



دانشگاه اصفهان

دانشکده علوم و فناوری های زیستی، گروه زیست شناسی سلولی مولکولی و میکروبیولوژی،
آزمایشگاه میکروبیولوژی



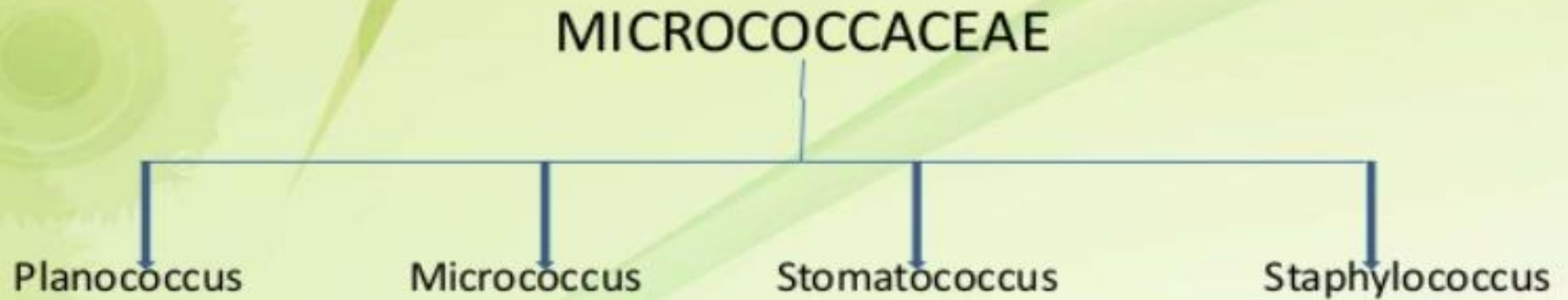
آزمایشگاه باکتری شناسی ۱

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- ۱- رنگ آمیزی و مشاهده کوکسی های گرم مثبت شامل استافیلوکوکوس اورئوس و استافیلوکوکوس اپیدرمیدیس به روش میکروسکوپی
- ۲- انجام آزمون های بیوشیمیایی جهت شناسایی استافیلوکوکوسی

TAXONOMY OF STAPHYLOCOCCI & RELATED GPC

- According to 1986 edition → Bergey's Manual of Systemic Bacteriology :



- According to new edition, **Staphylococcus** belongs to phylum : *FIRMICUTES* & comprise Genus I in family V ("*Staphylococcaceae*")



History

- **Robert Koch (1878)**- first to see staphylococci in pus specimen
- **Louis Pasteur (1880)**- first to cultivate in liquid medium
- **Sir Alexander Ongston (1881)**- named the bacteria as “staphylococcus”

IMPORTANT GENERA & SPECIES

➤ Staphylococcus aureus

➤ Coagulase-Negative Staph. (most commonly encountered)

- S.epidermidis
- S.haemolyticus
- S.saprophyticus
- S.lugdunensis
- S.schleiferi

➤ Coagulase-Negative Staph. (less commonly encountered)

- S.capitis
- S.caprae
- S.warneri
- S.hominis
- S.auricularis
- S.cohnii
- S.xylosum
- S.simulans

➤ Micrococcus species & related genera

Classification

- Based on pigment production:

- *S.aureus* :-golden-yellow pigmented colonies
- *S.albus* :- white colonies
- *S.citrus* :-lemon yellow colonies



S. albus , *S. aureus* , *S. citrus* on Nutrient Agar

- Based on pathogenecity:

- Pathogenic:- includes only one i.e., *S.aureus*
 - Non-pathogenic:- includes *S.epidermidis*, *S.saprophyticus*, *S.albus*, *S. citrus*, *S.hominis*, etc.
- Based on coagulase production:
 - Coagulase positive: *S. aureus*
 - Coagulase negative: *S. epidermidis*, *S. saprophyticus*

Staphylococcus aureus

Staphylococcus aureus

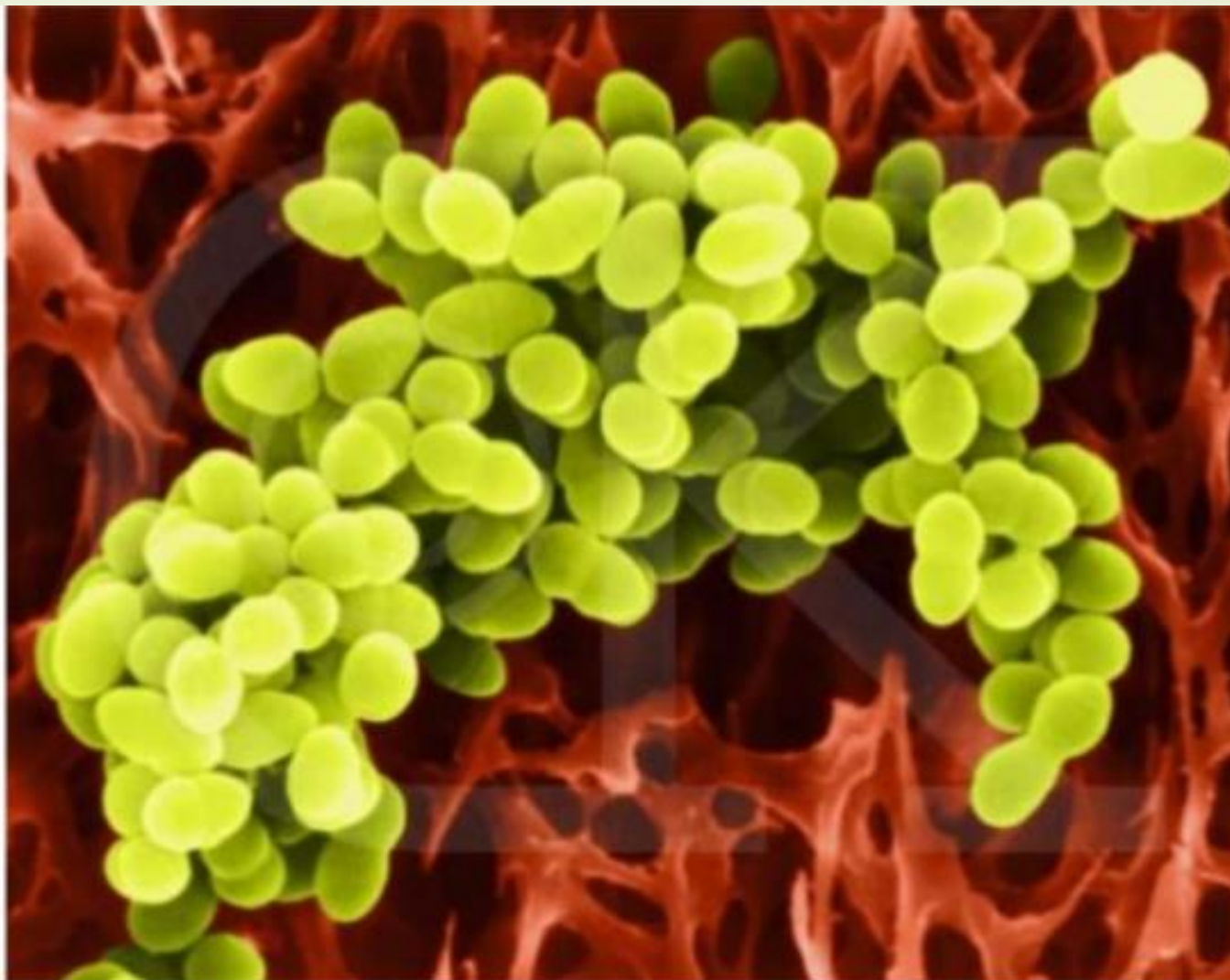
MORPHOLOGY

- Gram positive ,Arranged in grape like clusters,Non motile, Non sporing, Non capsulated
- 1 micrometer in diameter

