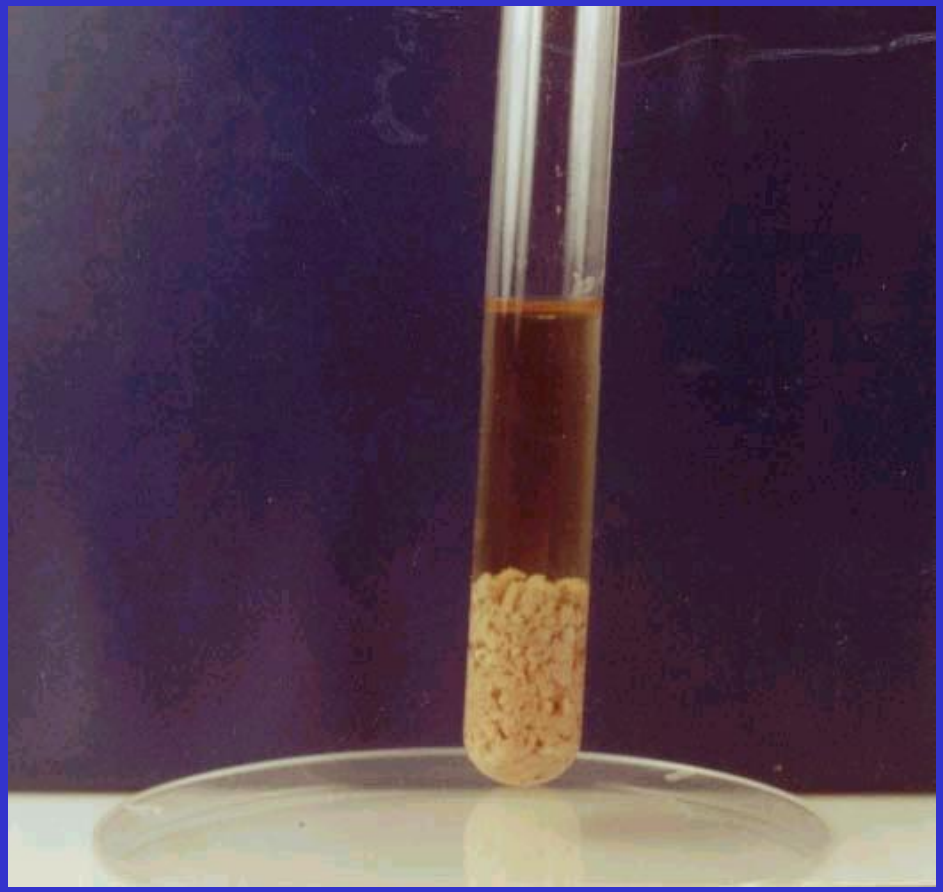
Practical examination

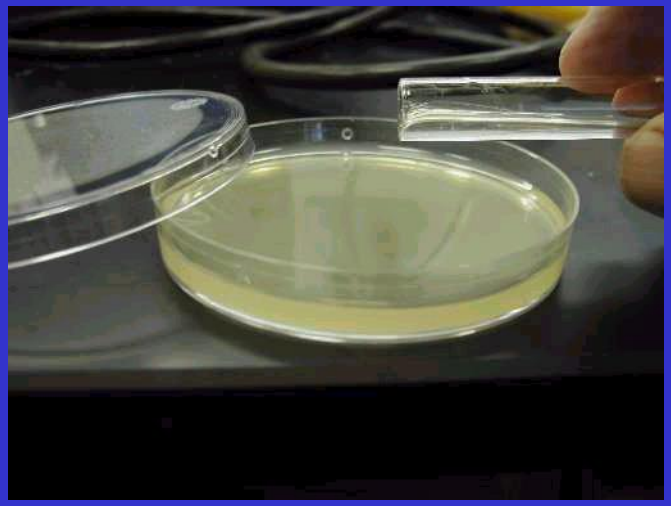
I. Sterile media

1. Bouillon, 2. Slant agar, tube agar

4. Enrichment media: meat bouillon



3., 5., 6.: Agar, blood agar and chocolate agar plates



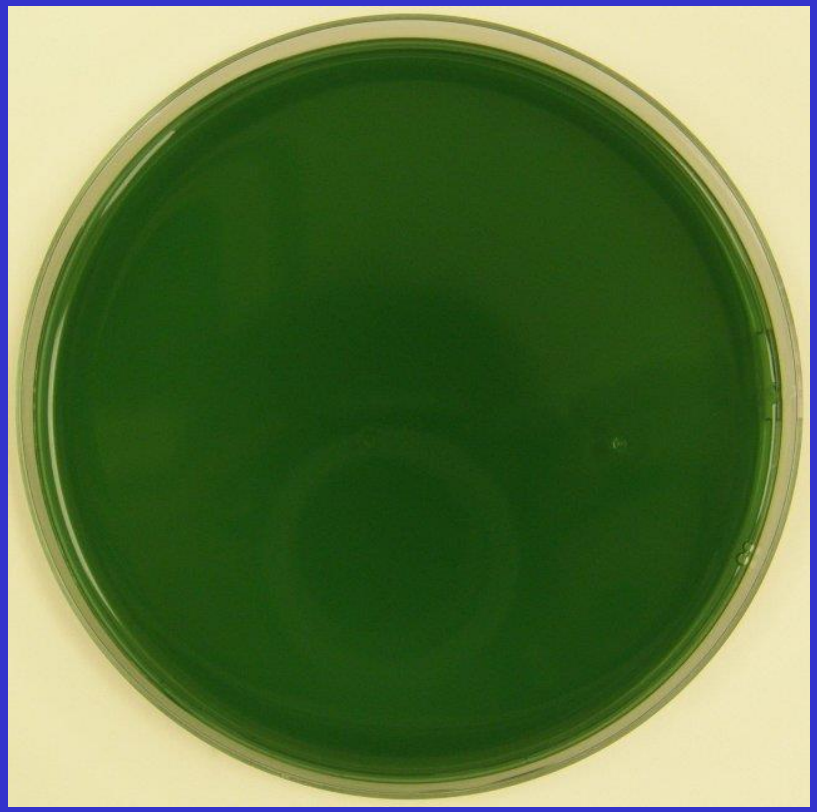
Uninoculated chocolate agar plate

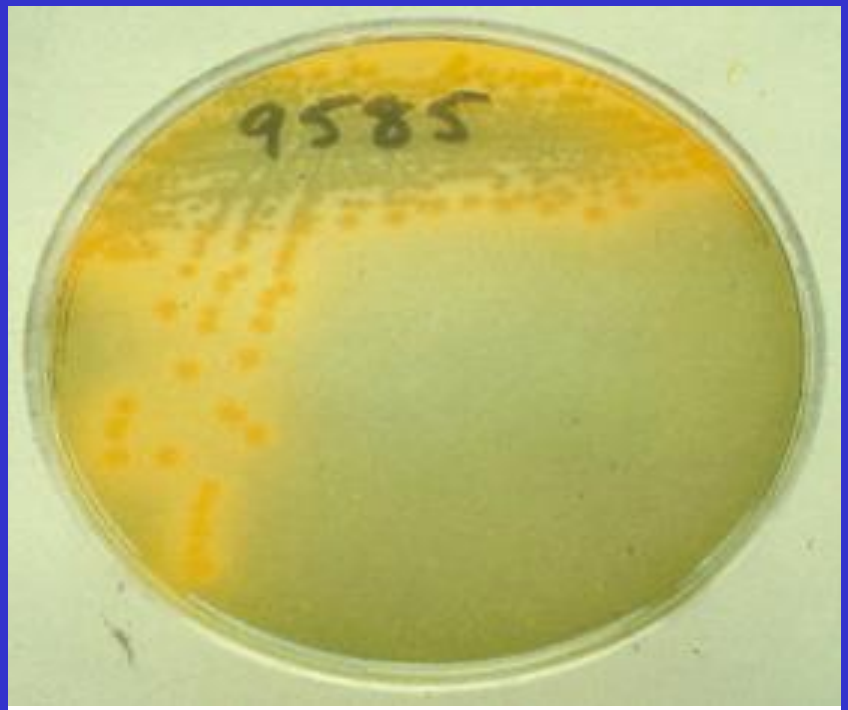
7. Selective and differentiating media
c. Eosin methylene blue (EMB)

