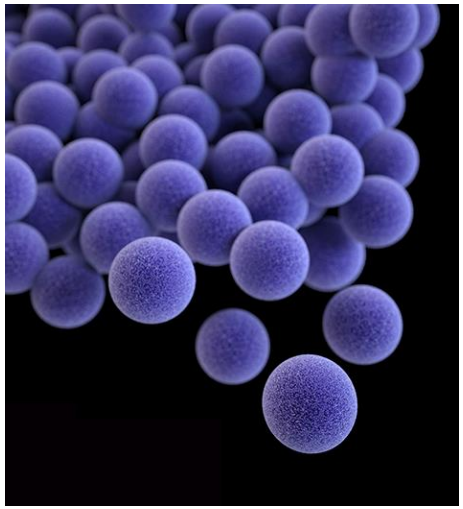
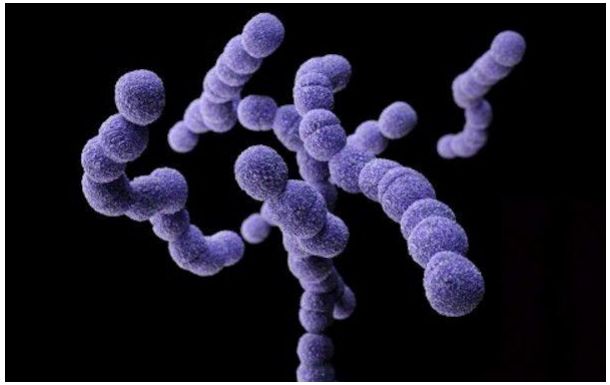


