

Microbiology Laboratory Aspects in the Diagnosis of Musculoskeletal and Skin Infections

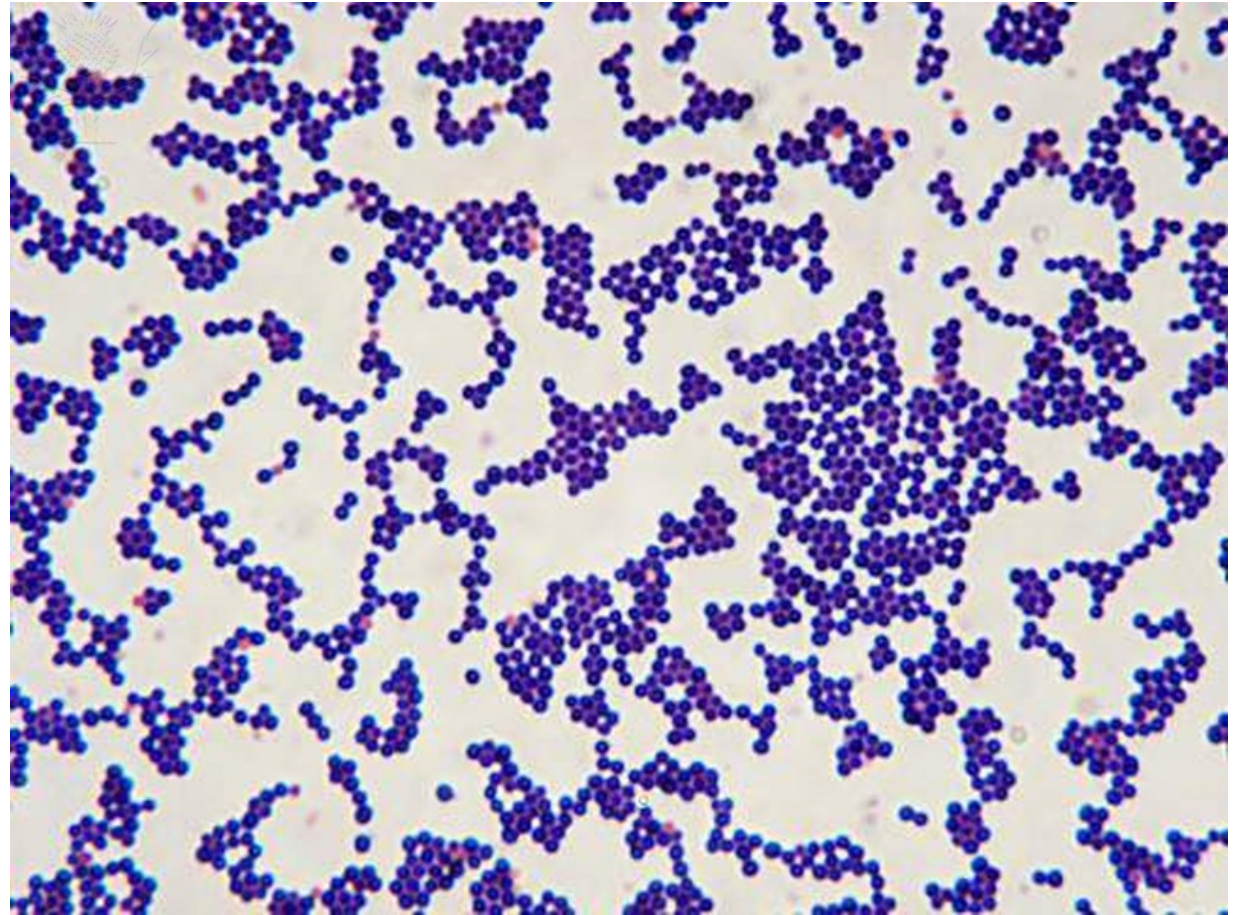
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The University of Jordan

MSS conditions caused by *Staphylococcus aureus*

- Toxic shock syndrome
- Cellulitis
- Folliculitis, furuncles, and carbuncles
- Infectious endocarditis
- Impetigo
- Scalded skin syndrome
- Burn and wound infections including surgical site infections (SSIs)
- Infective endocarditis
- Osteomyelitis
- Septic arthritis

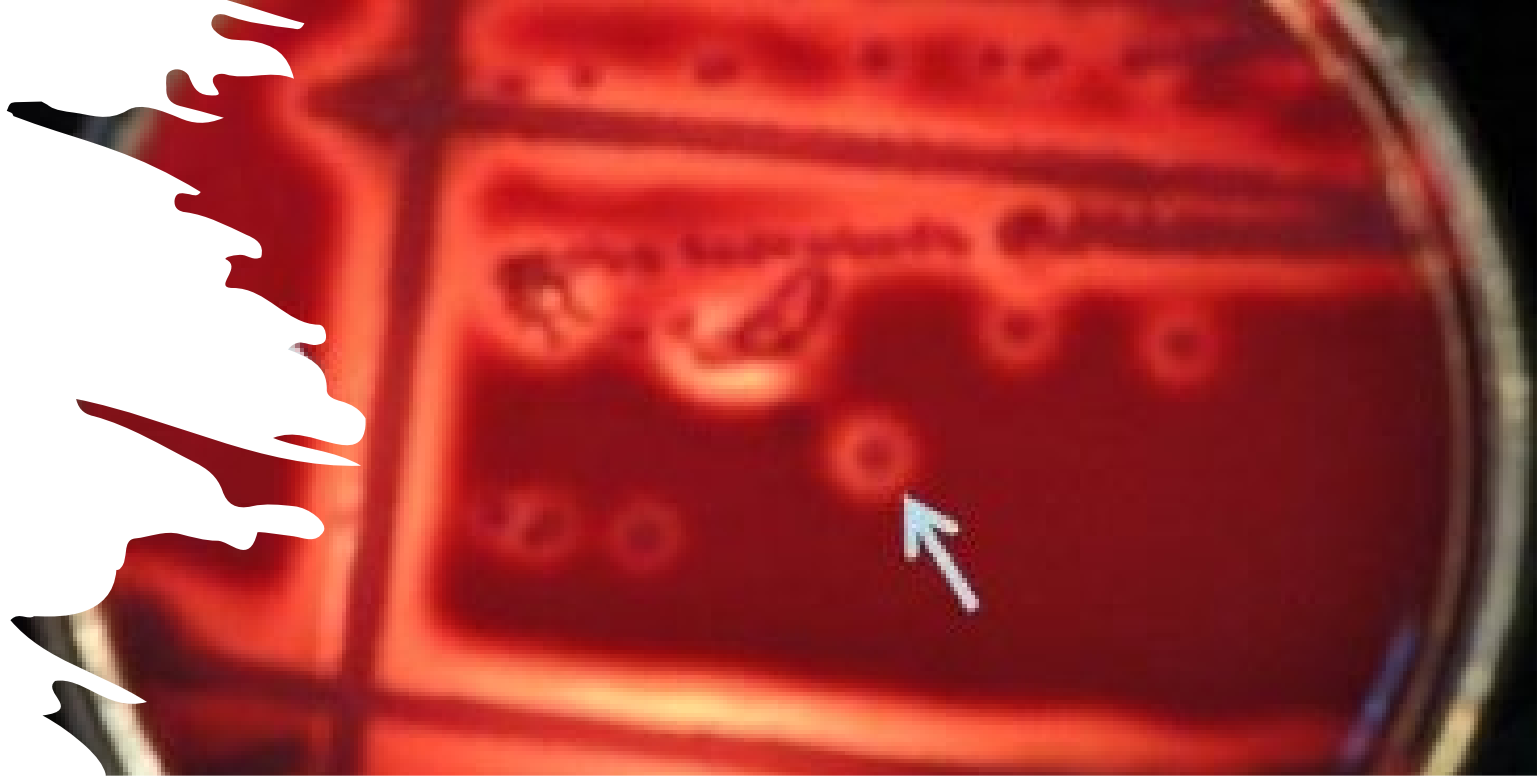


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Laboratory identification of *Staphylococcus aureus*