Lactose + colonies

Lactose - colonies

7. Selective and differentiating media
g. TCBS





Vibrio cholerae

8. Transport media

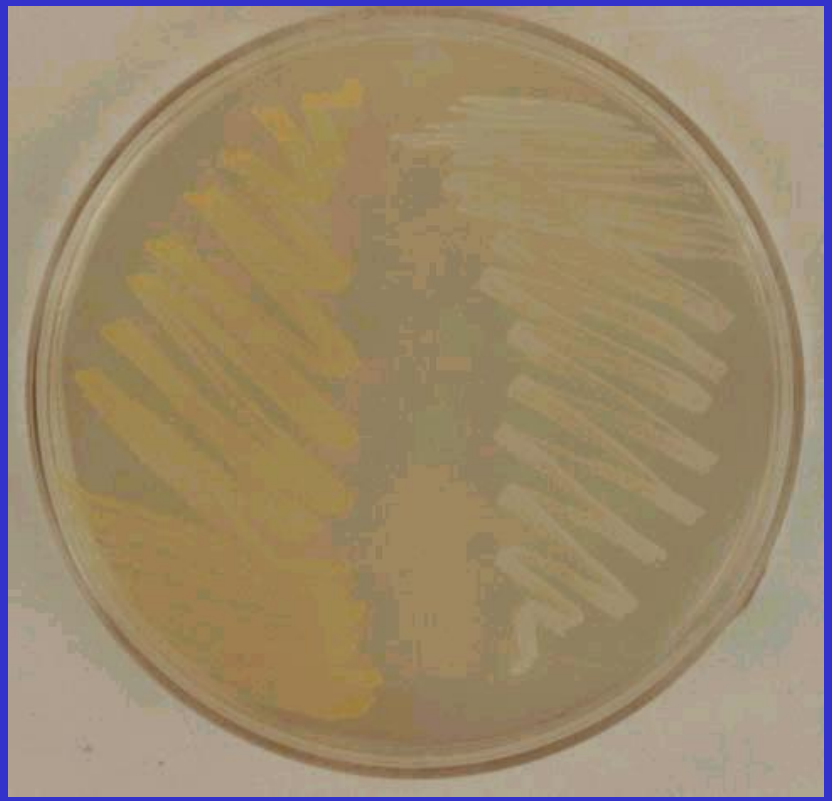
Transport swabs
(e.g. Stuart)

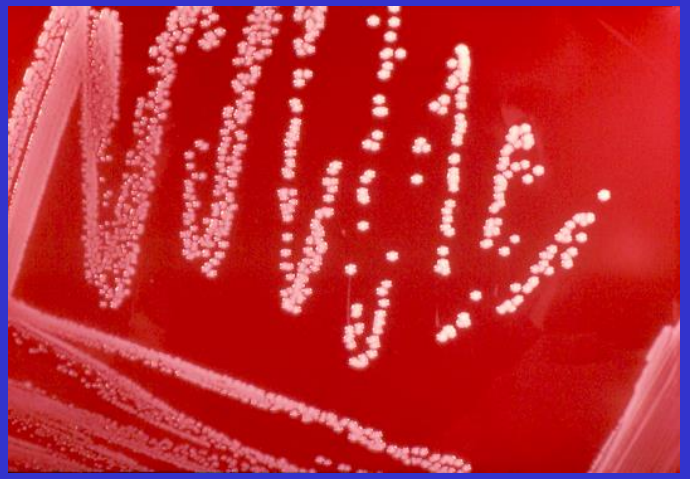
9. Blood culture

10. Virus transport medium

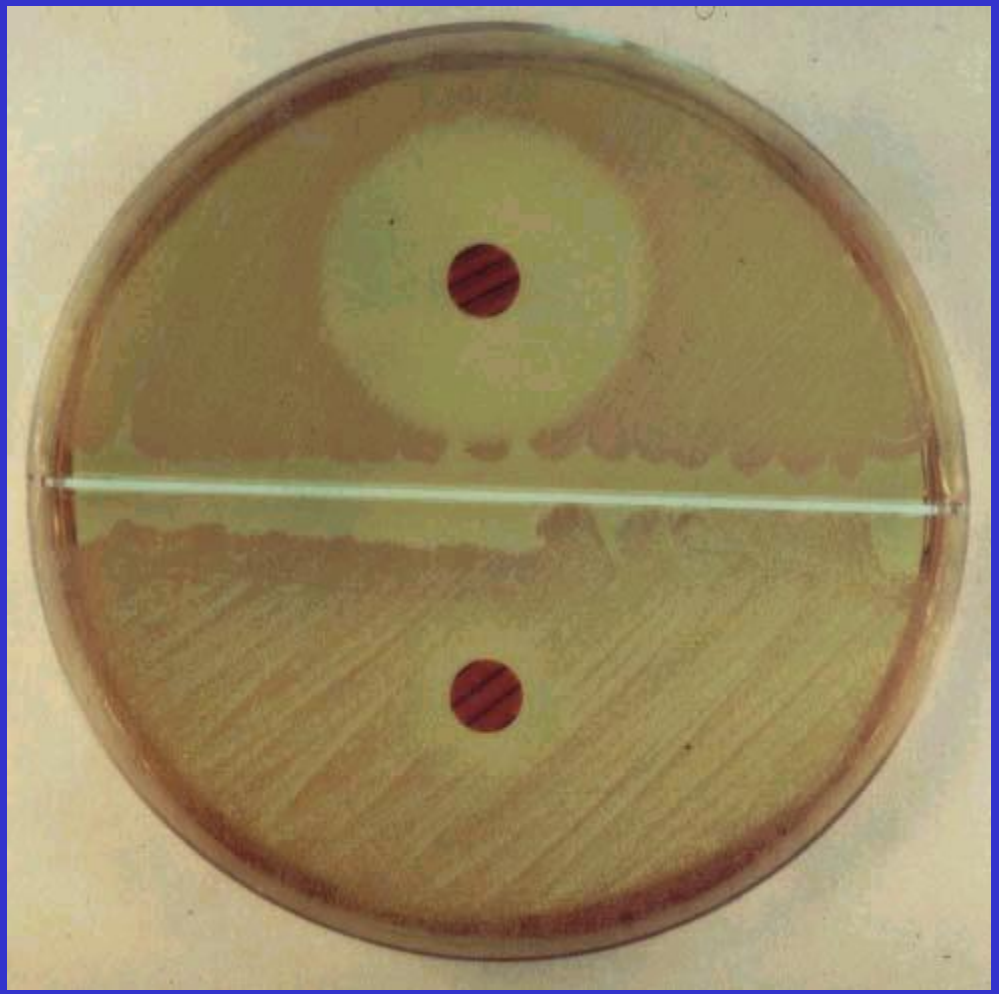
II. Cultures

1-2-3. *Staphylococcus aureus* and *S. epidermidis* on agar plate and blood agar plate





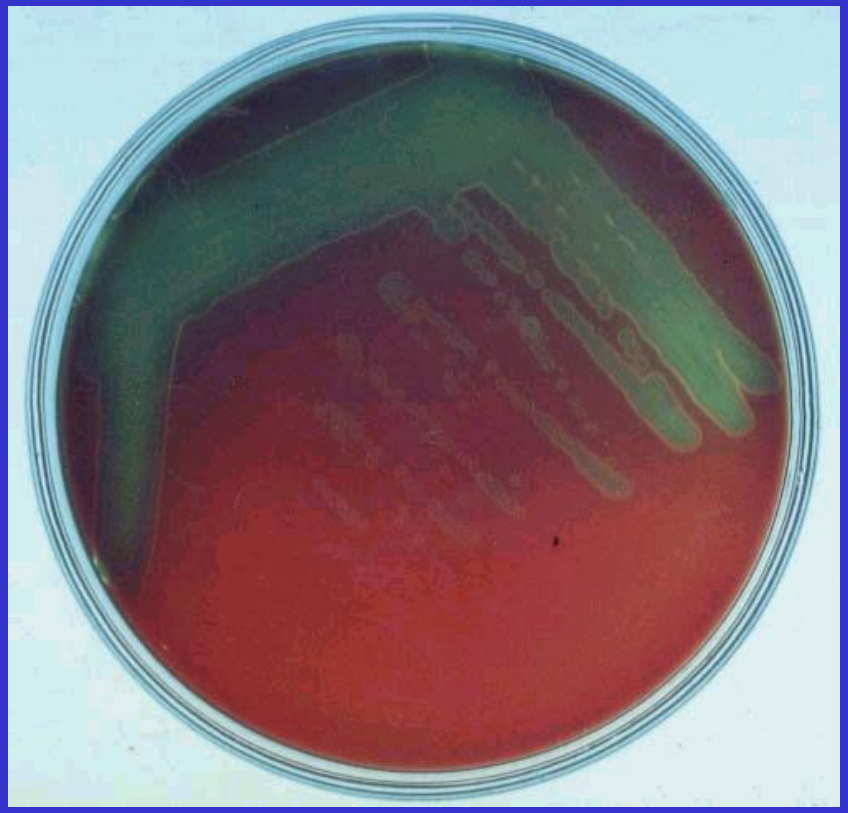
4. *S. epidermidis* (S) and *S. saprophyticus* (R) with novobiocin disc