MORPHOLOGY

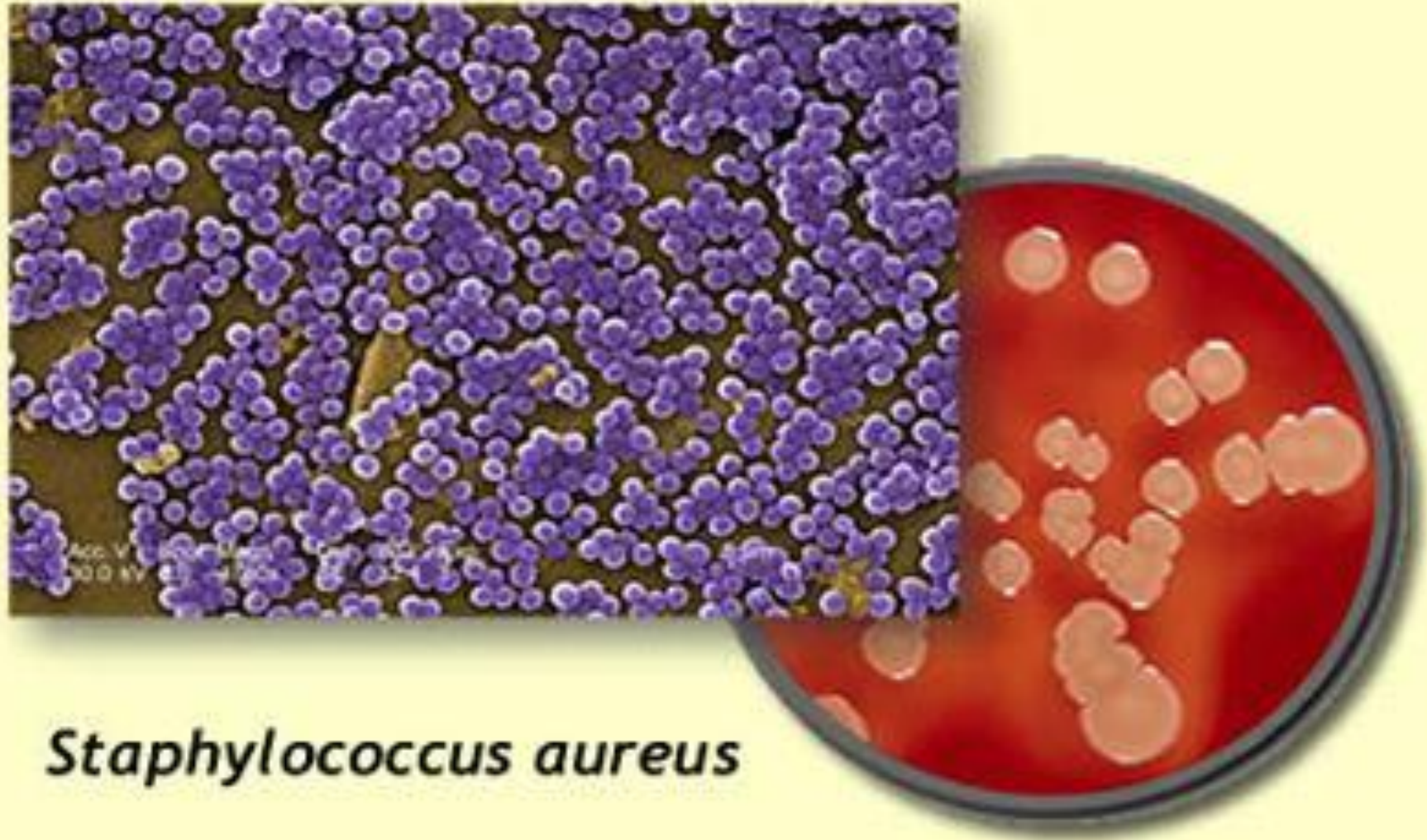
- Spherical, 1 μm dia.
- Arrangement : Grape like clusters.
- Non-motile, Non-sporing
- Non-capsulated {Few strains possess microscopically visible capsule (young cultures)}.
- Stain readily with aniline dye & are uniformly Gram Positive.