Staph e Strep

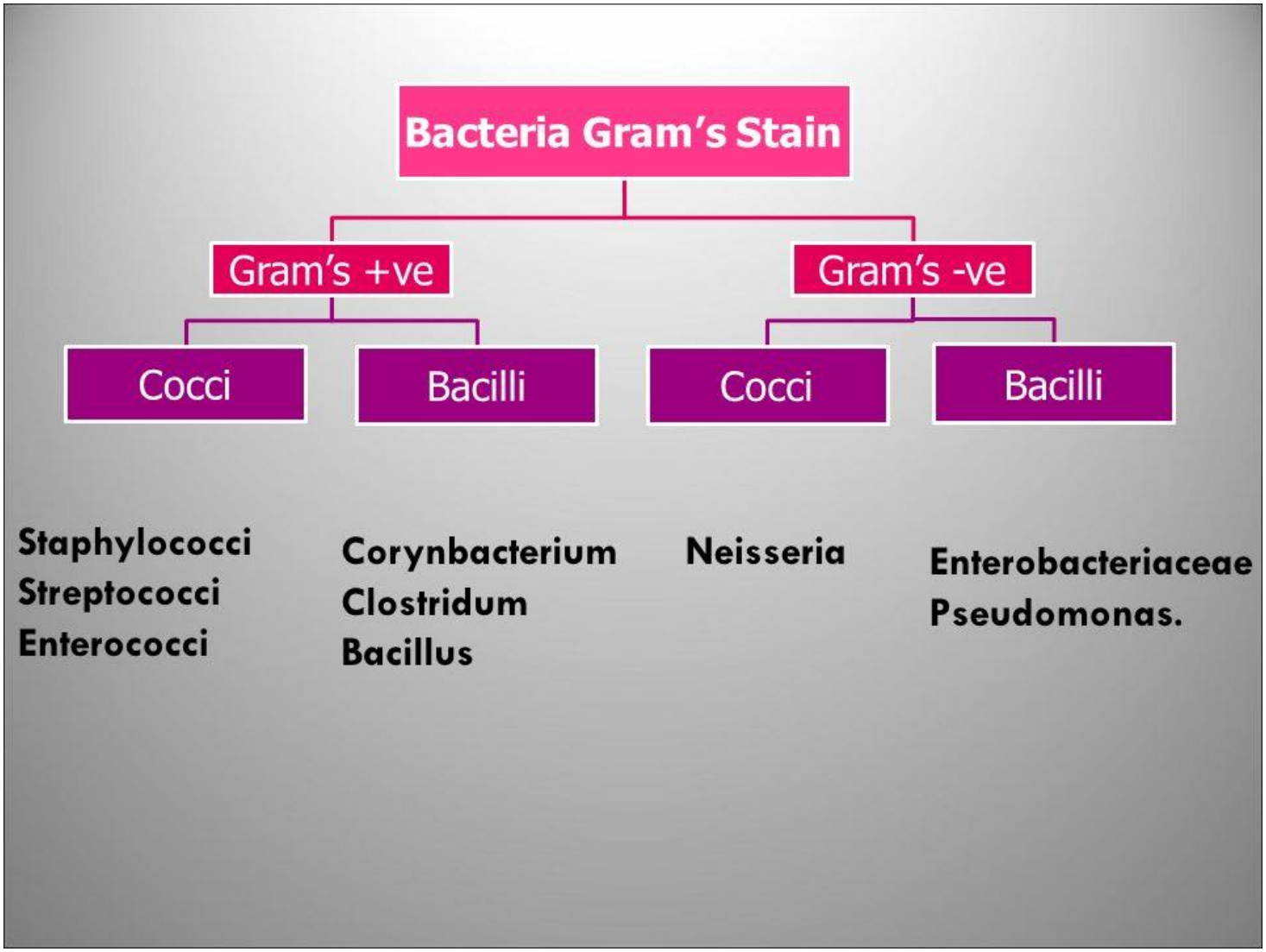


Blood agar plates
Left: *Staphylococcus*; Right: *Streptococcus*

www.bacteriainphotos.com

Hans N.

Mannitol Salt Agar


Bacteria Gram's Stain
Gram's +ve
Gram's -ve
Cocci
Bacilli
Cocci
Bacilli
Staphylococci
Streptococci
Enterococci
Corynbacterium
Clostridum
Bacillus
Neisseria
Enterobacteriaceae
Pseudomonas.

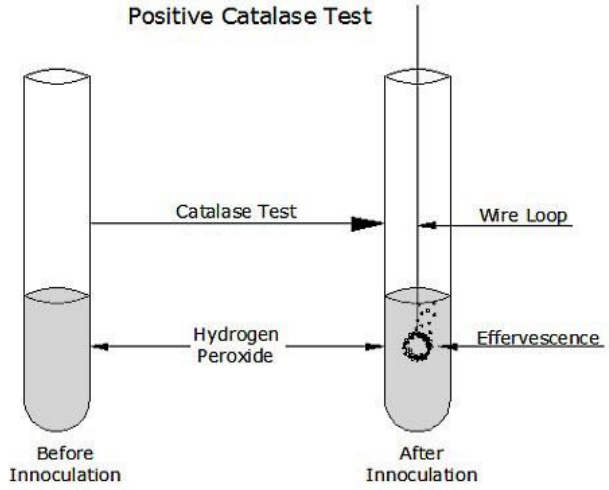
Catalase test

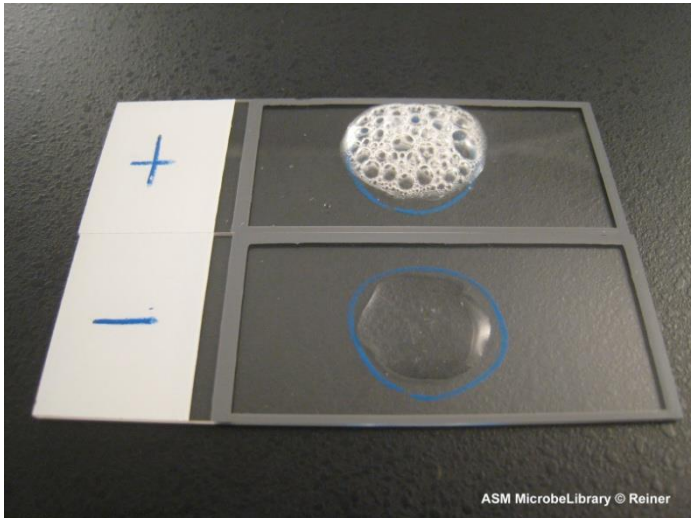
The catalase test is used to detect the presence of the enzyme catalase in bacteria.