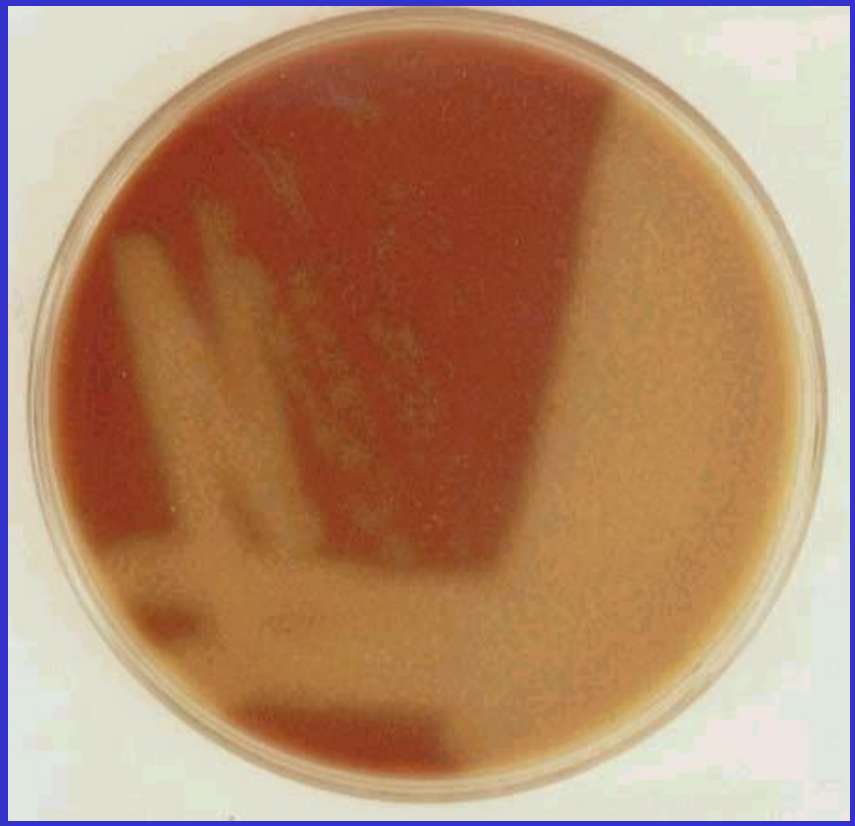


5. *Streptococcus pyogenes* on blood agar

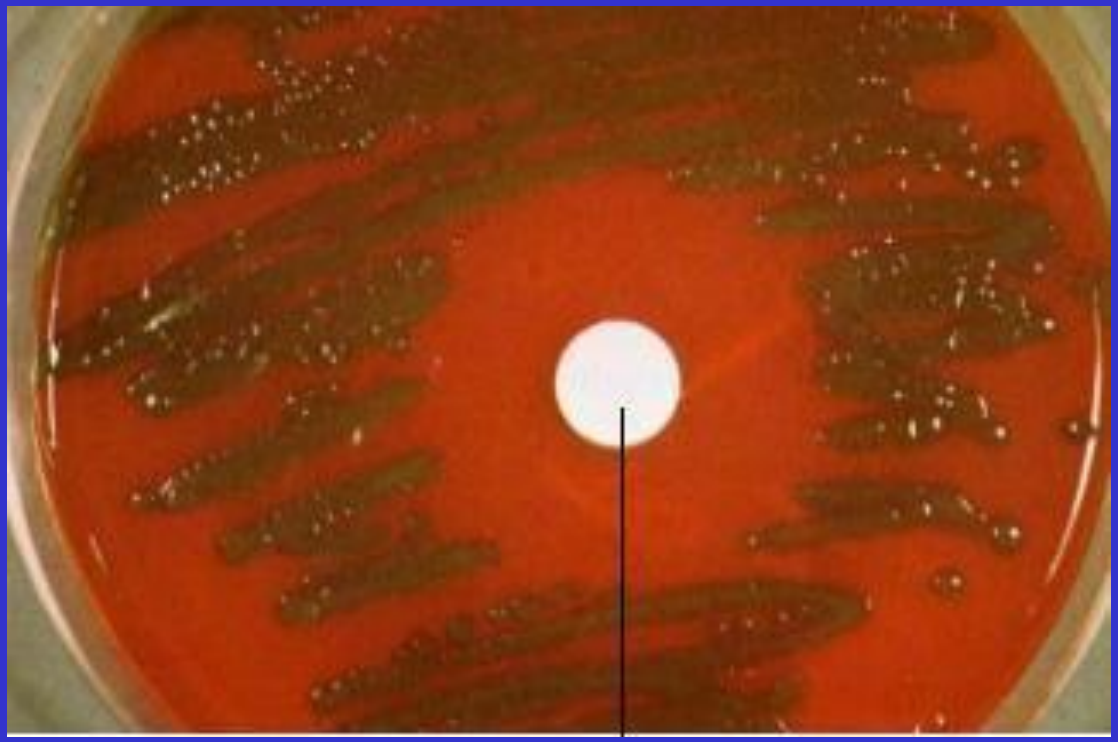
Pinpoint colonies,
strong β-hemolysis!

6. *Str. mitis* on blood and chocolate plates





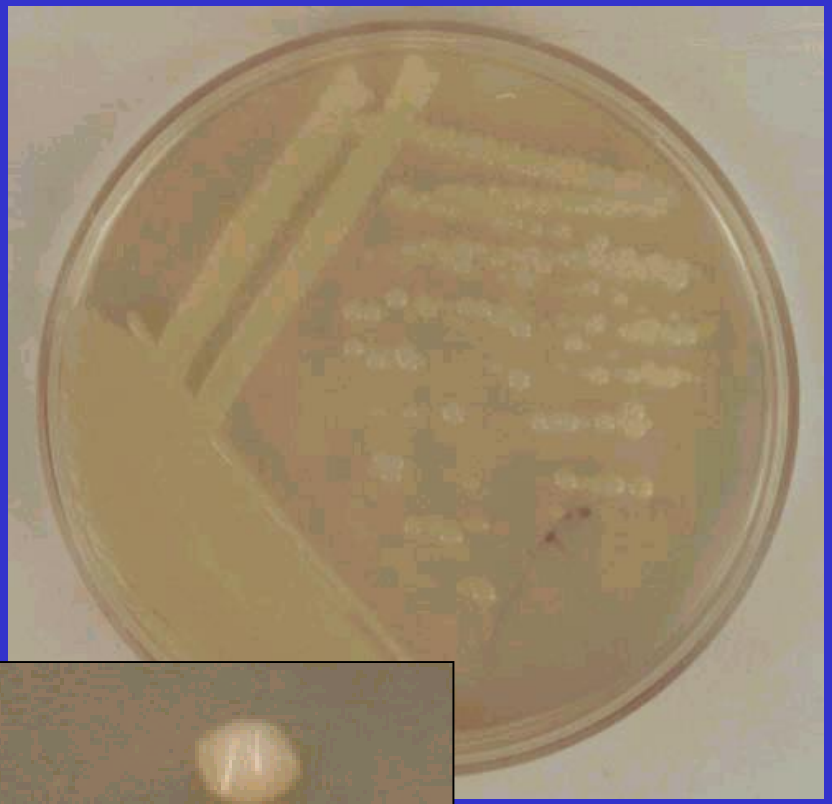
7. *Streptococcus pneumoniae* on blood agar plate (optochin S)