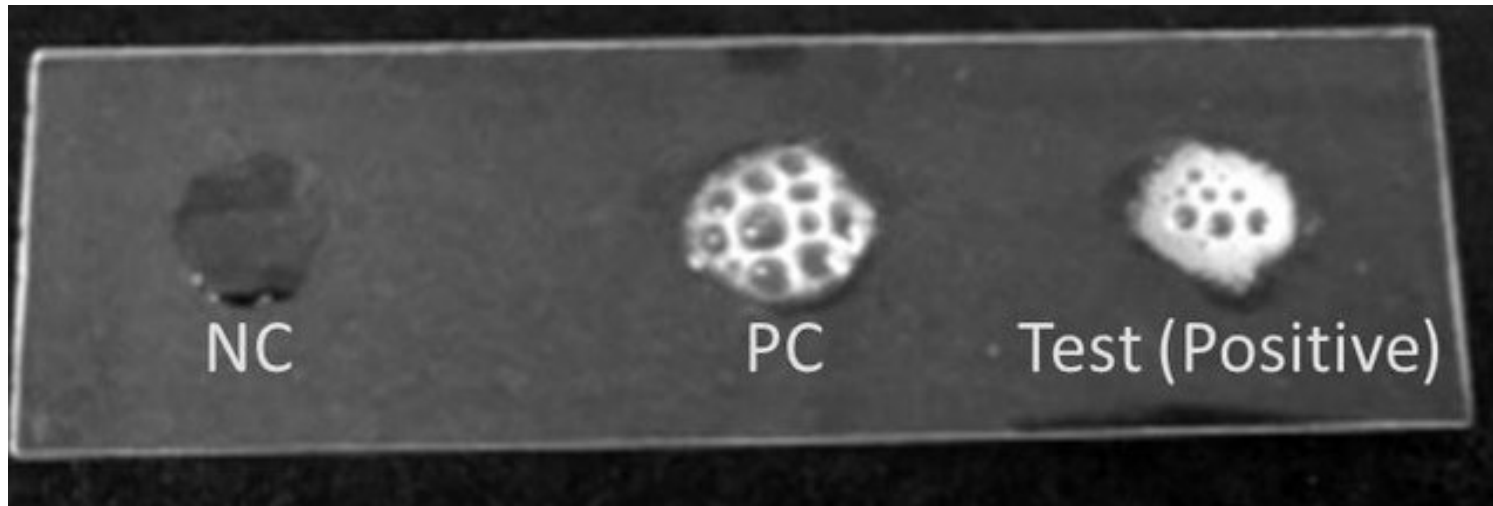
- *S. aureus* produces **beta hemolysis** on sheep blood agar plates and is usually **golden yellow** in color. Gram staining reveals Gram-positive cocci in clusters.
- The first test to differentiate staphylococci from other Gram-positive cocci is the **positive catalase test**
- To differentiate *S. aureus* from coagulase negative staphylococci (CoNS) the following tests are used:
 - **Positive tube coagulase test**
 - **Positive slide coagulase test**
 - **Fermentation of mannitol in 10% mannitol salt agar**

Staphylococcus aureus
yellow colonies on 5%
sheep blood agar
showing beta hemolysis
(complete lysis)



Credit:
<https://www.jfmed.uniba.sk/fileadmin/jlf/Pracoviska/ustav-mikrobiologie-a-imunologie/VLa/STAPHYLOCOCCI.pdf>

Jahan et al.
<http://dx.doi.org/10.5455/javar.2015.b47>

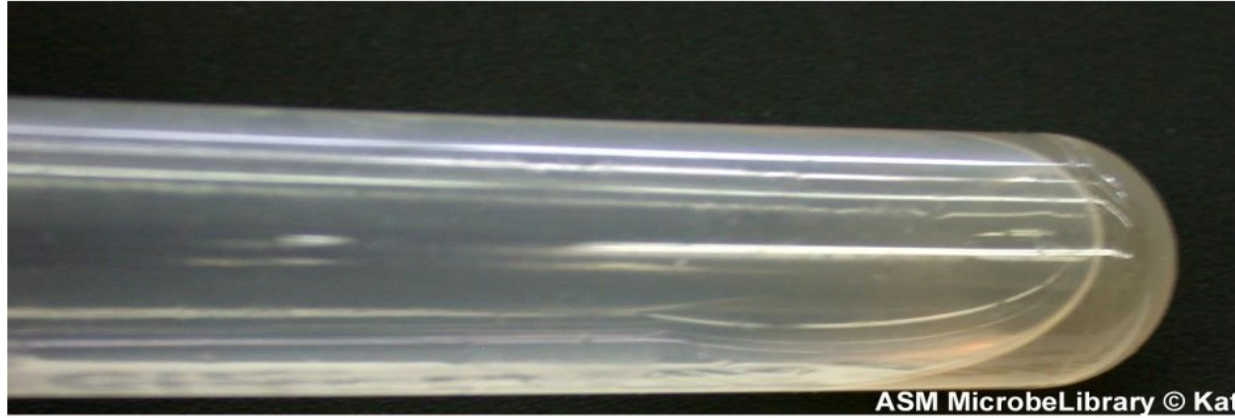


Catalase test

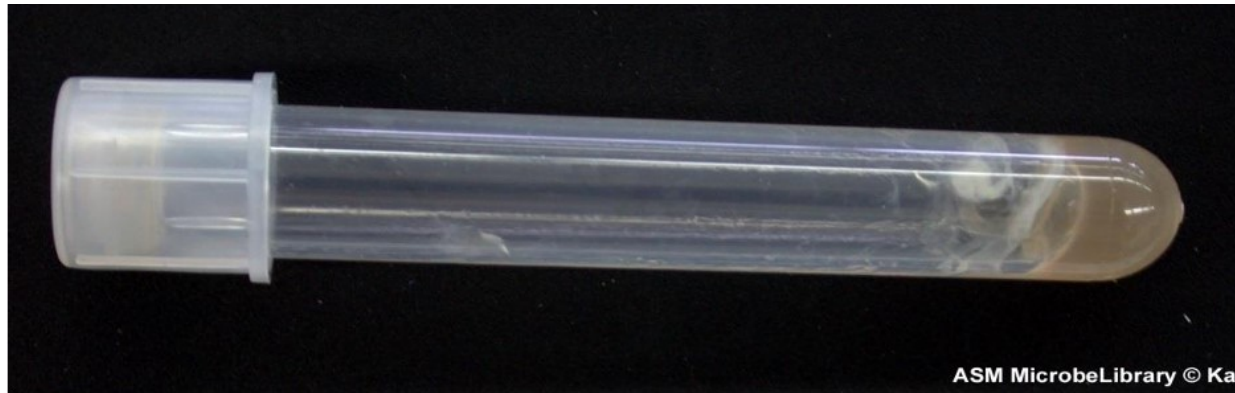


Slide Coagulase test

FIG. 1. Slide test. Coagulase-negative staphylococci are present on the left side of the slide, while coagulase-positive staphylococci are present on the right side of the slide.