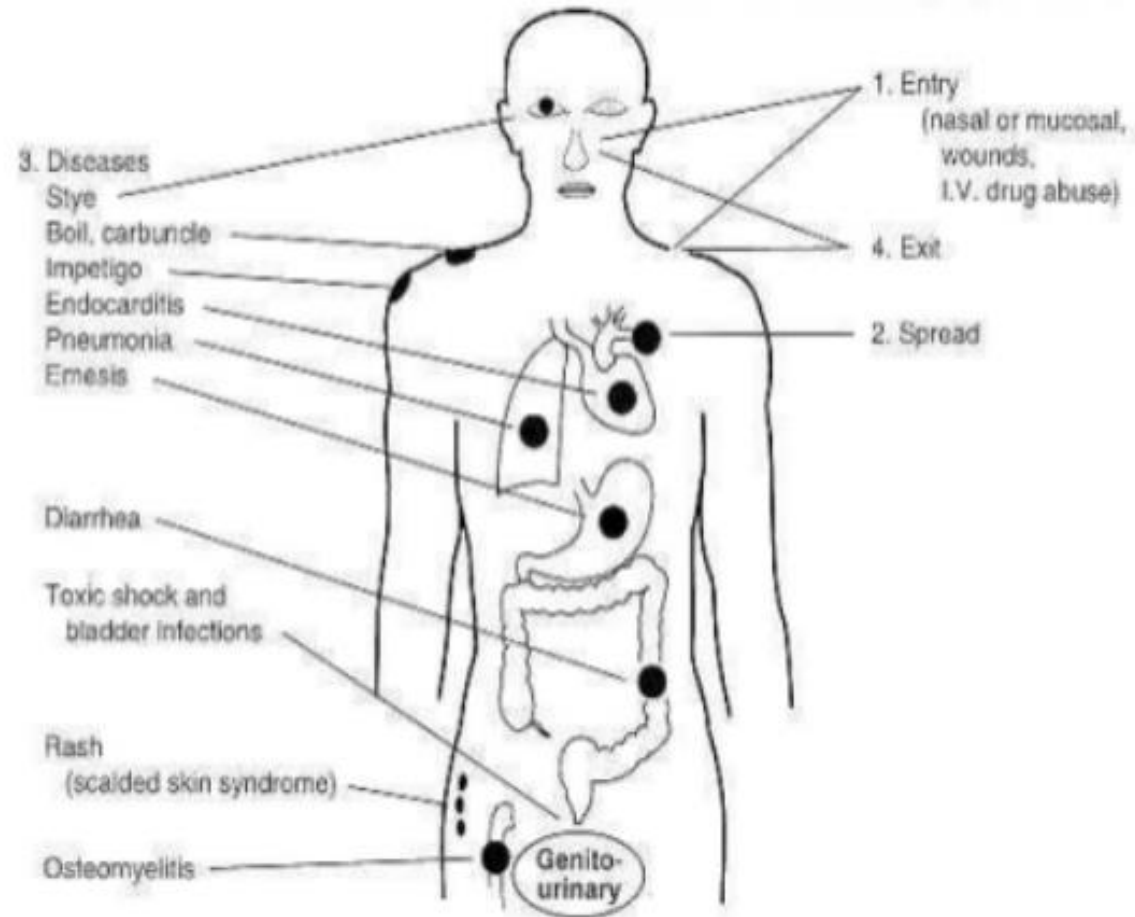


STAPHYLOCOCCUS

تهیه کننده: سهیلا عباسی



Staphylococcus aureus



Staphylococcal infections

LAB DIAGNOSIS

SPECIMENS

Specimens are to be depend on the type of lesions

1. Pus(suppurative lesions)
2. Sputum(respiratory infections)
3. Blood(endo vascular)
4. CSF(CNS infections)
5. Urine (UTI)
6. Feces,vomit,remains of suspected food(food poisoning)
7. Nasal swab(carriers)

METHODS

Direct microscopy

Smears of material shows gram positive cocci in clusters

Culture

Inoculated on to **BLOOD AGAR ,NUTRIENT AGAR** etc

Look for typical colony morphology, hemolysis, pigmentation etc

CULTURE CHARACTERISTICS

- Aerobes and facultative anaerobes
- Temperature = 10°C to 42°C (37°C)
- pH = 7.4 - 7.6

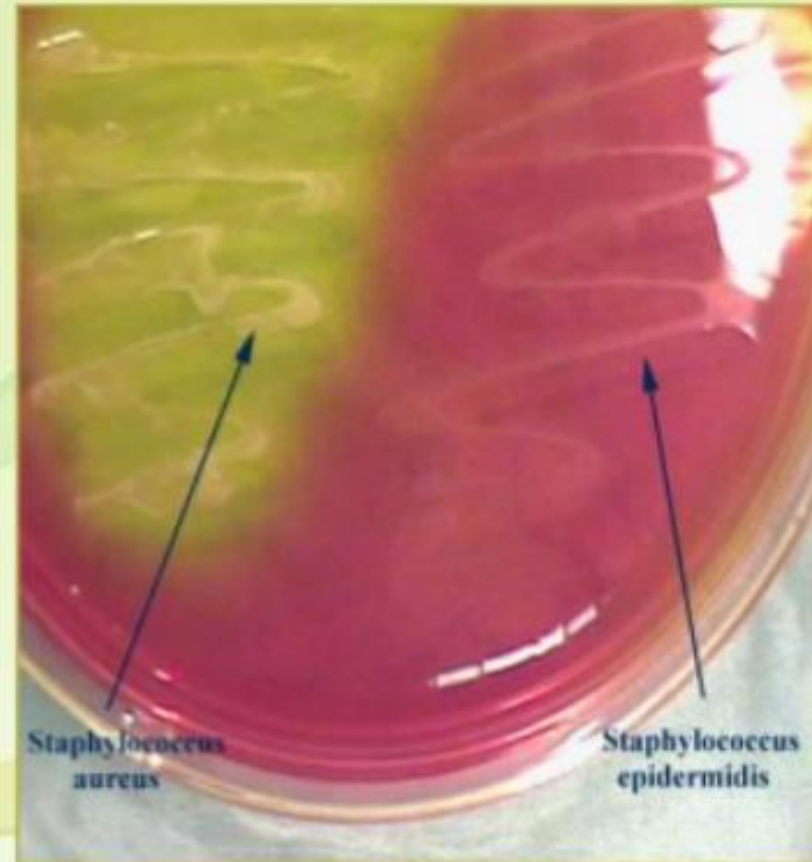
- **On Blood agar**
 - golden yellow colonies, surrounded by a clear zone of hemolysis (beta-hemolysis), esp. when incubated in sheep or rabbit blood agar in atmosphere of 20% CO₂



- **On MacConkey agar**
 - Smaller colonies than those on Nutrient agar and are pink coloured due to lactose fermentation.

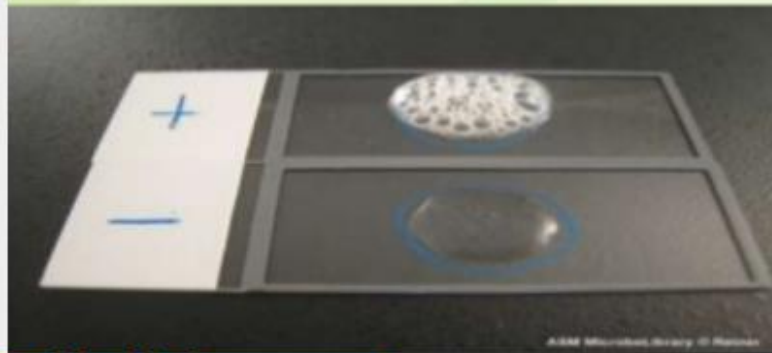


- On Mannitol salt agar
 - *S. aureus* ferments mannitol and appear as yellow colonies
 - MSA is a useful selective medium for recovering *S. aureus* from faecal specimens, when investigating food poisoning.



BIOCHEMICAL PROPERTIES

- Catalase positive
- Oxidase negative
- Ferment glucose, lactose, maltose, sucrose and mannitol, with production of acid but no gas
- Mannitol fermentation carries diagnostic significance.



- Indole test= negative
- MR test= positive
- VP test= positive
- Urease test= positive
- Hydrolyse gelatin
- Reduces nitrate to nitrite
- Phosphatase= positive
- DNA-ase test= positive
- Coagulase test= positive



Slide test (clumping factor)



Tube test (free coagulase)

RESISTANCE

- Remain viable for 3-6 months (isolated from dried pus after 2-3 months).
- May withstand 60°C for 30 minutes (thermal death point: 62°C for 30 minutes)
- Some require heating at 80°C for 1 hour to be killed.
- Heat resistant strains can grow at high temperature (45°C).