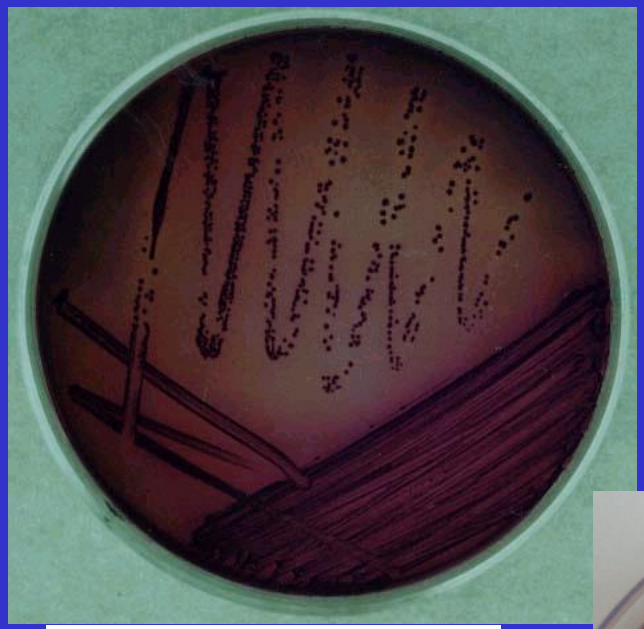
8. *Haemophilus influenzae* on chocolate agar

„Morning dew” colonies,
running nose smell!

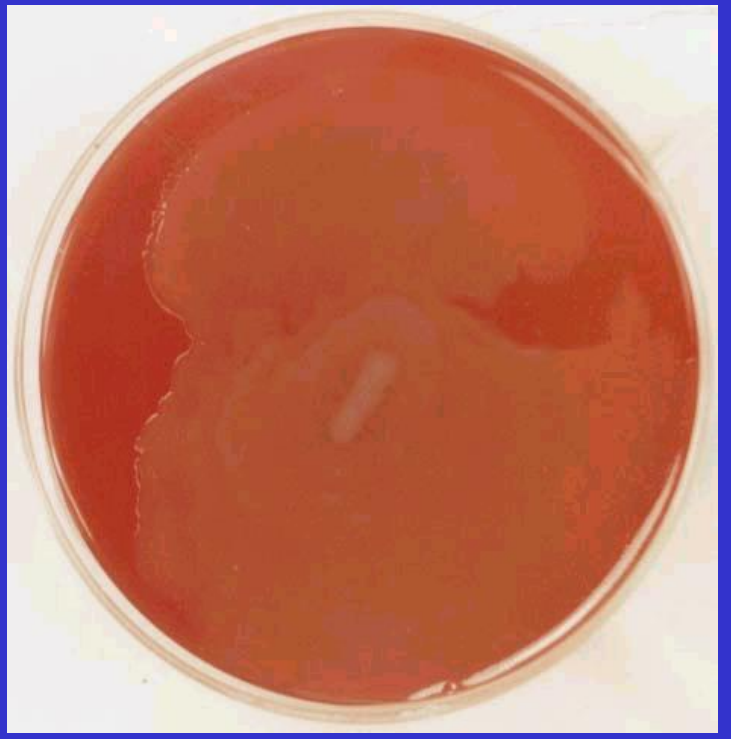
9/12. *E. coli* and *Klebsiella* on agar plate



9/12. *E. coli* and *Klebsiella* on EMB plate

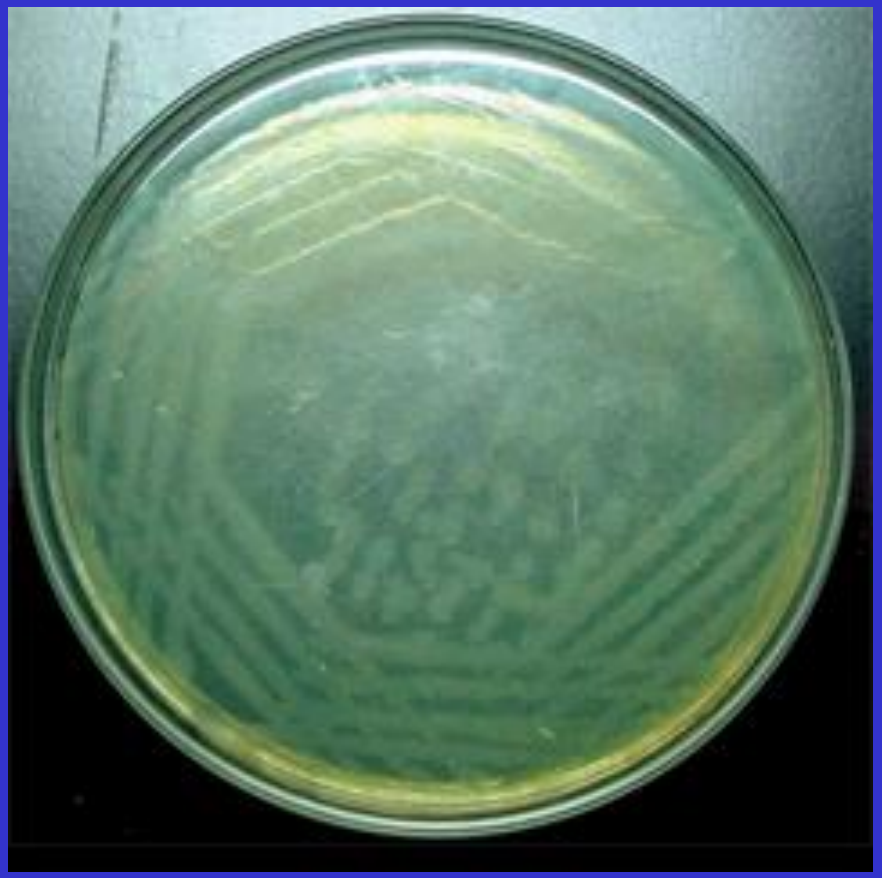


10. *Proteus* on agar and blood agar (swarming!)



11. *Pseudomonas* on agar plate

Pseudomonas aeruginosa



- Green pigment!!!
- Pleasant odour
- Mucoid colonies

13. *Salmonella* on bismuth sulphite medium

14. *E. coli* and *Salmonella* on brillant green medium

15. *E. coli* and *Shigella* on DC medium

16. Faeces of patient with dysentery on DC medium (*E. coli* + *Shigella*)

18. Urease test

- NH_2-CO-NH_2 (ureum) → CO_2 + NH_3 (ammonia)
- Christensen medium: indicator (phenol red)
  - urease +: purple
  - urease -: citric yellow