A



B

FIG. 3. (A) A negative tube coagulase test reaction indicating coagulase-negative cells. (B) A positive tube coagulase test reaction indicating coagulase-positive cells.

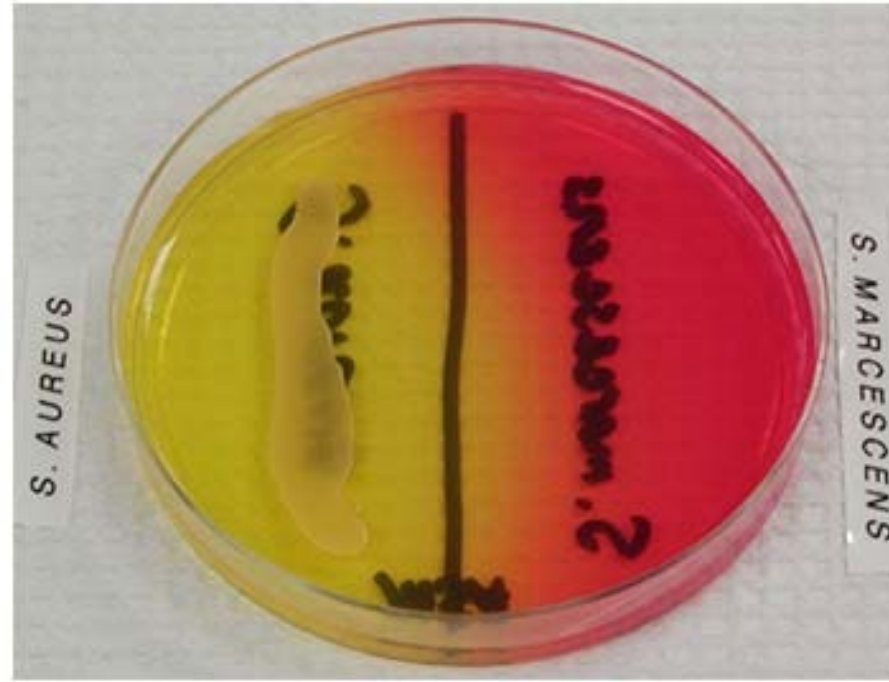
Credit: <https://asm.org/protocols/coagulase-test-protocol>

Tube Coagulase test

Mannitol Salt Agar (MSA) for the isolation of *Staphylococcus aureus*



Yellow colonies of *Staphylococcus aureus*

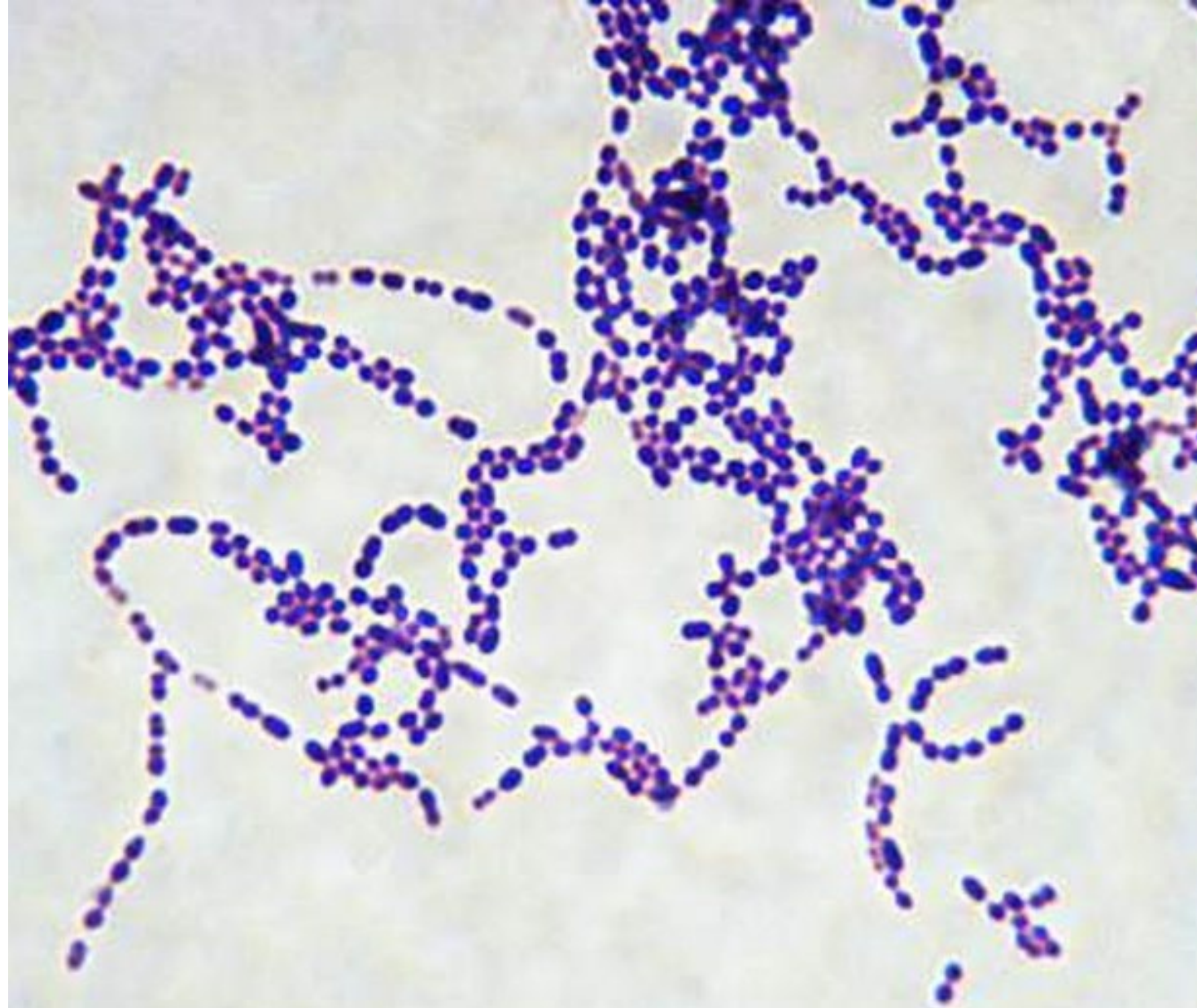


Staphylococcus aureus and *Serratia marcescens* on MSA

**Growth on
MSA**

MSS conditions caused by *Streptococcus pyogenes*

- Scarlet fever
- Toxic shock syndrome
- Cellulitis
- Erysipelas
- Subcutaneous necrotizing infections