PENICILLIN RESISTANCE

- 3 types:

- Production of Beta lactamase
- Alteration in penicillin binding protein PBP2a
- Development of tolerance to penicillin

Bacterium is only inhibited but not killed

ANTIGENIC STRUCTURE & VIRULANCE FACTORS



ANTIGENIC STRUCTURE

Capsule

- Some strains have a capsule. That inhibit phagocytosis, they inhibit opsonisation
- Capsulated strains are more virulent

Peptidoglycan

- Give rigidity to the cell wall, activates complement

Teichoic acid

- Major antigenic component
- Protect cell from complement mediated opsonisation

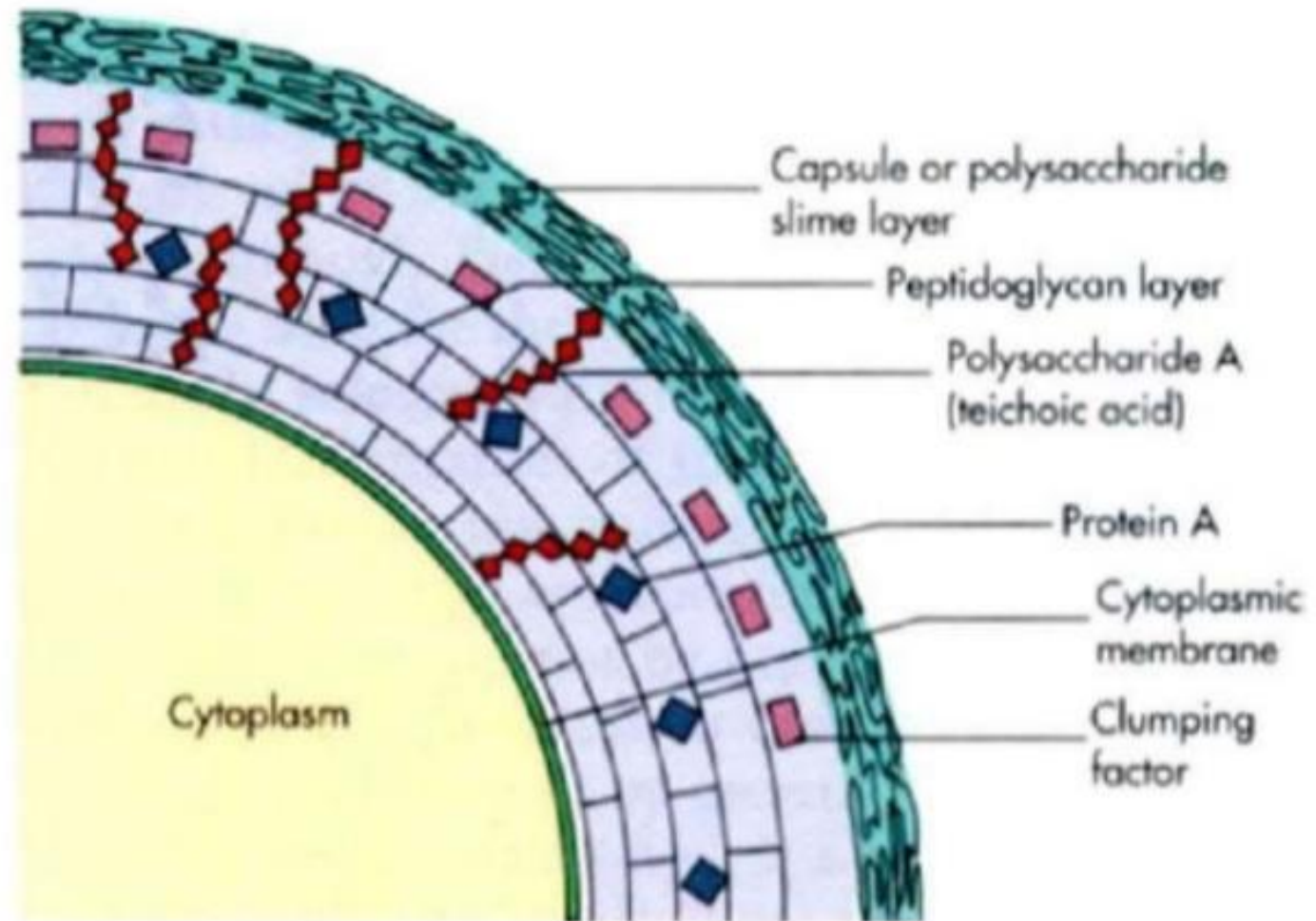