Urease +: *Proteus, Klebsiella, Helicobacter*

19. *Corynebacterium* on Clauberg and Löffler medium

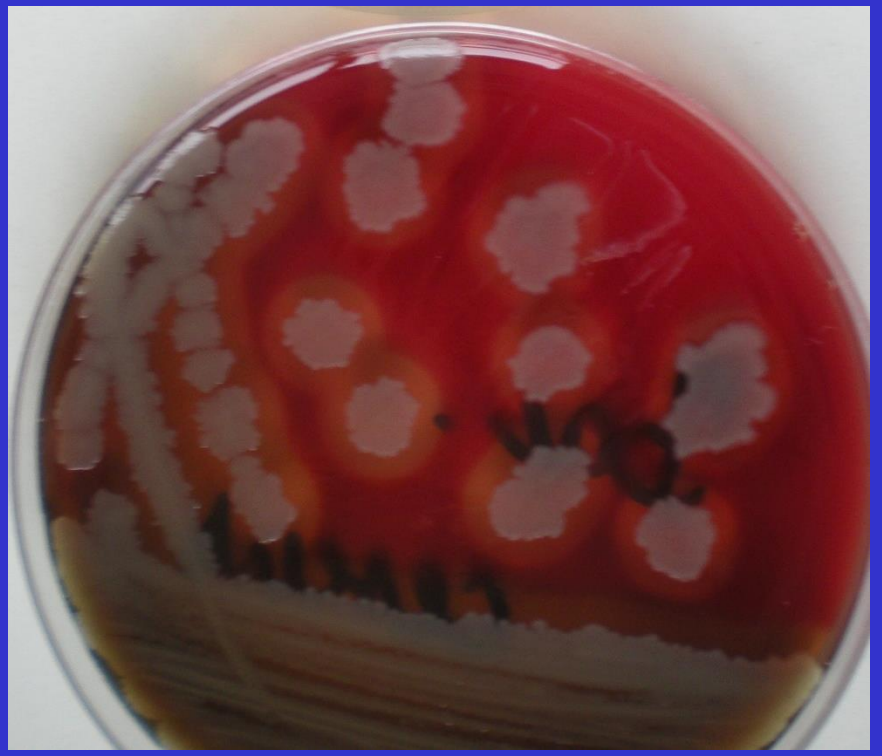
Clauberg

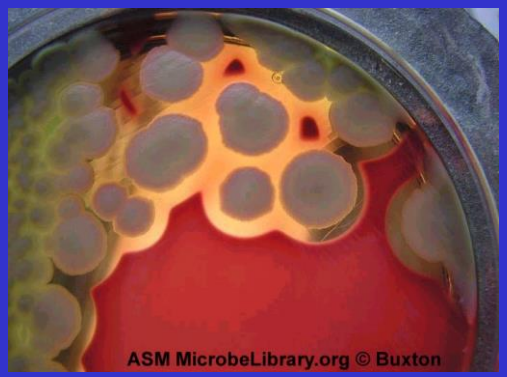
Löffler

20. *Mycobacterium tuberculosis* on Löwenstein-Jensen medium

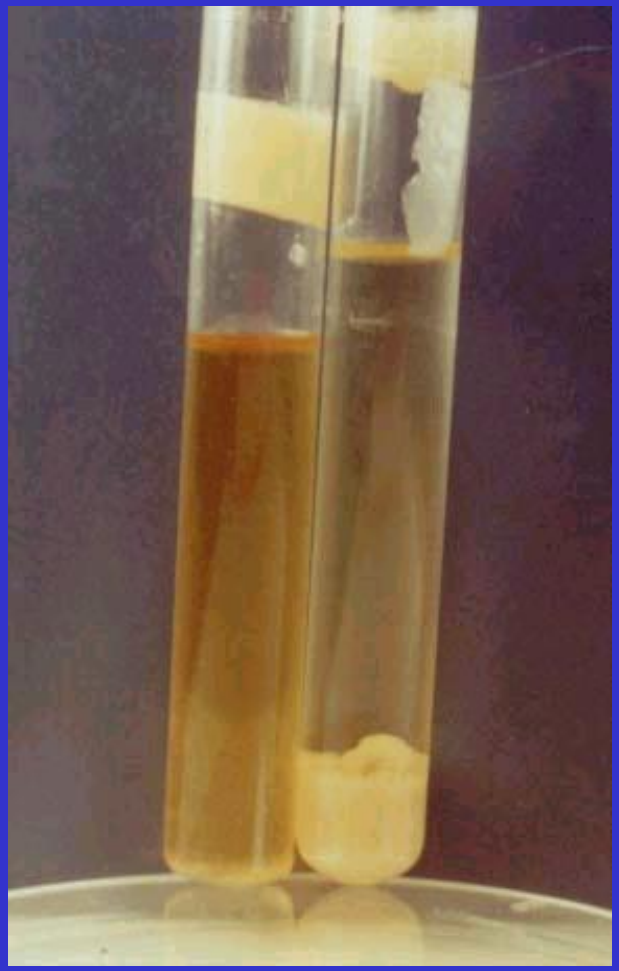
21. *Leptospira* in Korthof medium

22. *Bacillus cereus* on agar and blood agar plate





23-24. *Clostridium tetani* and *C. perfringens* in Holman and thioglycolate media

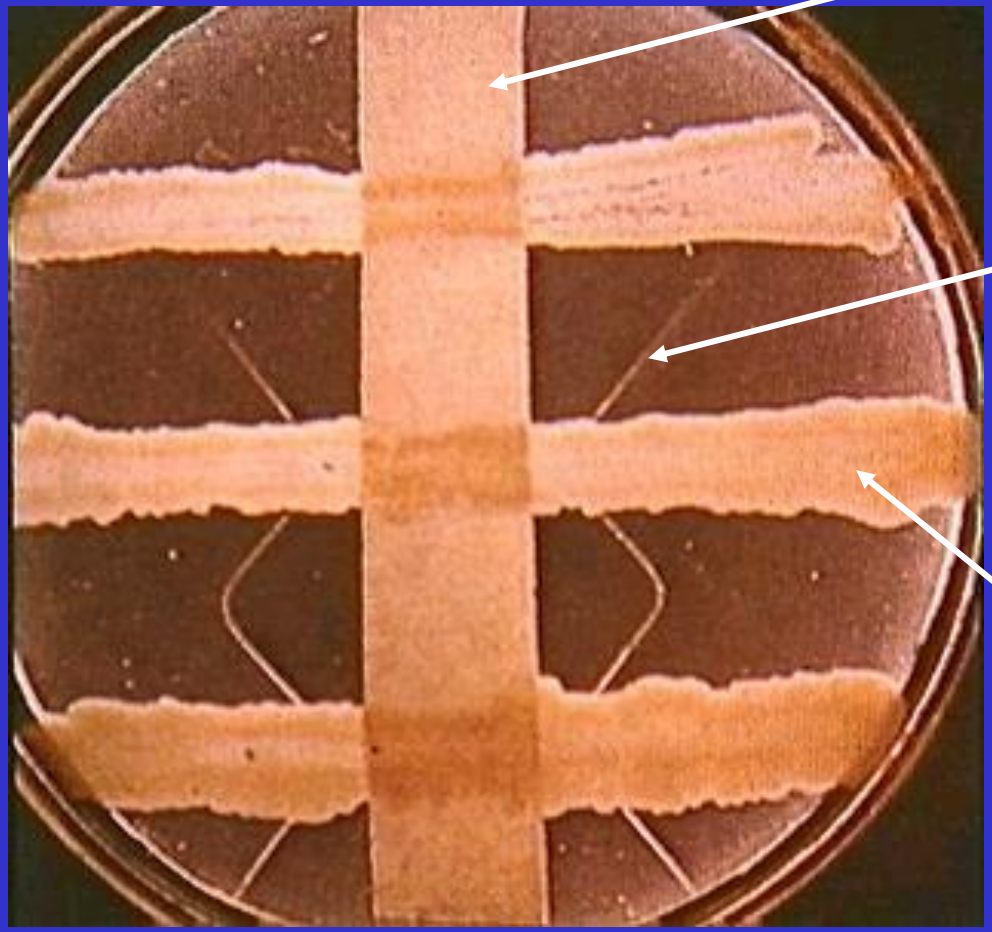


GasPak

III. Reactions and methods

1. Elek test

Aim: test for toxin production



Filter paper with antitoxin

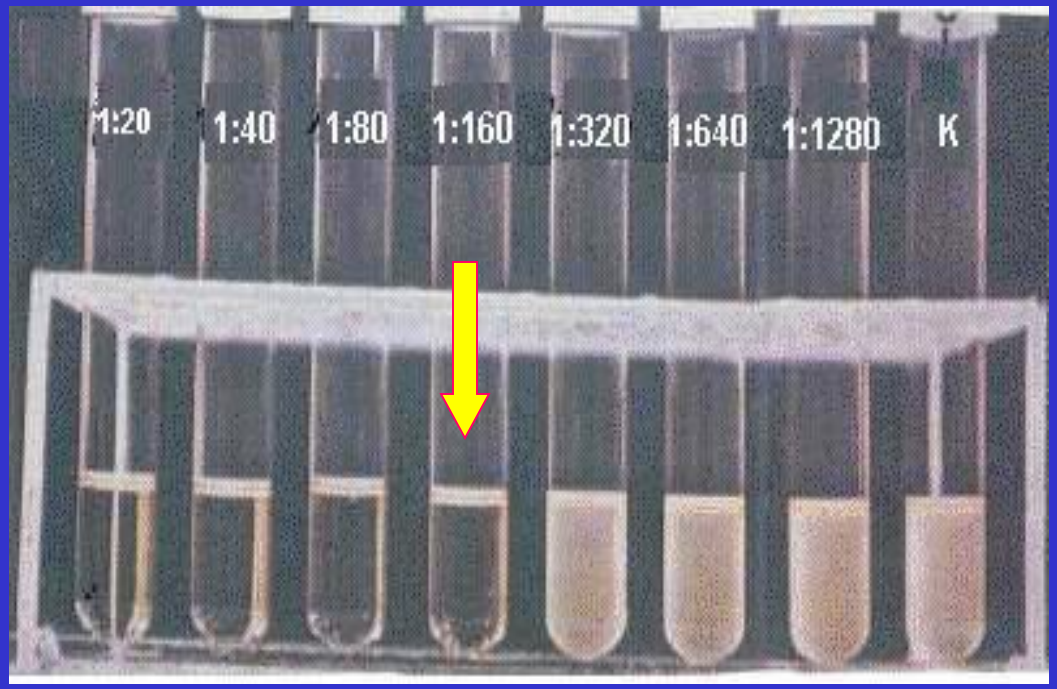
Precipitation arch

Corynebacterium

2. Widal-type reaction

- Gruber-Widal: abdominal typhus (*Salmonella*)
- Weil-Felix: epidemic typhus (*Rickettsia*)
- Wright: *Brucella* / *Francisella*

With *Proteus* OX19 antigen!