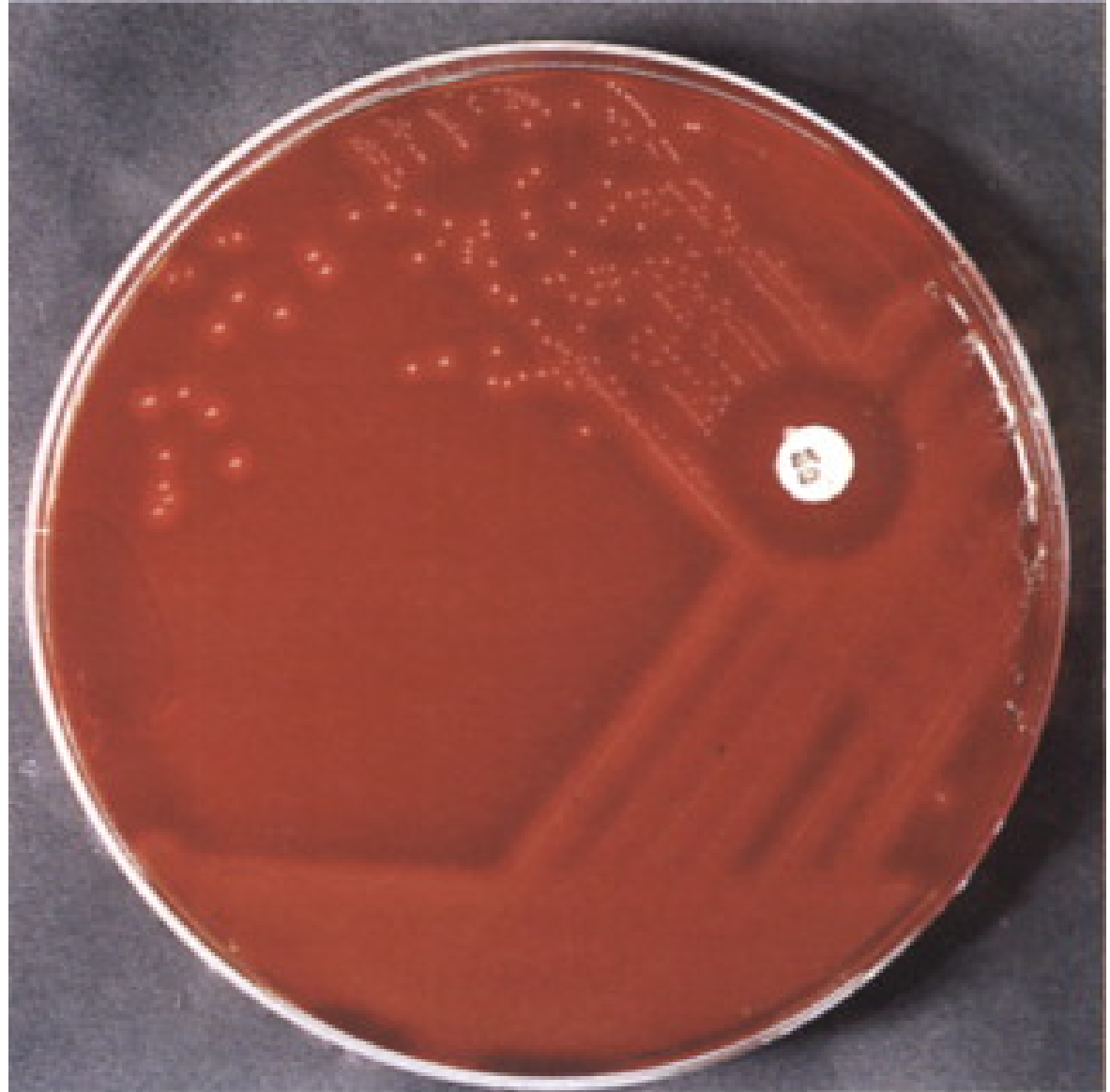


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Laboratory identification of *Streptococcus pyogenes*

- *S. pyogenes* produces **beta hemolysis** on sheep blood agar plates. Gram staining reveals **Gram-positive cocci** in chains.
- The first test to differentiate streptococci from other Gram-positive cocci is the **negative catalase test**
- To differentiate *S. pyogenes* from other beta hemolytic streptococci:
 - **Bacitracin sensitivity compared to bacitracin resistance for group B**
 - **Positive Lancefield group A antigen using latex agglutination test**

**Bacitracin
sensitivity and
beta hemolysis of
*Streptococcus
pyogenes***



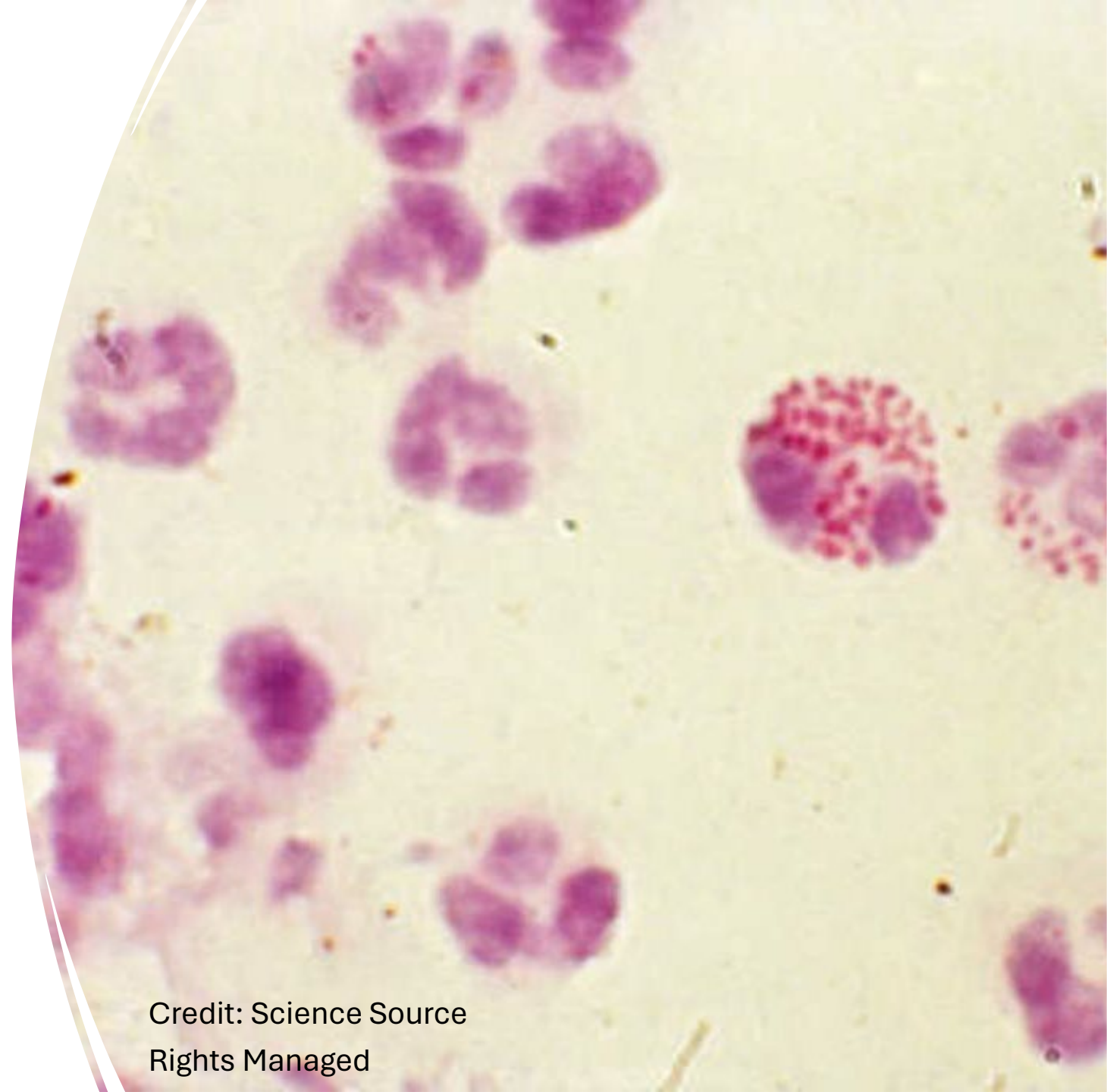
Agglutination tests for identification of β -hemolytic streptococci



Credit: <https://www.bio-rad.com/en-jo/product/pastorex-strep?ID=OT2MTO7OP/>

MSS conditions caused by *Neisseria gonorrhoea*

- Gonococcal septic arthritis
- Gonococemia



Credit: Science Source
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Laboratory identification of *Neisseria gonorrhoea*

- Identification of *N. gonorrhoeae* depends on the isolation of an oxidase-positive, **Gram-negative** diplococci grown on non-selective chocolate agar and on selective media such as Thayer Martin agar.
- The inoculated plates should be incubated at 35°C to 37°C in a moist atmosphere enriched with CO₂.