Protein A

- It has chemotactic, antiphagocytic & anti complementary action

Antigenic Structure

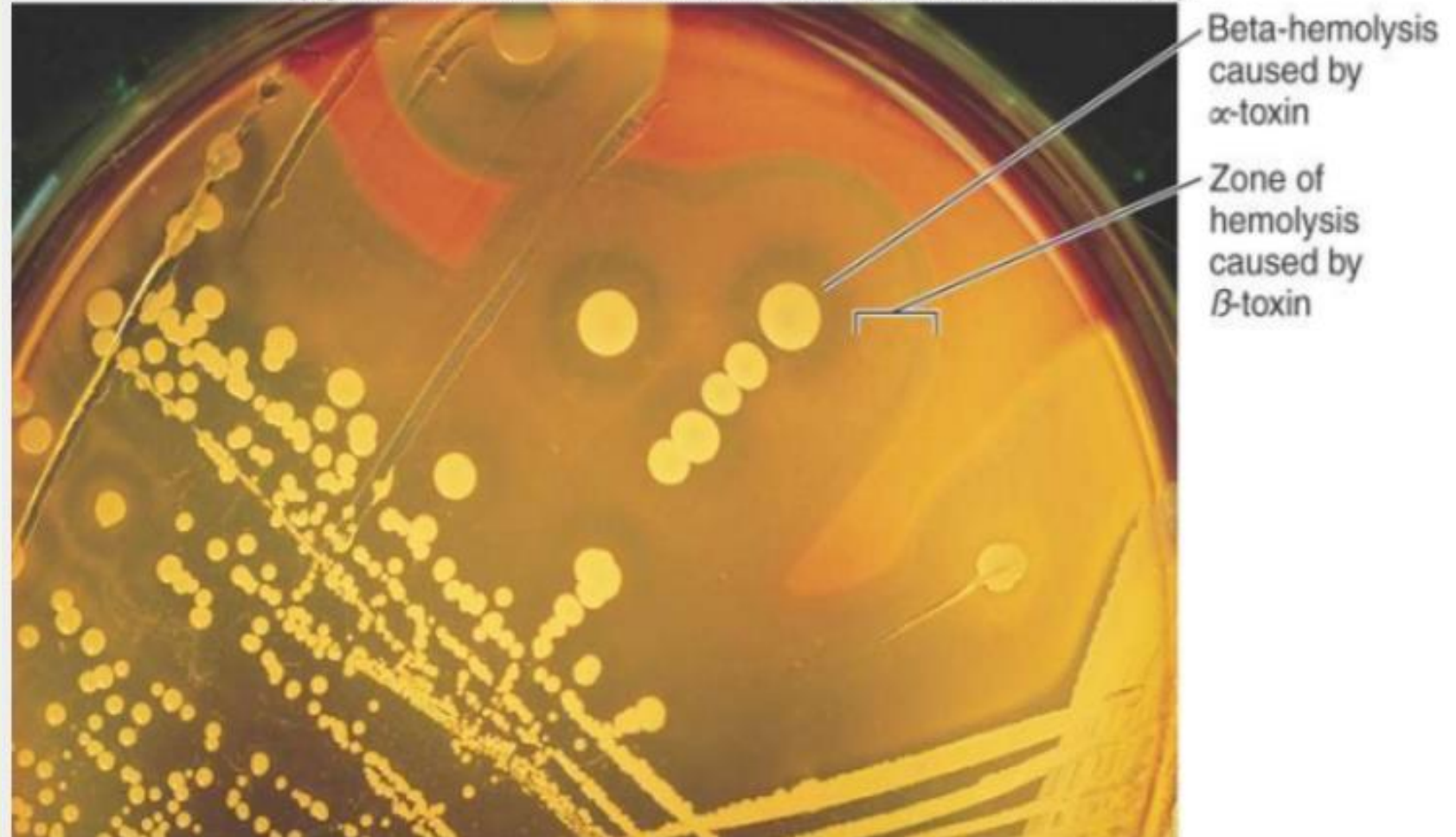


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Blood agar plate, *S. aureus*

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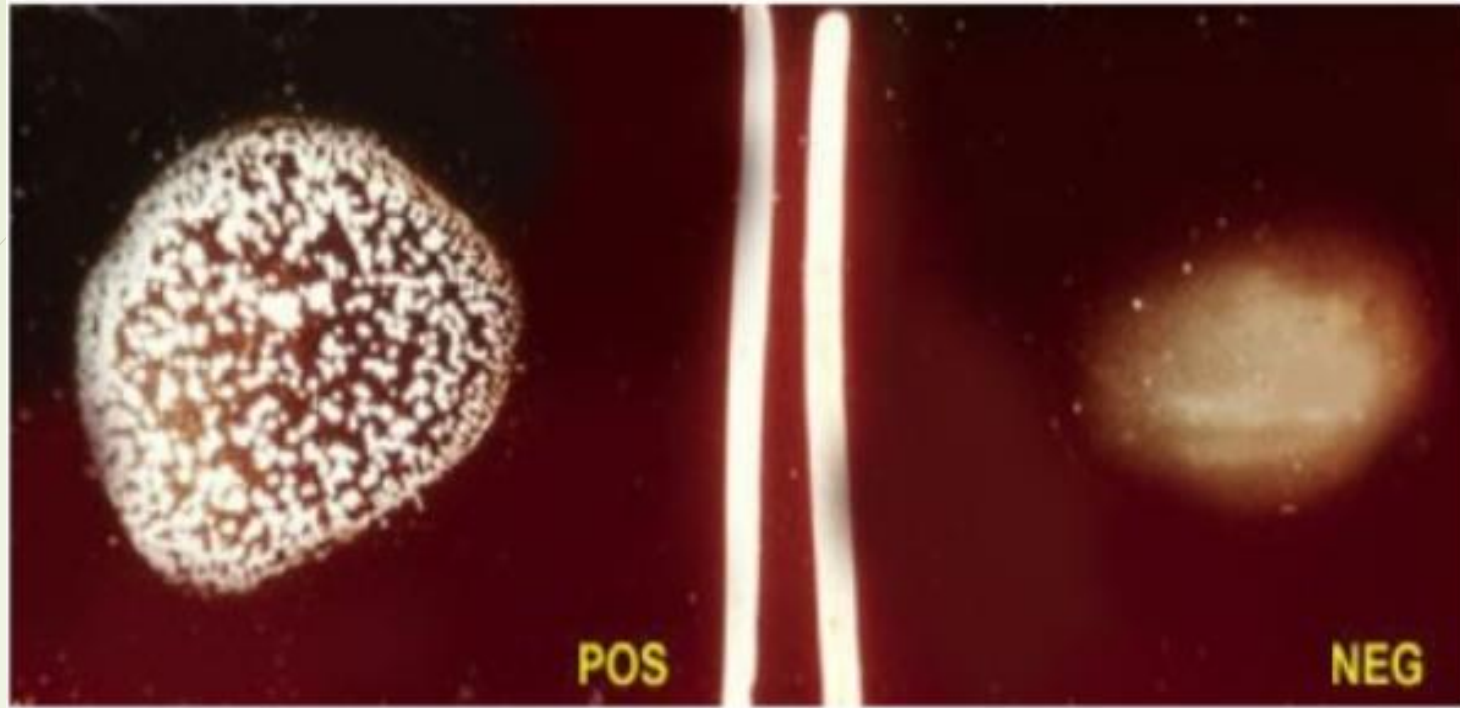
COAGULASE TEST

- It is the main lab test used to differentiate staph aureus from other sps of
- 2 types –slide test & tube test

Slide coagulase test

Few colonies are emulsified in a drop of normal saline on a slide and mixed with a drop of rabbit or human plasma.