Purpose:-

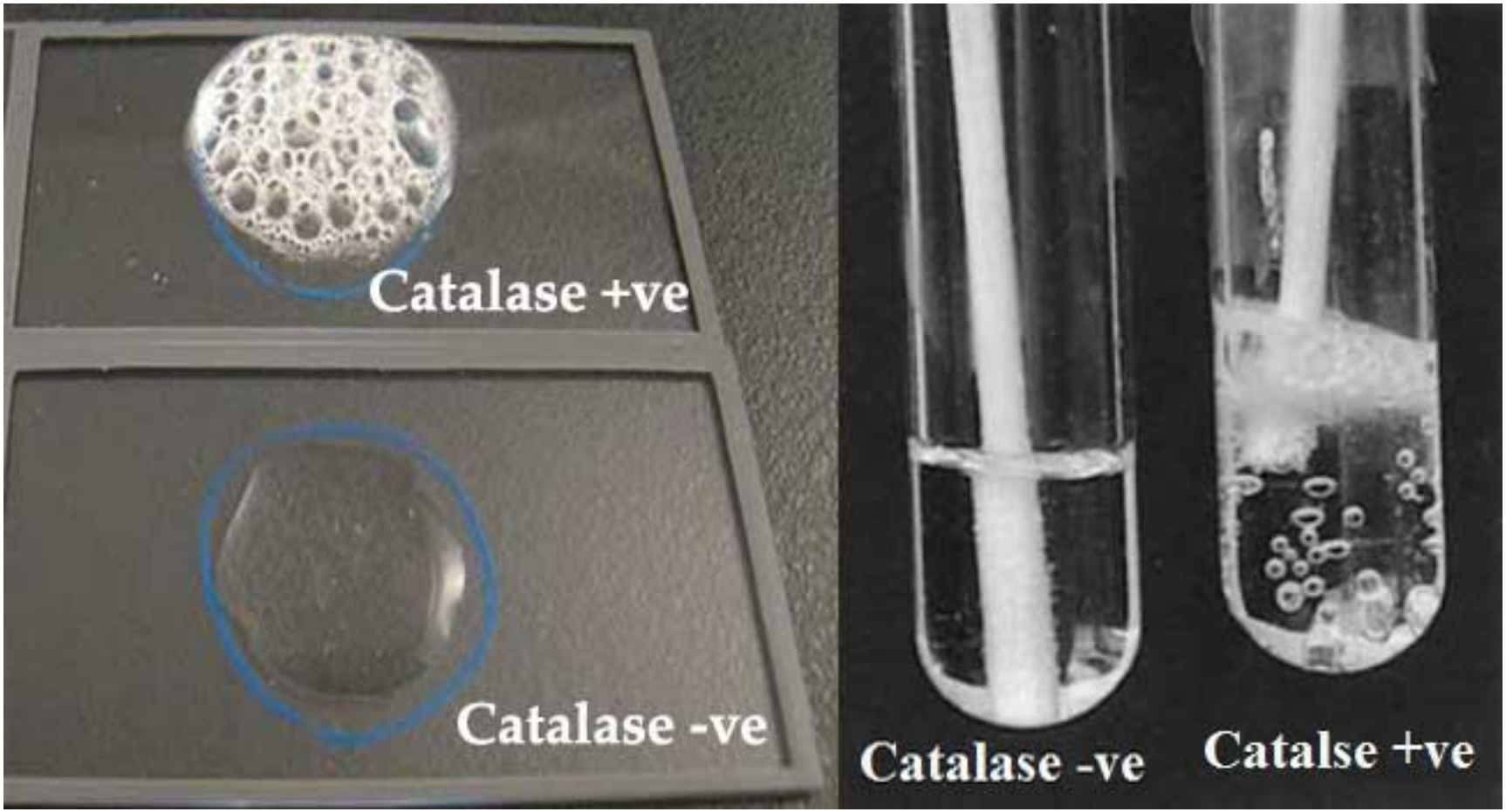
- Identification for gram-positive & gram-negative organisms.
- It is a primary test used in the differentiation of staphylococci and streptococci.