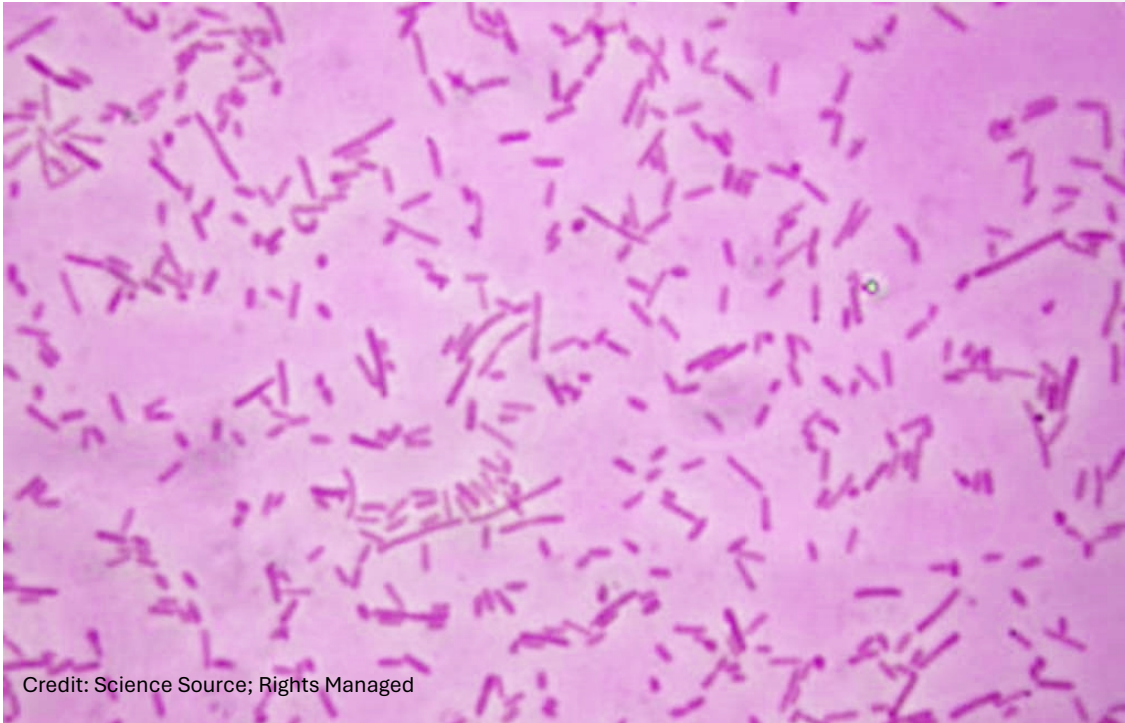
Cervical Specimen Thayer-Martin Medium



Positive Oxidase Test

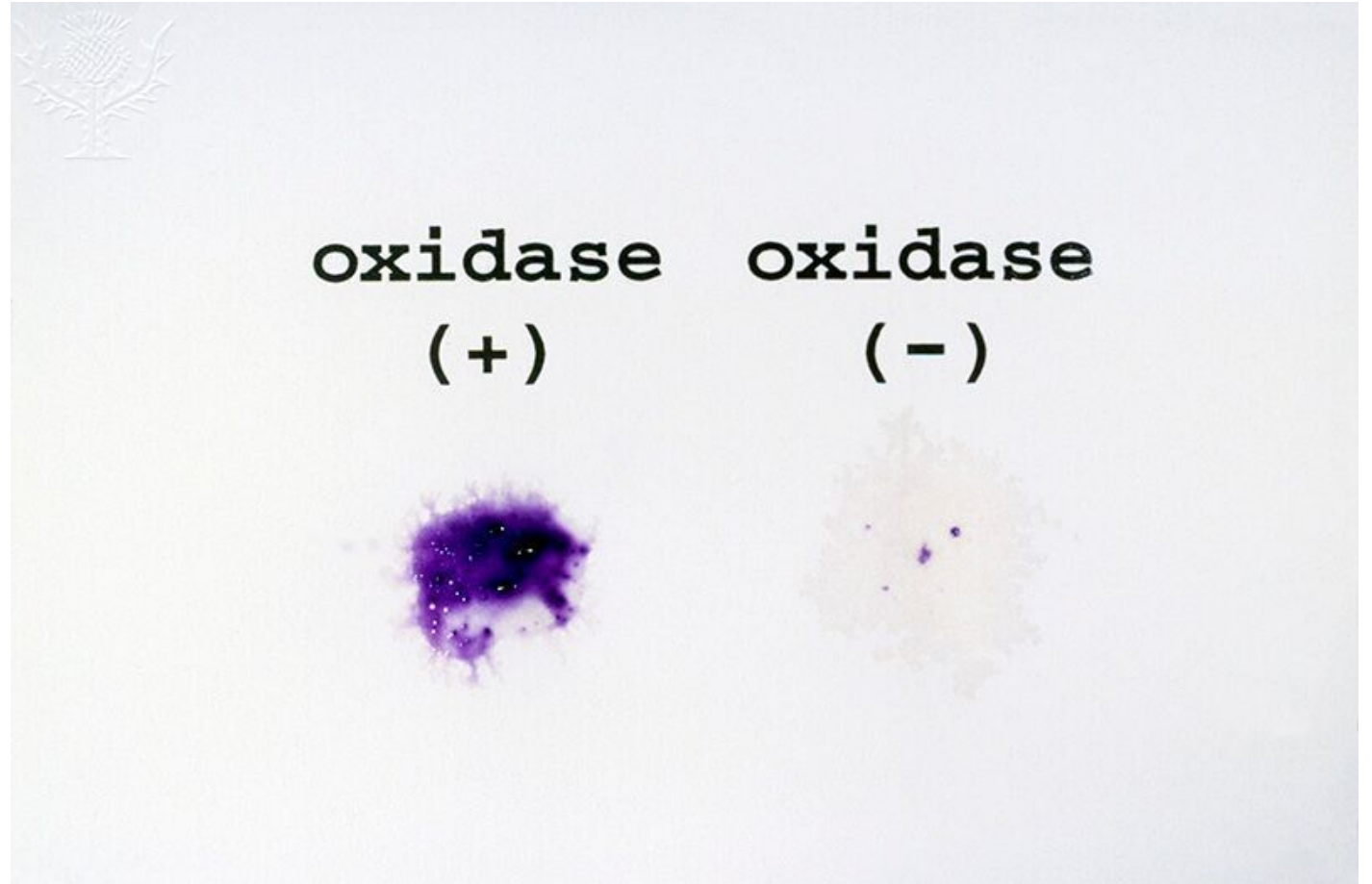
MSS conditions caused by *Pseudomonas aeruginosa*

Cellulitis
Hot-tub folliculitis
Burn and wound infections
Ecthyma gangrenosum
Infectious endocarditis



Laboratory identification of *P. aeruginosa*

- *P. aeruginosa* grows well on most laboratory media and commonly is isolated on blood agar plates or MacConkey agar or CLED agar.
- *P. aeruginosa* is identified based on its **Gram-negative** rod morphology, inability to ferment lactose or glucose, a positive oxidase reaction, its fruity odor, and the ability to produce green or blue or yellow pigments.