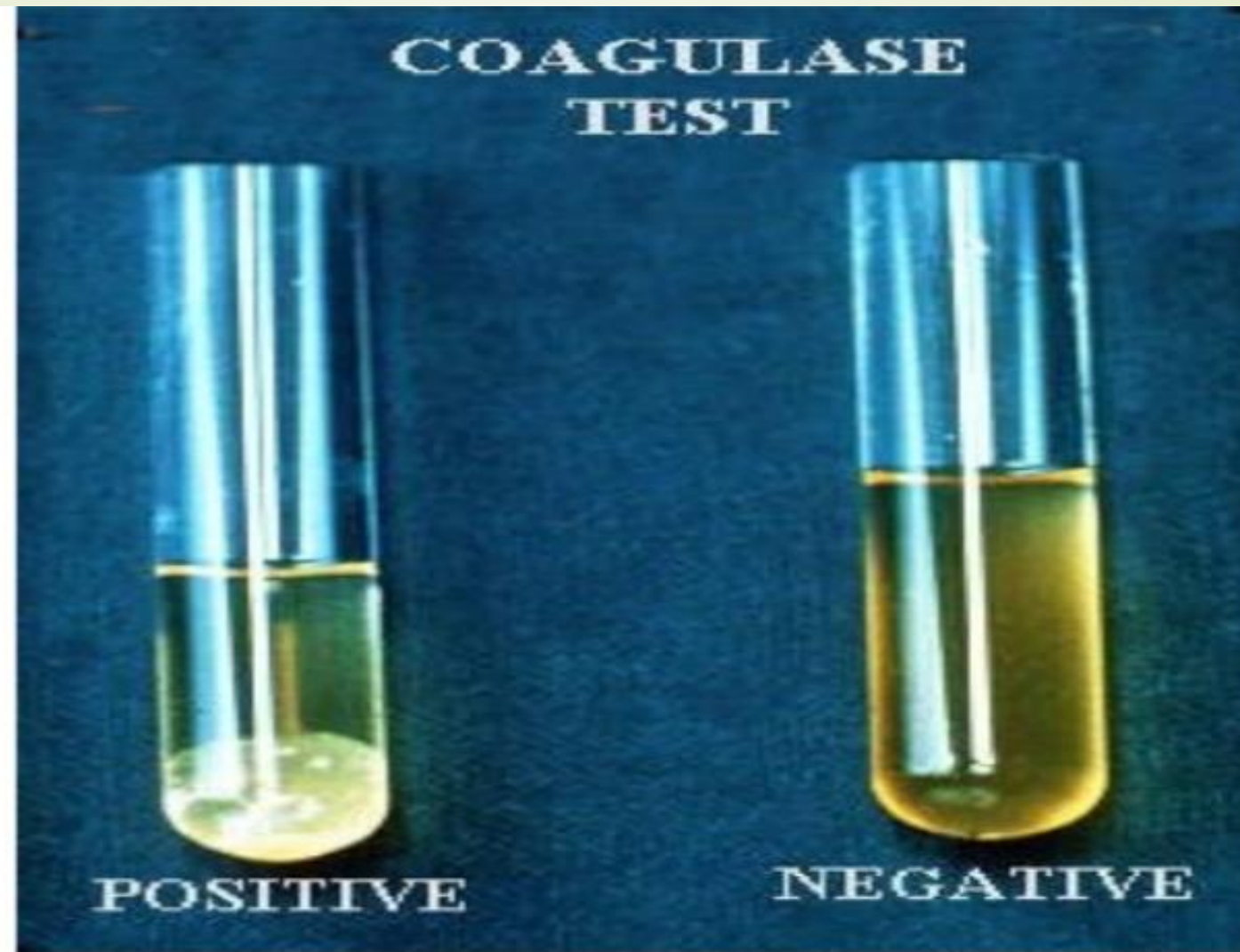
Clumping indicates positive reaction



Slide coagulase test

TUBE COAGULASE test

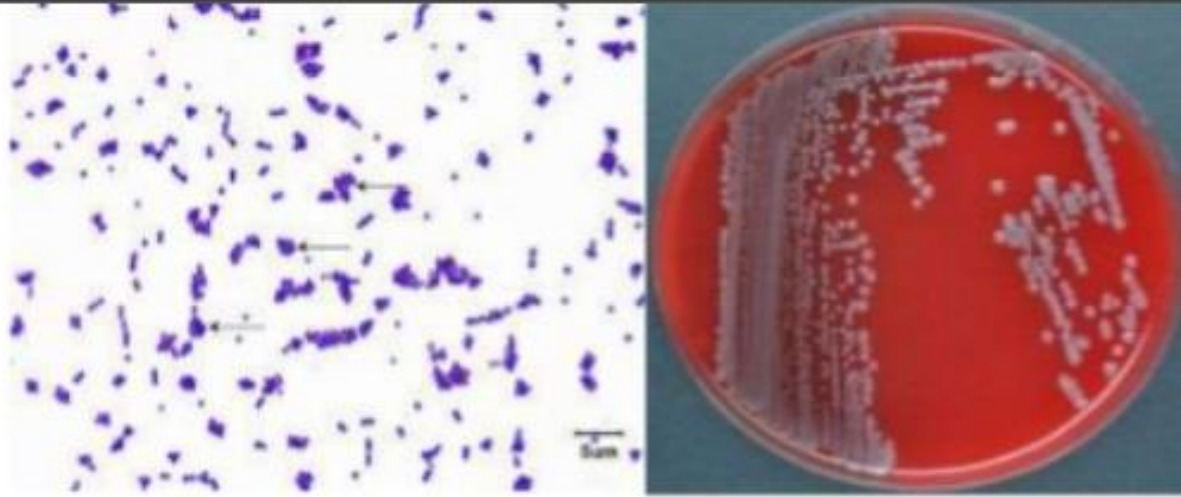
- Done to detect free coagulase
- 0.1 ml broth culture of the isolate is added to 0.5 ml of human or rabbit plasma in a narrow test tube.
- The tubes incubated at 37 degree Celsius for 3-6 hrs
- If positive ,the plasma clots & does not flow when the tube is tilted



Tube coagulase test



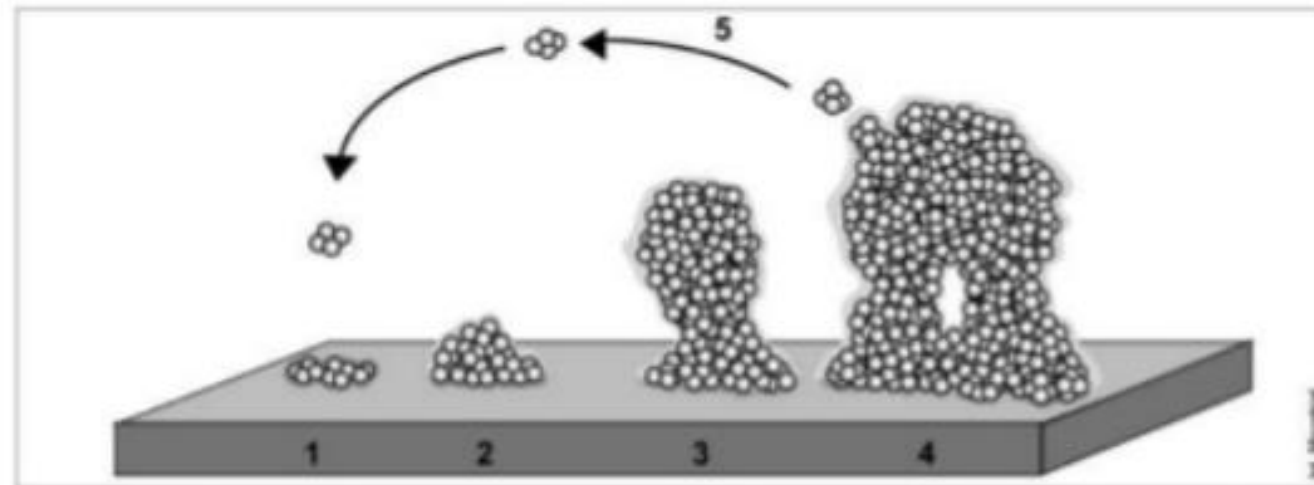
Mannitol fermentation



Staphylococcus epidermidis

- Gram positive, coagulase negative cocci
- Skin commensal
- True opportunistic pathogen, as it requires a major breach in the host's innate defenses.
- Leading pathogens of nosocomial infections
- Associated with infection of IV lines, prosthetic heart valves, shunts
- Cause UTI in catheterized patient
- facultative anaerobe, it grows best in aerobic conditions.