Staph and Strep





Biochemical differentiation between Staphylococci and Streptococci with CATALASE test


Catalase +ve
Catalase -ve
Catalase -ve
Catalse +ve

Background

- Enzyme name ⟶ catalase

important enzyme in protecting the cell from oxidative damage by reactive oxygen species (ROS).

- Substrate name ⟶ hydrogen peroxide
- Enzyme action ⟶ breakdown of toxic H_2O_2 producing oxygen gas and water

- $2H_2O_2 \xrightarrow{\text{catalase}} 2H_2O + O_2$

- Hydrogen peroxide produce due to the aerobic respiration of the cells and have to be breakdown to prevent it's toxic action on DNA and cell membrane

La maggior parte dei batteri gram negativi possiede la catalasi (catalasi +).
La ricerca della catalasi nei gram positivi permette di differenziare gli stafilococchi ed i micrococchi (catalasi+) dagli enterococchi (catalasi -)

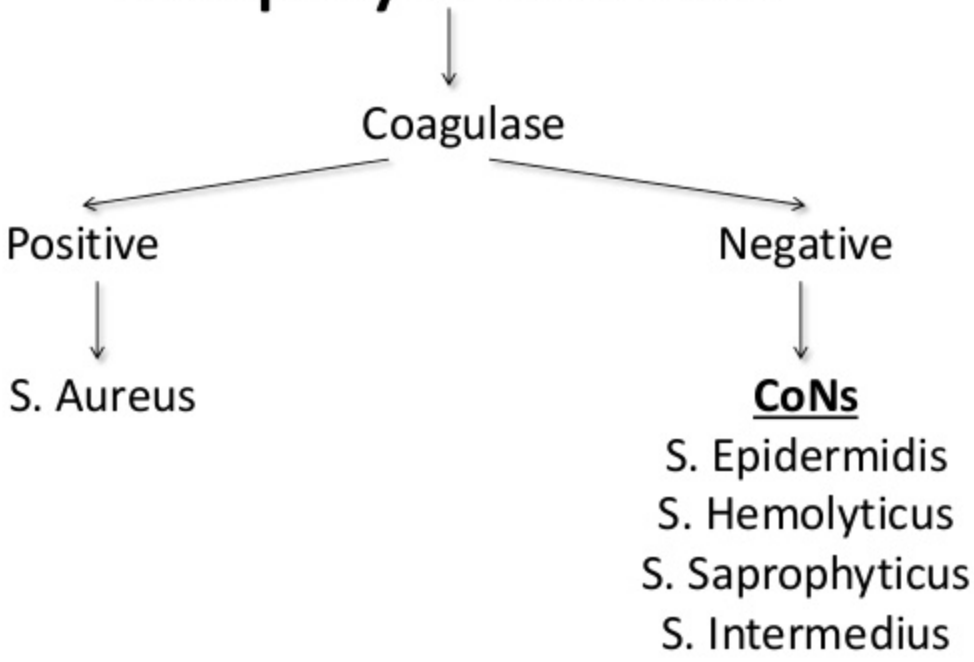
Esempi:

Batteri Gram + catalasi + :
Stafilococco, Micrococco (antica famiglia delle Micrococcaceae)
Bacillo;

Batteri Gram + catalasi - :
Streptococco
Enterococco

Per effettuare il test in laboratorio si utilizza il plasma citratato di coniglio. A 0,5 ml di plasma si aggiunge una colonia e, dopo aver stemperato si incuba in termostato a 37° e va contrallata ogni 30' per 2h circa, fino ad un massimo di 4h. La formazione di un coagulo solido conferma o meno la positività della reazione.