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Laboratory diagnosis of Infectious Mononucleosis

Monospot test

IgM + Sheep RBCs = Agglutination



Test is no longer recommended today

Sensitivity is only 75% in the first week but increases to 90% afterwards

Both false positives (cancer, early HIV, autoimmune disorders) and false negatives (young age) are common

STATPEARLS

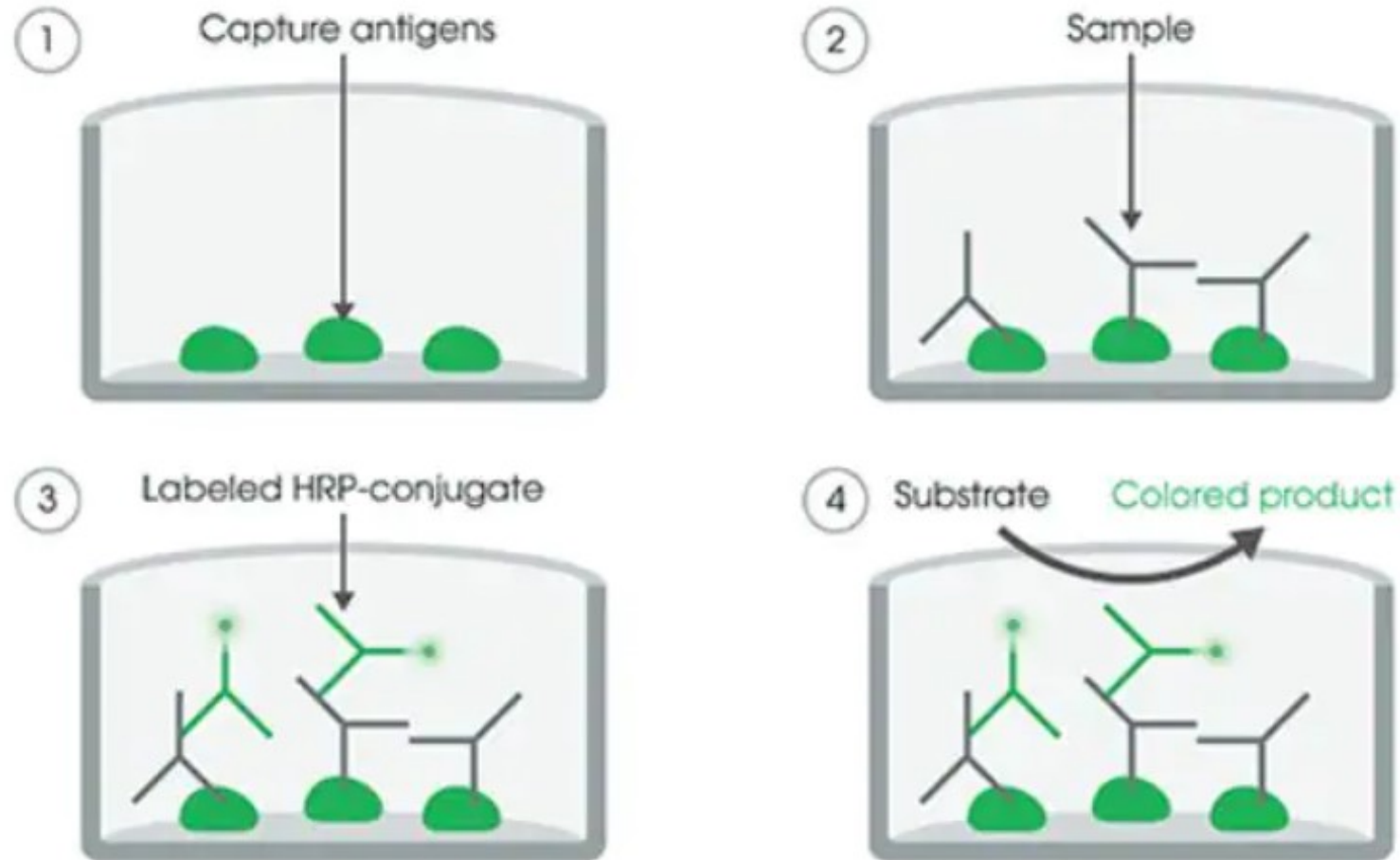
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Laboratory diagnosis of Infectious Mononucleosis

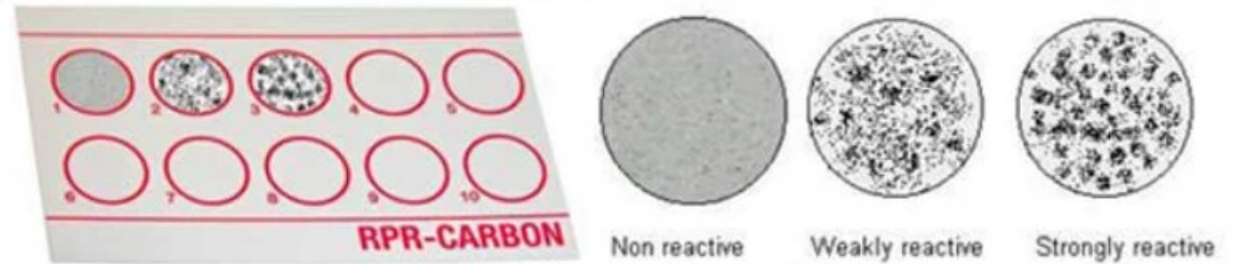
ELISA for VCA antibody



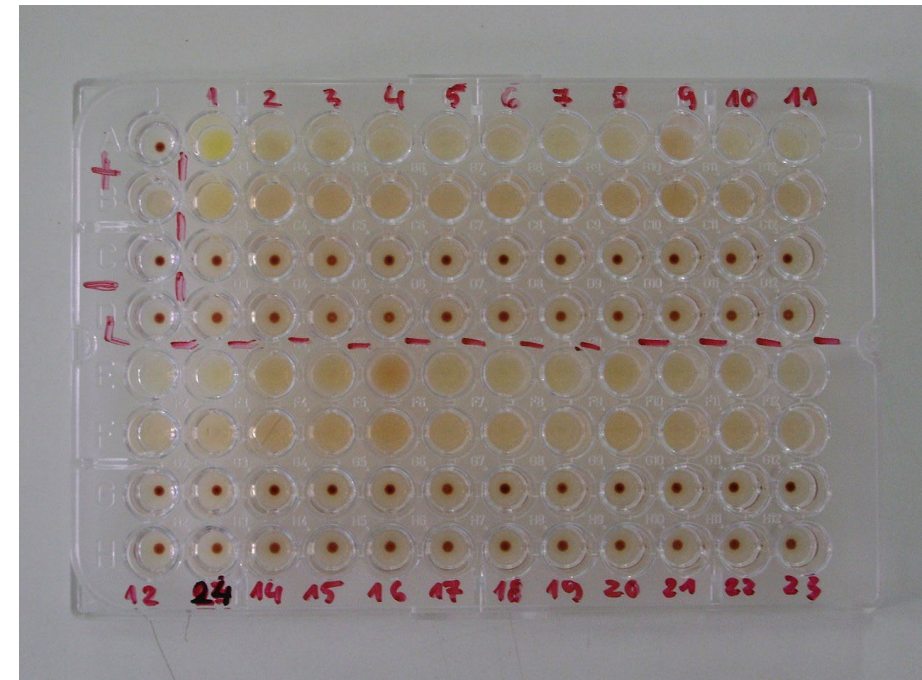
Secondary Syphilis Diagnosis

- Screening by non-treponemal serologic tests including the venereal diseases research laboratory (VDRL) and rapid plasma reagin (RPR) tests.
- Confirmatory or treponemal tests include the fluorescent treponemal antibody absorption (FTA-ABS), and microhemagglutination assay for *T. pallidum* (MHA-TP).