- produces slime layers, which forms a hydrophobic biofilm.
- adhesive to hydrophobic biopolymers of prosthetics, creating diseases such as endocarditis



Diseases caused by staph. epidermidis

- Most infections are hospital acquired
- Opportunistic pathogen in immunosuppressed
- Strongly associated with presence of foreign bodies
 - Prosthetic heart valves (endocarditis)
 - IV catheters (Bacteremia)
 - Urinary catheters (UTI in elderly)
 - CSF shunts (meningitis)
 - Peritoneal dialysis catheter (Peritonitis)

- As *S. epidermidis* is part of the human normal flora, it has developed resistance to many common antibiotics such as methicillin, novobiocin, clindamycin, and benzyl penicillin.
- As a result, **vancomycin** or rifampin is used to treat an infection

• **Staphylococcus epidermidis**

- **Skin commensal**
- **Has predilection for plastic material**
- **Ass. With infection of IV lines, prosthetic heart valves, shunts**
- **Causes urinary tract infection in catheterized patients**
- **Has variable ABS pattern**
- **Treatment should be aided with ABST**



Staphylococcus saprophyticus

. Staphylococcus saprophyticus

- Skin commensal
- Imp. Cause of UTI in sexually active young women
- Usually sensitive to wide range of antibiotics



Staphylococcus saprophyticus

- Gram Stain : Gram Positive Cocci
- Aerobic
- Extracellular
- Features
 - Morphology : Cocci
 - Arrangement : Clusters, Tetrads
 - Colonies: White /yellow , rounded on blood agar plate
- Non motile
- No capsule
- No Glycocalyx

Importance of *S.saprophyticus*

- *S. saprophyticus* frequently isolated in rectum and genitourinary tract of young women
- Can be causative agent in UTI in young healthy women
- 2nd most common urinary pathogen (other than *E. coli*) in uncomplicated cystitis in young women
- Colony counts of $\geq 10^5$ CFU/ml usu. indicative of significant Bacteriuria

Diseases caused by staph. saprophyticus

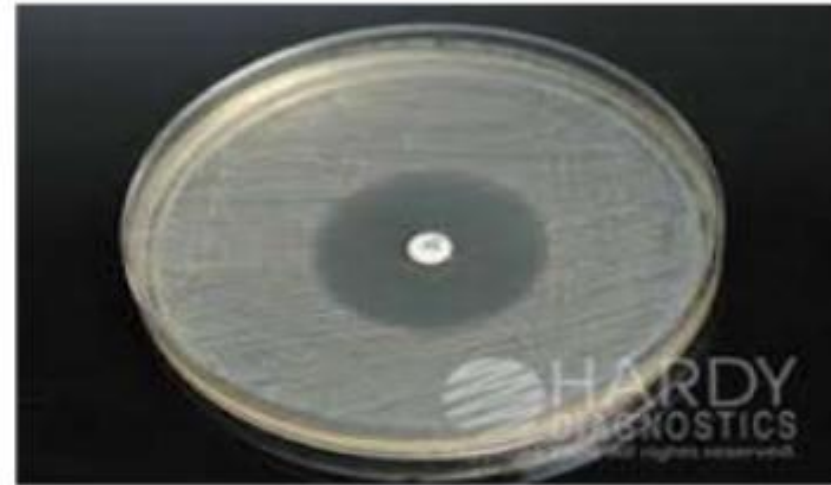
- *S. saprophyticus* is second only to *E. coli* as the most frequent causative organism of **uncomplicated UTI in women**
- The vast majority of infections occur in young sexually active women.
- It can also cause **UTI in males of all ages.**
- Organism has been isolated in young boys, male homosexuals, and elderly men with indwelling urinary catheters

-Novobiocin Susceptibility Test

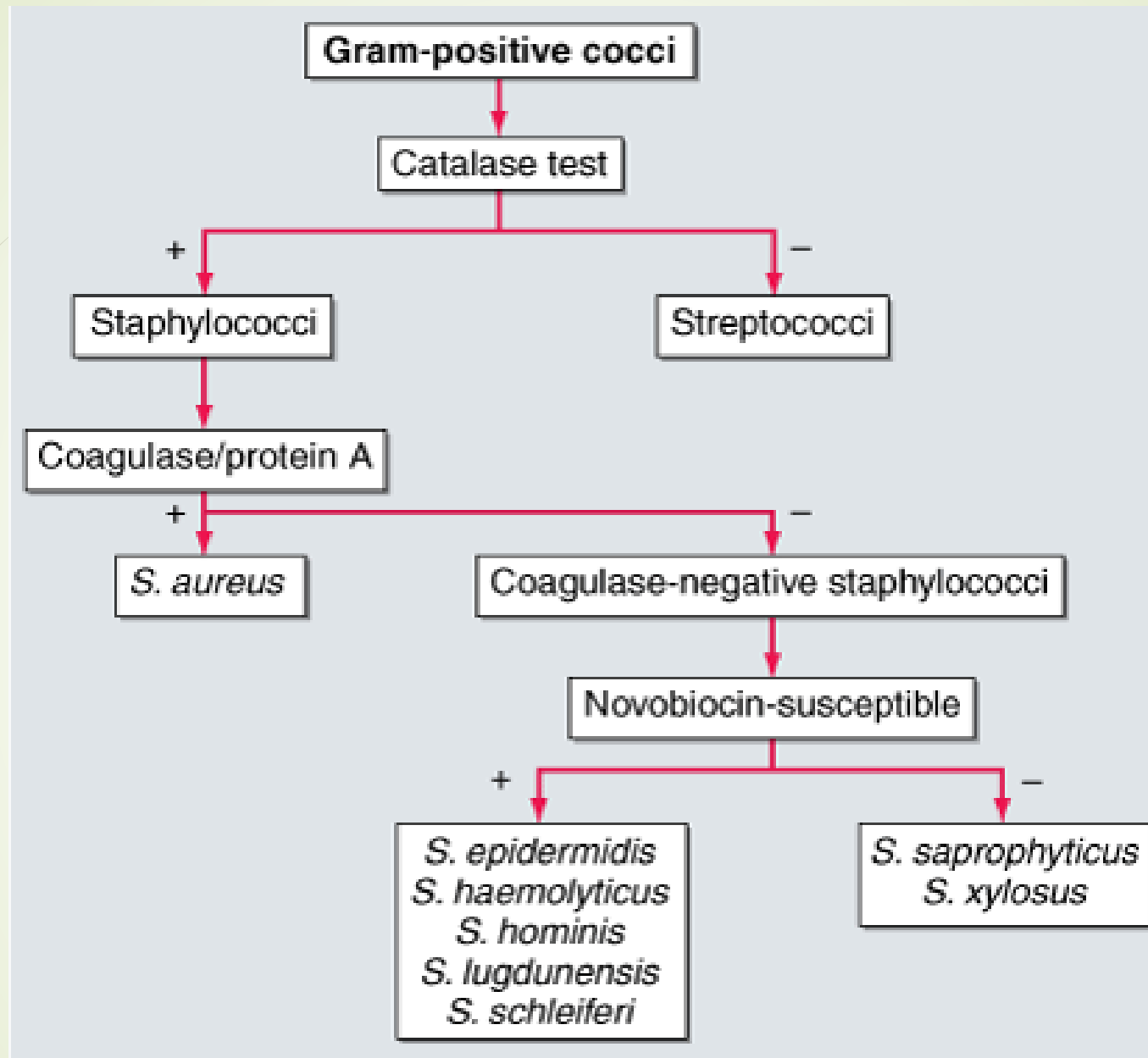
- This test is used to differentiate coagulase-negative staphylococci.