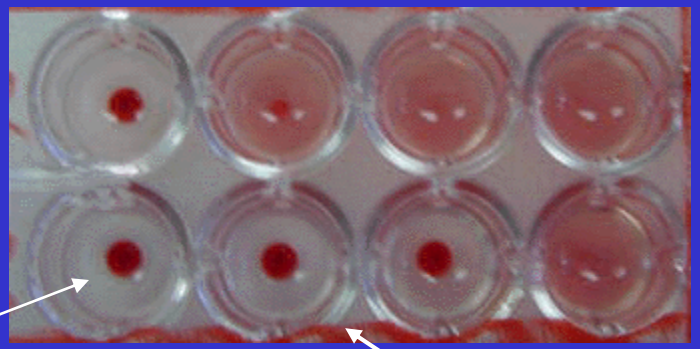


titre: the smallest amount of antibody, which can cause positive reaction**!!!**

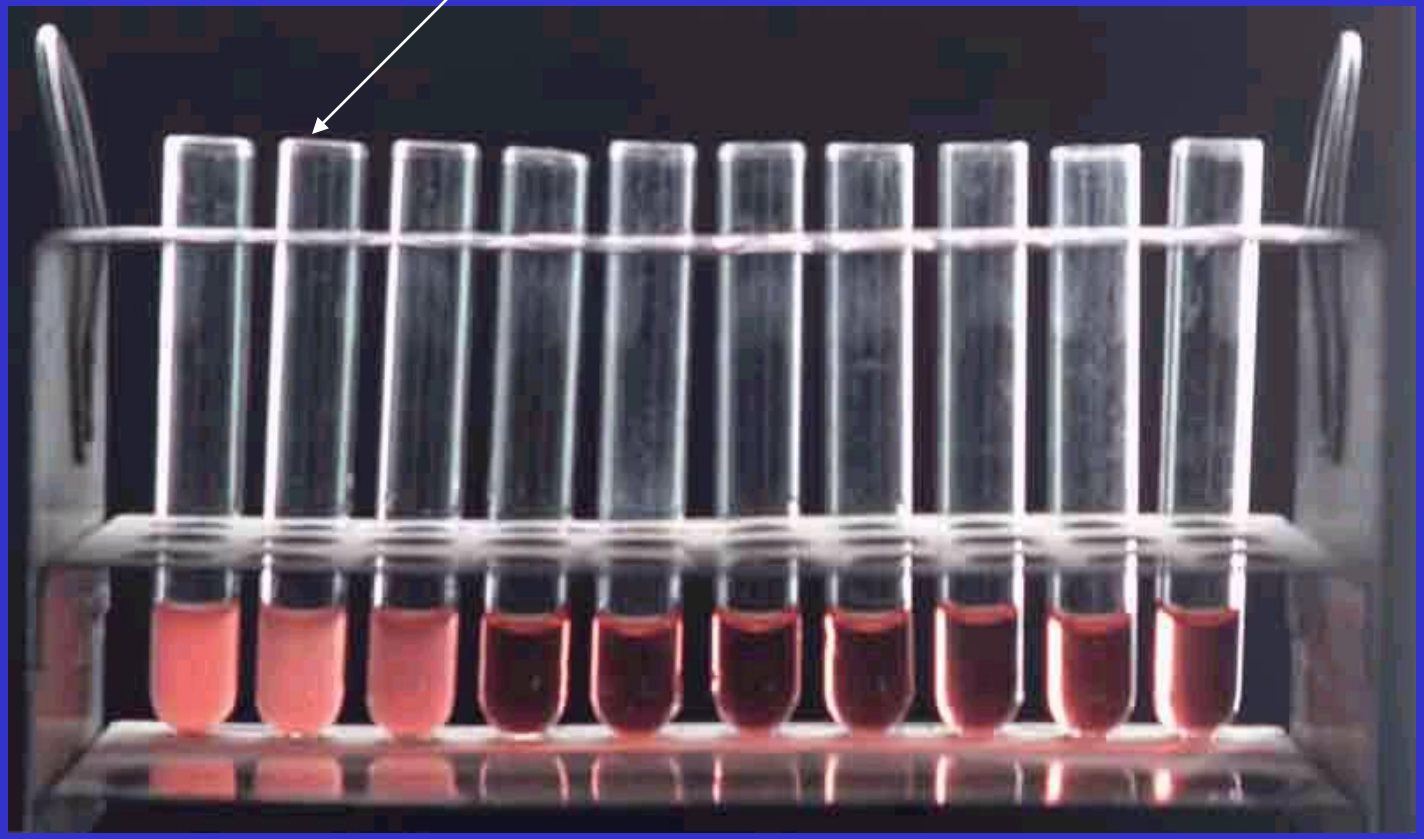
3. Wasserman test for syphilis (CF)

1

2



Positivity: no hemolysis



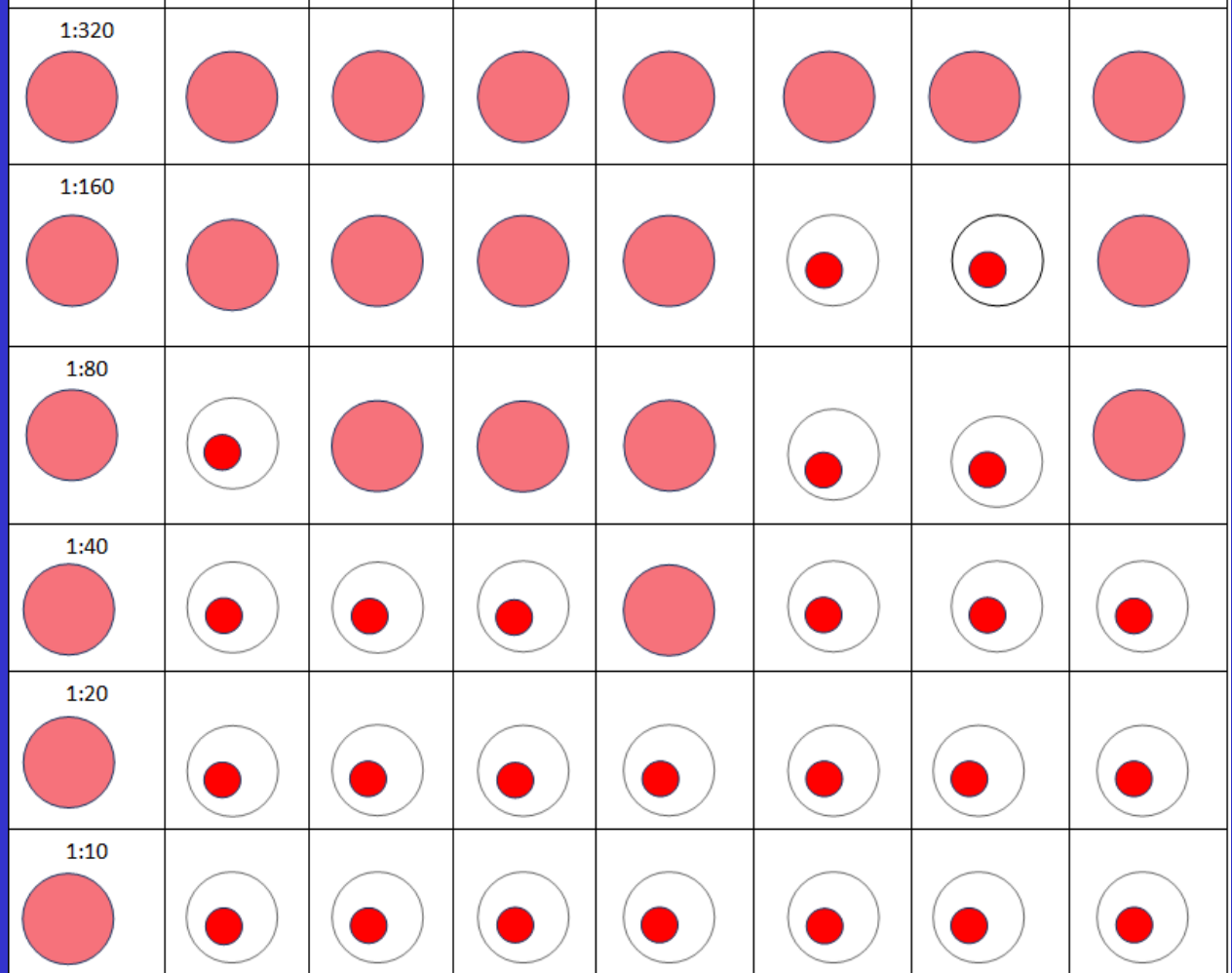
2: 4-fold increase in titre compared to **1** - indicates infection