Rapid Plasma Reagin (RPR) Test for the diagnosis of Syphilis



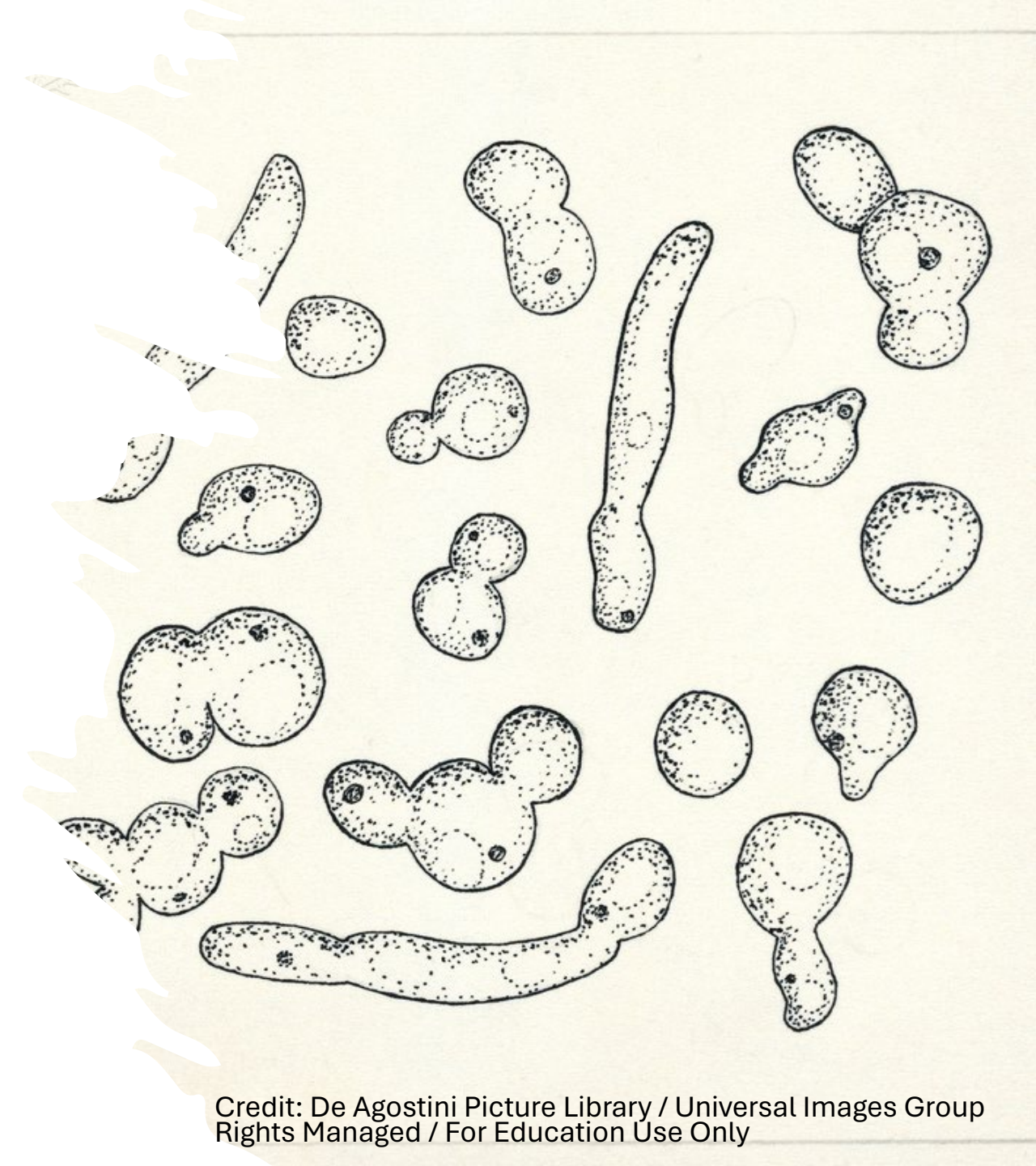
Credit: <https://microbiologyinfo.com/rapid-plasma-reagin-rpr-test-for-the-diagnosis-of-syphilis/>



Credit: dr. David Csaba Levente

Cutaneous candidiasis

- *Candida* species grow readily in cultures and results are usually available in 48–72 hours usually producing white creamy colonies.
- Wet preparation reveal budding yeast.



Cutaneous candidiasis

- The 10% KOH (potassium hydroxide) procedure is a method used to examine specimens for yeast.
- KOH serves as an enzymatic agent that breaks down debris in a specimen, such as epithelial cells and WBCs, to view yeast or pseudohyphae.



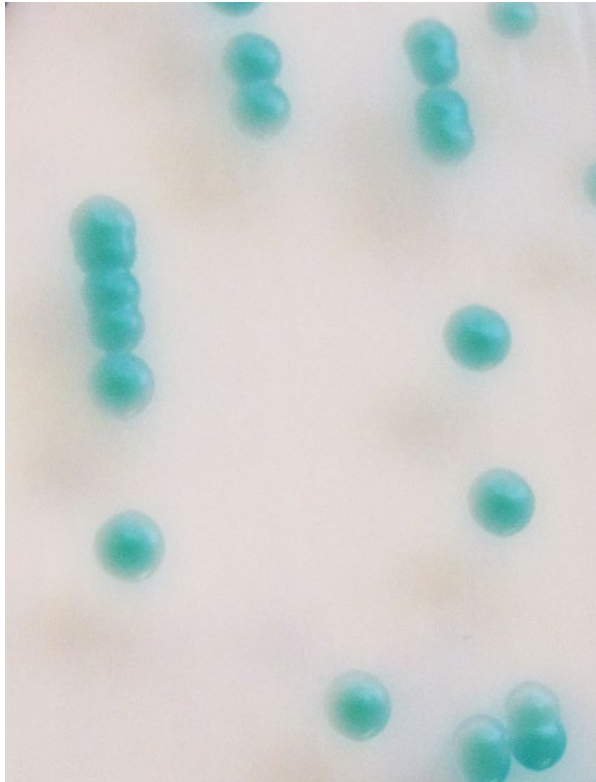
Species identification of *Candida*

- Species identification of *Candida* is required for all infections because of the variable susceptibility to antifungal drugs that is species specific.
- CHROMagar, a culture media utilized to rapidly identify many common *Candida* species, employs a colorimetric reaction on special agar that allows distinction among *C. albicans*, *C. glabrata*, *C. krusei*, *C. tropicalis*, and several other non-*albicans* *Candida* species.