Staphylococcus

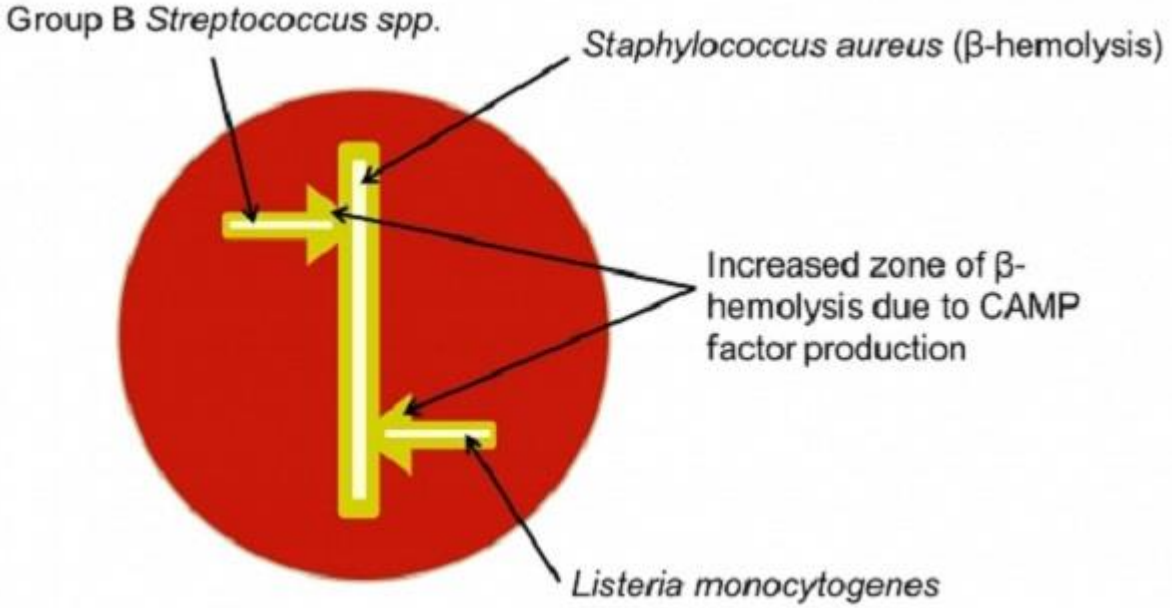


STAPHYLOCOCCUS

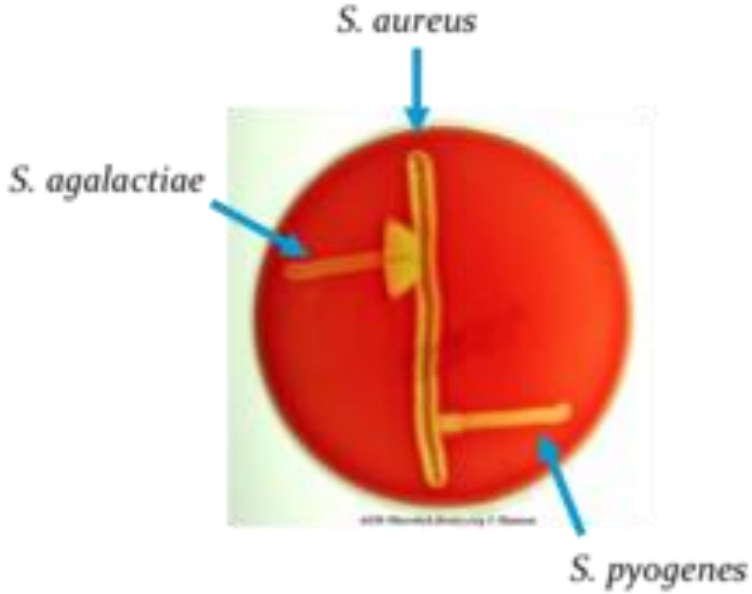
Important Classification

Species	Catalase	Coagulase	Novobiocin Sensitivity	DNAse
Staph. aureus	Positive	Positive	Sensitive	Positive
Staph. epidermidis	Positive	Negative	Sensitive	Negative
Staph. saprophyticus	Positive	Negative	Resistant	Negative
Staph. lugdunensis	Positive	Positive (Slide) Negative (Tube)	Sensitive	Negative

