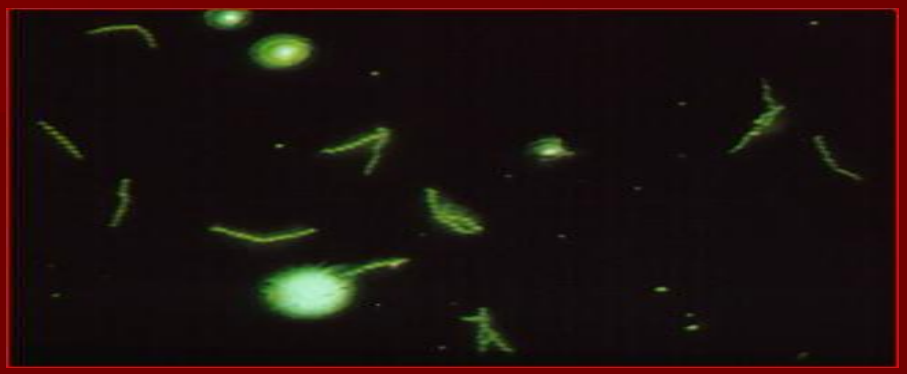
Direct identification of T.pallidum

- Direct microscopic identification of T.pallidum by dark field microscopy
- Direct antigen detection tests
- Nucleoside amplification techniques (PCR)

Serological tests to detect IgG/Igm antibodies

- Non Treponemal tests (for determining the disease activity)
- Treponemal tests (for disease confirmation)
- Detection of Treponemal IgM antibodies (to detect early infection)

T. PALLIDUM - DIRECT IDENTIFICATION



Results

- T.pallidum in dark field microscopy is identified by its typical morphology and characteristic movements
- T.pallidum is differentiated from the other treponemes by the tightness of spirals and characteristic cork screw movements

T pallidum	Other non pathogenic treponemes
Has 6-14 regularly wounded coils	Irregularly wounded coils And may be longer & thicker
Shows a slow, deliberate forward & backward movement, rotating on its long axis, soft bending and twisting from side to side	Lack a characteristic mobility

DIRECT SMEARS

Darkfield microscopy

Darkfield microscopy is used to demonstrate *Treponema pallidum* in material from lesions or lymph nodes. The presence of *T. pallidum* constitutes a definitive diagnosis of syphilis. Since *T. pallidum* is identified by characteristic spiral morphology and its motility, the preparation must be fresh and the organisms actively motile.

Direct fluorescent antibody (DFA-TP)

As an alternative to darkfield microscopy, fixed smears from lesions, serous fluid or lymph node aspirates may be sent to reference laboratories for staining with fluorescein-conjugated antibody to *T. pallidum*.

Laboratory Testing

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Nontreponemal Reagin Tests

- Non-specific or non-treponemal serological test to detect reagin, utilized as screening test only.
- Reagin is an antibody formed against cardiolipin.
- Found in sera of patients with syphilis as well as other diseases.
- Non treponemal tests become positive 1 to 4 weeks after appearance of primary chancre.
- in tertiary 25% are negative, after successful treatment will become nonreactive after 1 to 2 years

VDRL: Venereal Disease Research Laboratory

- the standard test against which other nontreponemal tests are compared

- Utilizes an antigen which consists of cardiolipin, cholesterol and lecithin.
- The antigen particles appear as short rod forms at magnification of about 100x.
- Aggregation of these particles into large or small clumps is interpreted as positivity

RPR cardiolipin test: Rapid Plasma Reagin (performed in STD clinics)

- the most commonly used non-treponemal test for the diagnosis of syphilis.
- flocculation assay - a cardiolipin antigen and the patient's anti-cardiolipin antibodies form an antigen–antibody lattice - visualised when carbon particles are trapped within it.
- Clumping of the carbon particles indicates the person's serum contains nonspecific antilipid (reagin) antibodies

TREPONEMAL TESTS

- Fluorescent Treponemal Antibody-Absorption - FTA-ABS

- *Treponema pallidum* Hemaglutination Assay - TPHA

- Microhemagglutination *Treponema pallidum* - MHA-TP

- *T. pallidum* particle agglutination - TP-PA

FTA-ABS

- Its greatest value is to distinguish true-positive nontreponemal test results from false-positive results

- to establish the diagnosis of late latent or late syphilis

- reactive FTA-ABS test result confirms the reactivity of the nontreponemal test used as the initial test for syphilis

- reactive FTA-ABS test result suggests current or past infection with pathogenic treponemes

- reactive FTA-ABS test may also support the diagnosis of late syphilis or long-standing late latent syphilis

- nonreactive FTA-ABS test result suggests that the reactive nontreponemal test result is a false-positive reaction.

Treponema pallidum Hemaglutination Assay - TPHA

- red blood cells are sensitized with antigens from *T. pallidum* subsp*. Pallidum*
- cells then aggregate on the surface of a test dish if exposed to the serum of a patient with syphilis
- it is used as a confirmatory test for syphilis infection
- negative test result shows a tight button or spot of red blood cells on the surface of the test dish

- For primary syphilis, TPHA has a sensitivity of 85% to 100%, and a specificity of 98% to 100%
- In secondary and late-latent syphilis, TPHA has a sensitivity of 98% to 100%.

Treponema pallidum Particle Agglutination (TP-PA) test

Particle agglutination methods

- MHA test has been modified to use gelatin particles rather than erythocytes as the antigen carrier creating *T.pallidum* particle agglutination
- removal of preabsorption process
- procedure similar to MHA-TP
- Sensitivity and specificity similar to that of the FTA-ABS test

Thank you

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