Species identification of *Candida*

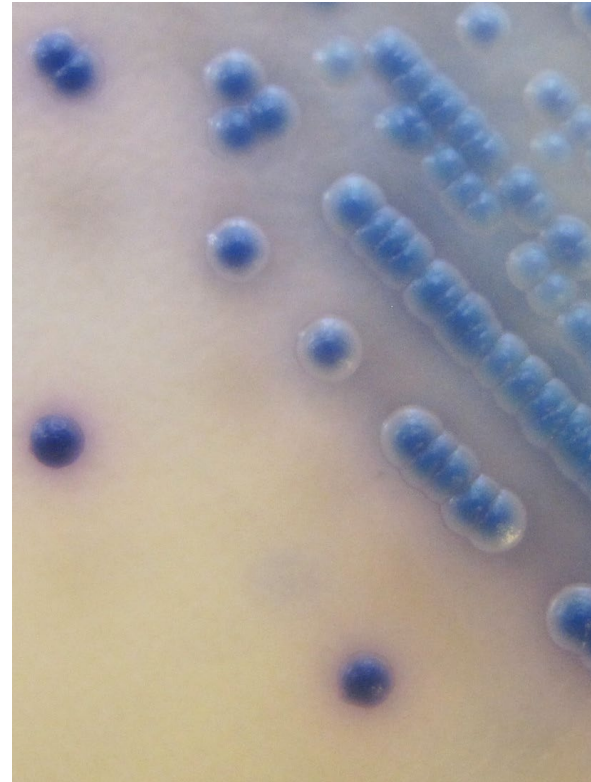
Candida albicans
green



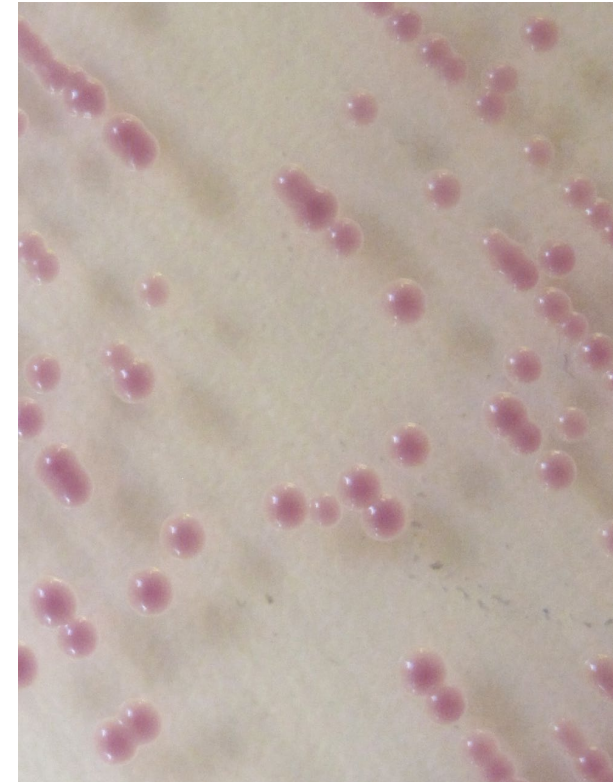
Candida krusei
pink, fuzzy



Candida tropicalis
metallic blue

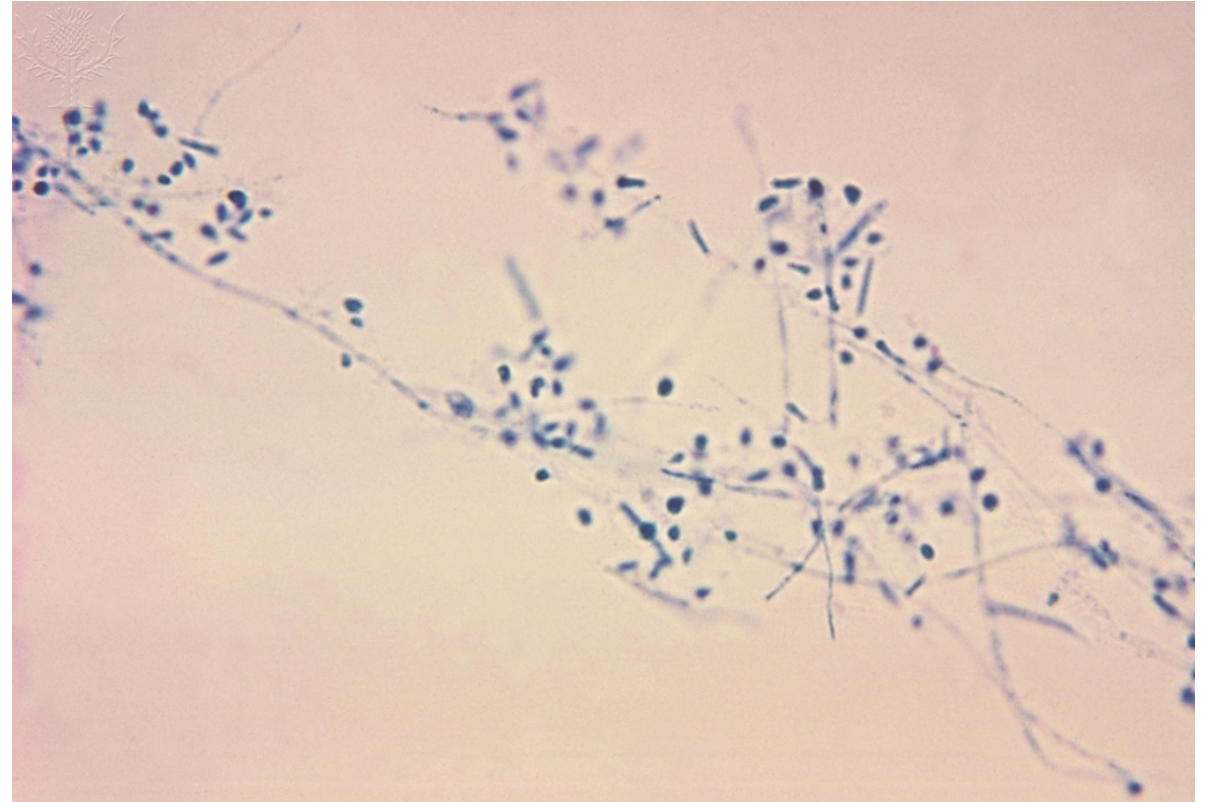


Candida glabrata
mauve



Tinea (Dermatophytoses)

- To isolate the causative agents of dermatophytoses, all specimens should be cultured on Sabouraud dextrose agar (SDA) supplemented with gentamicin and incubated for at least 2 weeks before ruling out dermatophytoses.
- Lactophenol blue stain is a mounting medium and staining agent used in the preparation of slides for microscopic examination of fungi.



Epidermophyton floccosum



Credit: Science Photo Library; Rights Managed



Credit: Macroconidia of the dermatophytic fungus en:Epidermophyton floccosum. Obtained from the CDC Public Health Image Library. Image credit: CDC/Dr. Libero Ajello (PHIL #4207), 1972.

Microsporium gypseum



Credit: Dr. Michael R. McGinnis, Medical Mycology Research Center, The University of Texas Medical Branch at Galveston, Texas, USA (<http://fungusweb.utmb.edu>).

Thanks for listening!