Paul-Bunnell test

- Mononucleosis infectiosa
- EBV: P-B positive
- CMV: P-B negative
- Patient’s antibodies agglutinate sheep rbc

3. Hemagglutination inhibition (HI) (Rubella)

NEG K	POZ K	A savópár 1. minta	A savópár 2. minta	B savópár 1. minta	B savópár 2. minta	B savó 2. minta össz-ellenanyag	B savó 2. minta IgM eltávolítás
1:320							
1:160							
1:80							
1:40							
1:20							
1:10							



4. Hemagglutination (HA) and -inhibition (HAI)

hemagglutination

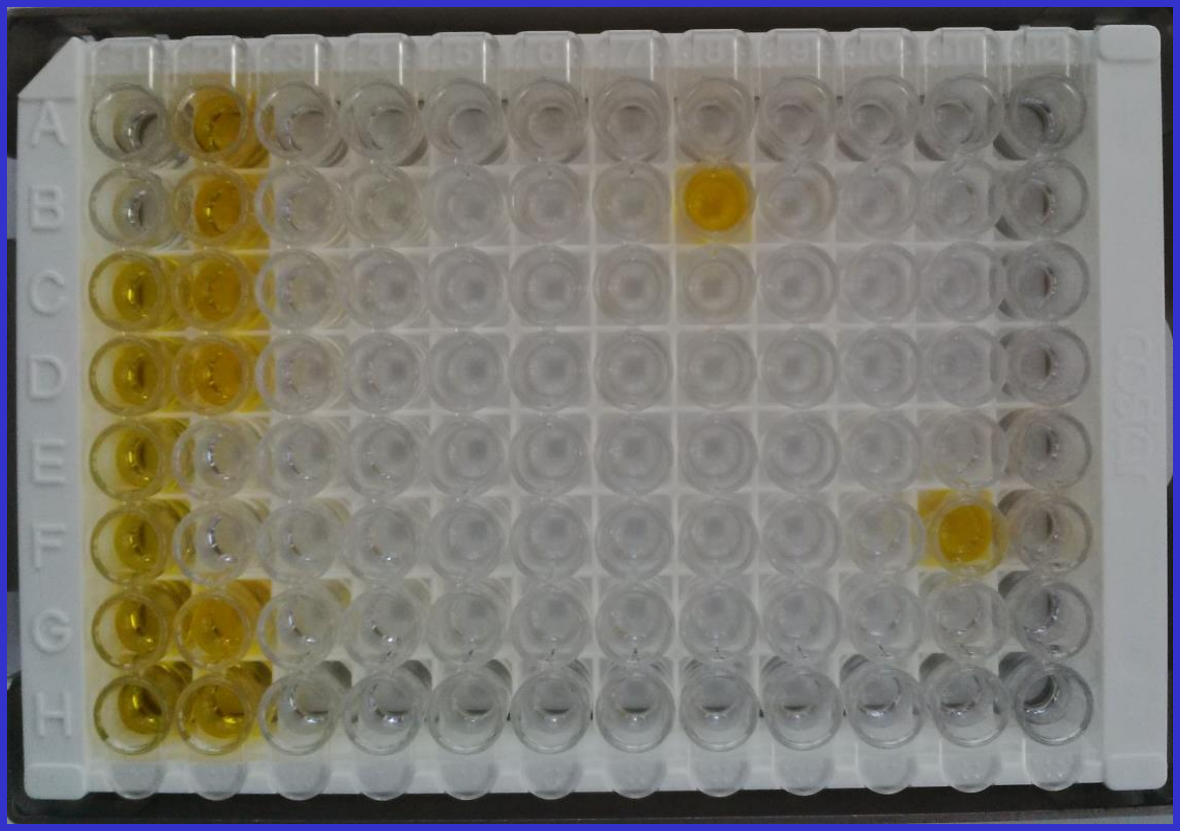
hemagglutination inhibition

CF test

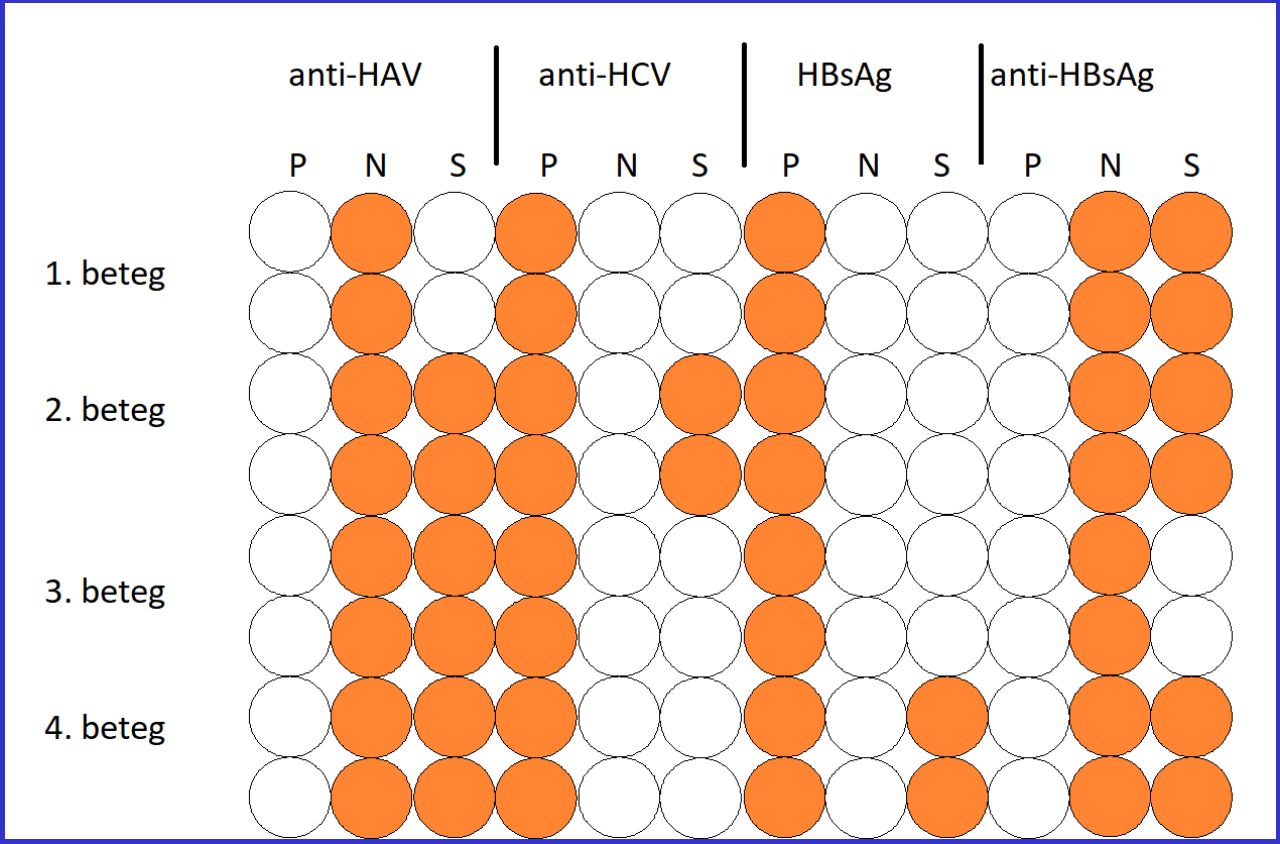
hemolysis (-)

no hemolysis (+)