	Characteristics	*Staphylococcus*	*Streptococcus*
1	**Arrangement**	Grape-like clusters.	A chain of round cells.
2	**Division**	Staphylococci divide in various directions (multiple axes).	Division occurs in one linear direction (single axis).
3	**Catalase Test**	Positive (Catalase is an enzyme to converts hydrogen peroxide to water and oxygen gas).	Negative
4	**Enriched Media**	Staph does not need enriched media (not fastidious).	Strep needs enriched media (fastidious).
5	**Habitat**	Staphylococci are found on the skin.	Streptococci are found in the respiratory tract.
6	**Hemolysis**	No hemolysis or beta hemolysis.	Either alpha, or beta or gamma hemolysis.
7	**Species Number**	About 40 staphylococcal species have been identified so far.	About 50 Streptococcal species have been identified so far.
8	**Pathogenesis**	Most of the staphylococcal species are non-pathogens.	Streptococcus cause many diseases.
9	**Common Diseases**	Food poisoning, bacterial conjunctivitis, skin diseases, community-acquired meningitis, Surgical Site Infection, Wound infection, impetigo, cellulitis, and toxic shock syndrome.	Strep throat, Scarlet fever, Impetigo, Toxic shock syndrome, Cellulitis and necrotizing fasciitis (flesh-eating disease), sinusitis, blood infections, pneumonia and meningitis in newborns.
10	**Pathogenic Species**	*Staphylococcus aureus,* *Staphylococcus epidermidis,* *Staphylococcus haemolyticus,* *Staphylococcus hominis*, *Staphylococcus saprophyticus*, etc.	*Streptococcus pyogenes,* *Streptococcus agalactiae,* *Streptococcus bovis,* *Streptococcus pneumoniae*, etc.
11	**Species Differentiation**	- Coagulase test - Novobiocin sensitivity test - Biochemical tests	- Type of hemolysis - Cell wall carbohydrate group (A, B, C, etc) - Bile Solubility Test - CAMPT Test - Optochin Sensitivity Test


Group B *Streptococcus spp.*
Staphylococcus aureus (β-hemolysis)
Increased zone of β-hemolysis due to CAMP factor production
Listeria monocytogenes


α
β
γ
ASM MicrobeLibrary.org © Buxton


S. aureus
S. agalactiae
S. pyogenes

IDENTIFICATION OF GRAM POSITIVE COCCI- IV ENTEROCOCCUS

- **Gram stain – Gram positive cocci in pairs**
- **Motility – Non-motile**
- **Catalase – Negative**
- **Oxidase – Negative**

So it may be Enterococcus (or) Pneumococcus (or) viridans streptococci

Note:

Enterococcus can be alpha or beta or gamma hemolytic on blood agar

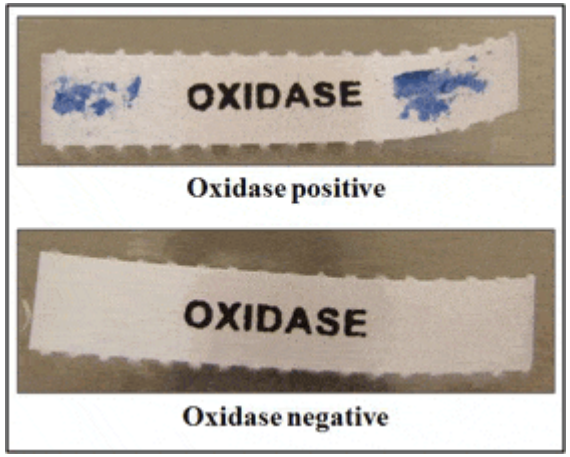
Enterococcus faecalis and Enterococcus faecium are important pathogenic members in Genus Enterococcus

Oxidase Test Strip

Rapid

10s

La **prova dell'ossidasi** è una prova usata in microbiologia per determinare se un batterio produca qualcuna delle citocromo-c ossidasi.
Per la prova si utilizzano supporti impregnati con un indicatore redox, in genere N,N,N',N'-tetrametil-p-fenilendiammina (TMFD) o N,N-dimetil-p-fenilendiammina (DMFD).
Il reattivo presente nella sua forma incolore ridotta subisce ossidazione da parte di citocromo-c ossidasi indicando batteri positivi alla prova dell'ossidasi



OXIDASE NEGATIVE

- All enterobacteriaceae are OXIDASE NEGATIVE
- Acinetobacter
- Staphylococci
- streptococci