Staphylococcus saprophyticus



Staphylococcus epidermidis



Summary Micrococcaceae

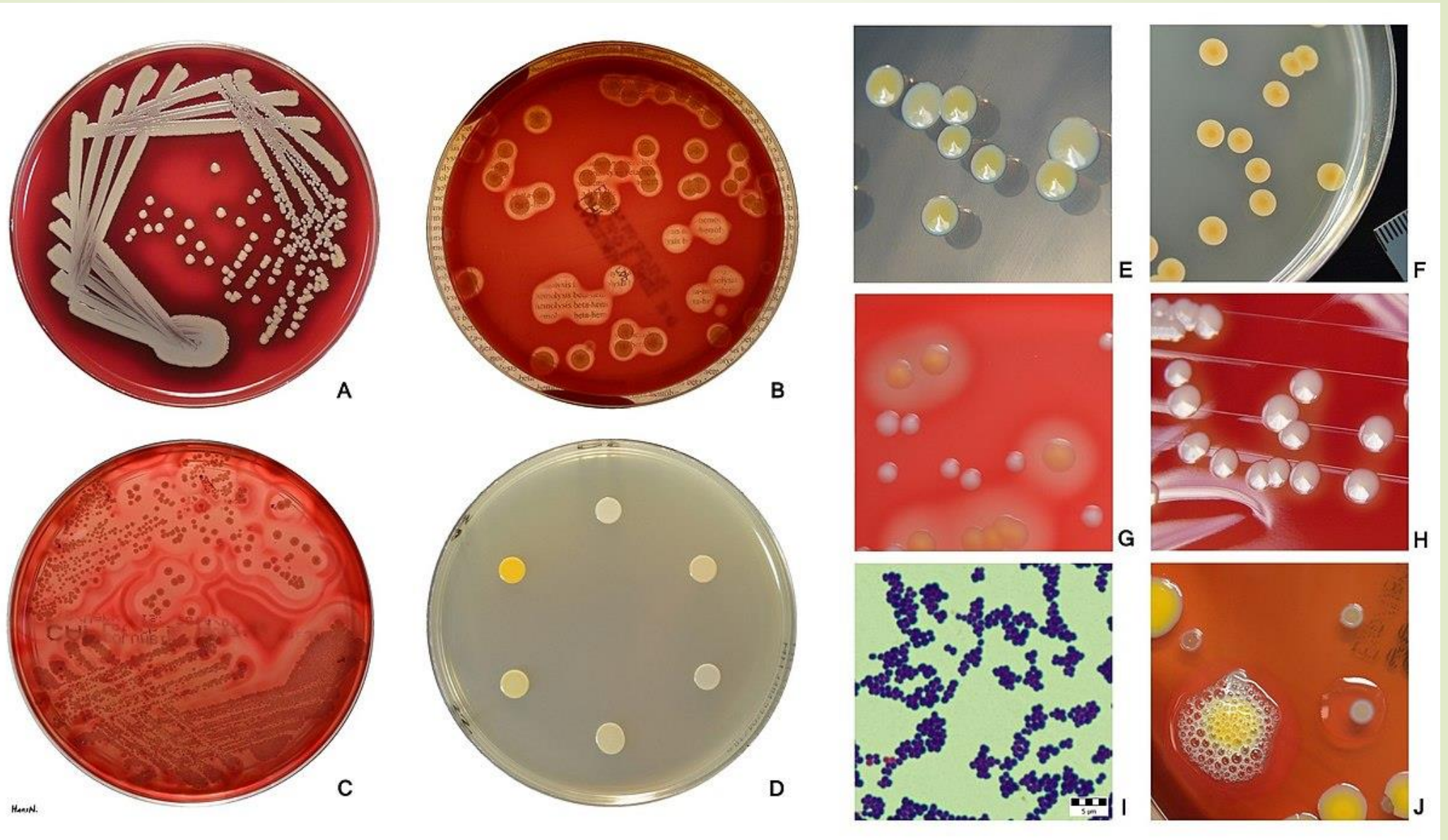
	Staph. aureus	Staph. Epidermidis	Staph. saprophyticus	Micrococcus
Colony Morphology	Opaque, smooth, raised, entire, white-golden(cream)	Opaque, smooth, raised, entire, gray-white	Opaque, smooth, raised, entire, butyrous, glossy, white-yellow	Opaque, smooth, raised, white, bright yellow
Hemolysis	Most are beta hemolytic	Non-hemolytic	Non-hemolytic	Non-hemolytic
Gram morphology	GPC in clusters, pairs, short chains or singly	GPC in clusters, pairs, short chains or singly	GPC in clusters, pairs, short chains or singly	GPC in pairs and tetrads
Catalase	Pos	Pos	Pos	Pos
Glucose fermentation	Fermenter	Fermenter	Fermenter	Oxidizer
Modified Oxidase	Neg	Neg	Neg	Pos
Coagulase Production (tube)	Pos	Neg	Neg	N/A
Clumping factor (slide or latex Coagulase test)	Pos	Neg	Neg	Neg

Biochemical tests	Results		
	<i>S.aureus</i>	<i>S.chromogenes</i>	<i>S.epidermidis</i>
Gram stain	Positive	Positive	Positive
Motility	Negative	Negative	Negative
Oxidase	Negative	Negative	Negative
Catalase	Positive	Positive	Positive
Growth on high salt containing media	Positive	Positive	Positive
Nitrat Reduction	Positive	Positive	Positive (weak)
Acetoin production	Positive	Negative	Positive (50-80%)
Mannitol fermentation	Positive	Variable (Positive 11-89%)	Negative
Growth on BPA	Black shiny colonies surrounded by zone of clearing	Not shiny orange-brown may produce clearing	Not shiny black and seldom produces clearing
Staphyloxanthin production	Positive	Positive	Negative
DNase	Positive	Negative	Negative
Coagulase & Clumping factor	Positive	Negative	Negative

Rapid diagnostic tests

- This techniques include Real-time PCR which is increasingly being employed in clinical laboratories.





تهیه کننده: سهیلا عباسی



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با سپاس از توجه شما
در سیمین / ایچ پی