OXIDASE POSITIVE ORGANISMS:

- Pseudomonas
- Neisseria
- Vibrio
- Campylobacter
- Aeromonas
- Alcaligenes
- Brucella
- Pasturella
- Eikenella
- Kingella
- Moraxella
- Legionella
- Helicobacter
- Chromobacter (oxidase variable)

QUALITY CONTROL:

- Positive control : Pseudomonas
- Negative control : E.coli

I batteri che posseggono l'enzima citocromo ossidasi, in presenza di ossigeno, ossidano il substrato contenuto nella striscia, incolore allo stato ridotto, con formazione di un composto colorato. **Il test dell'ossidasi è particolarmente indicato per la caratterizzazione dei batteri Gram negativi**

I ceppi con una attività citocromo ossidasi lenta (es. Pasteurella) possono apparire negativi al test.

IDENTIFICATION OF GRAM NEGATIVE BACILLI

(I)

ESCHERICHIA COLI /E.COLI

- Gram stain – Gram negative bacilli
- Motility – Motile
- Catalase – Positive
- Oxidase – Negative

So it is one of the members of Enterobacteriaceae

Enterobacteriaceae includes E.coli, Klebsiella, Citrobacter, Enterobacter, Serratia, Salmonella, Shigella, Proteus

IDENTIFICATION OF GNB (II)
KLEBSIELLA SPECIES

- Gram stain – Gram Negative bacilli
- Motility – Non-motile
- Catalase – Positive
- Oxidase – Negative

So it is one of the members of Enterobacteriaceae

IDENTIFICATION OF GNB (III)
CITROBACTER

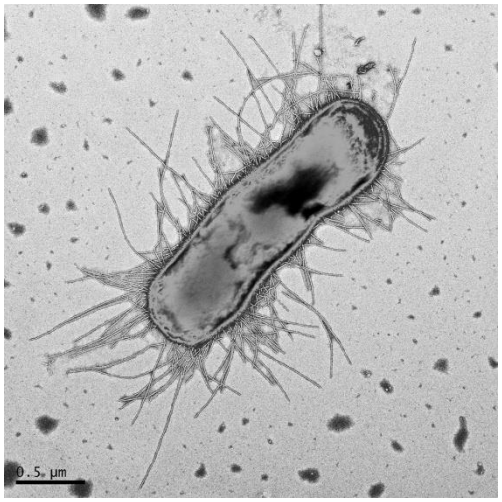
- Gram stain – Gram negative bacilli
- Motility – Motile
- Catalase – Positive
- Oxidase – Negative

So it is one of the members of Enterobacteriaceae

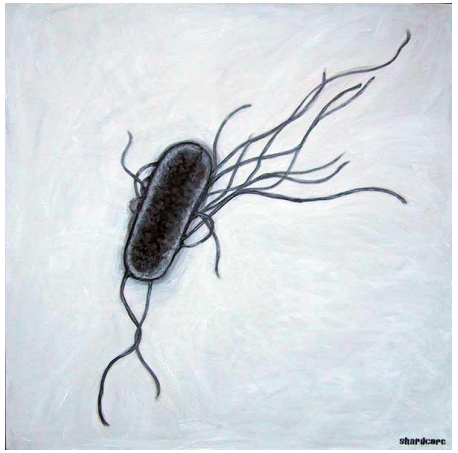
On Mac – LF colonies

Being motile

It may be E.coli or Enterobacter or Citrobacter



Escherichia coli e *Klebsiella pneumoniae* sono enterobatteri produttori di beta lattamasi a spettro esteso (ESBL) e rappresentano un problema di notevole rilievo clinico ed epidemiologico. Le ESBL sono enzimi in grado di idrolizzare una vasta gamma di antibiotici beta-lattamici inclusi penicillina e cefalosporine



IDENTIFICATION OF GNB – VIII
PSEUDOMONAS SPECIES

- Gram stain – Gram negative bacilli
- Motility – Motile
- Catalase – Positive
- Oxidase – Positive

So it is one of the members of

Non-enterobacteriaeceae like

Pseudomonas species

Vibrio species