5. ELISA tests: HIV-1 and HIV-2



5. ELISA tests: Hepatitis



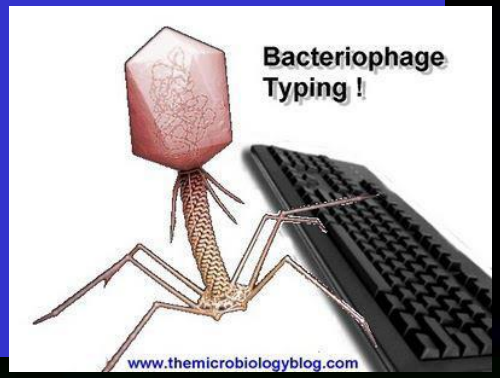
Anti-HAV (competitive ELISA)

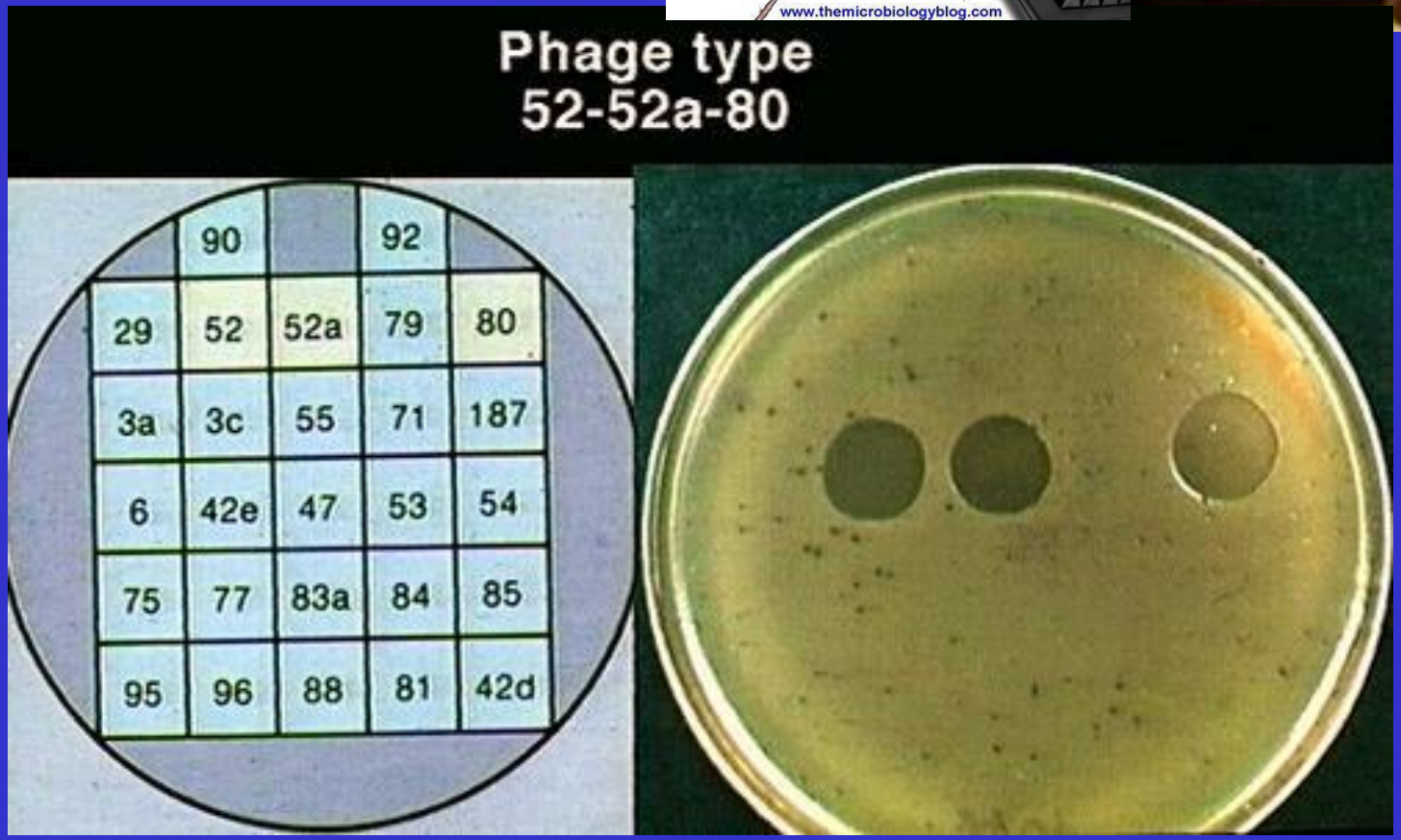
Anti-HCV (indirect ELISA)

HBsAg (sandwich ELISA)

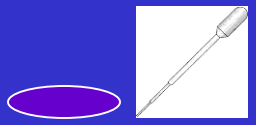
Anti-HBsAg (competitive ELISA)

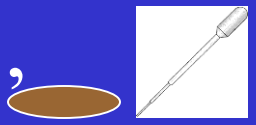
8. Phage typing





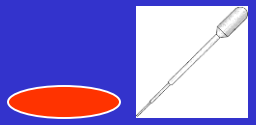
10. Steps of Gram staining

1/a. Crystal violet 2’ 

1/b. Lugol solution 1’ 

2. Differentiation with 96 % ethanol

3. Washing with water

4. Safranine 1’ 

5. Washing

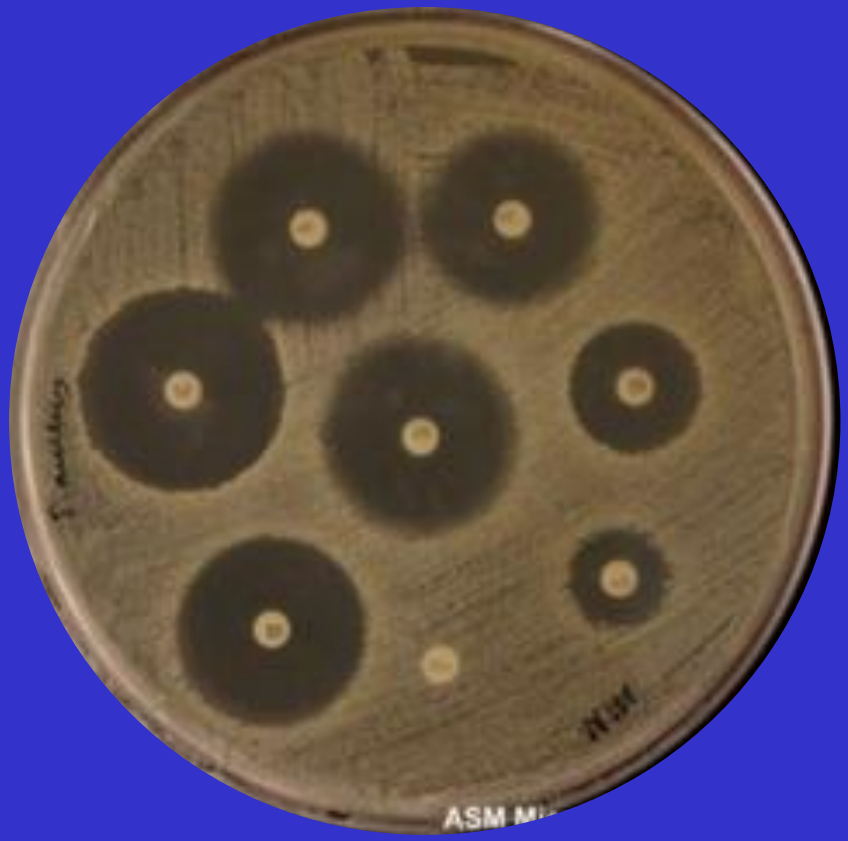
6. Drying

7. Microscopic examination (immersion)

11. Steps of Ziehl-Neelsen staining
"acid fast staining"

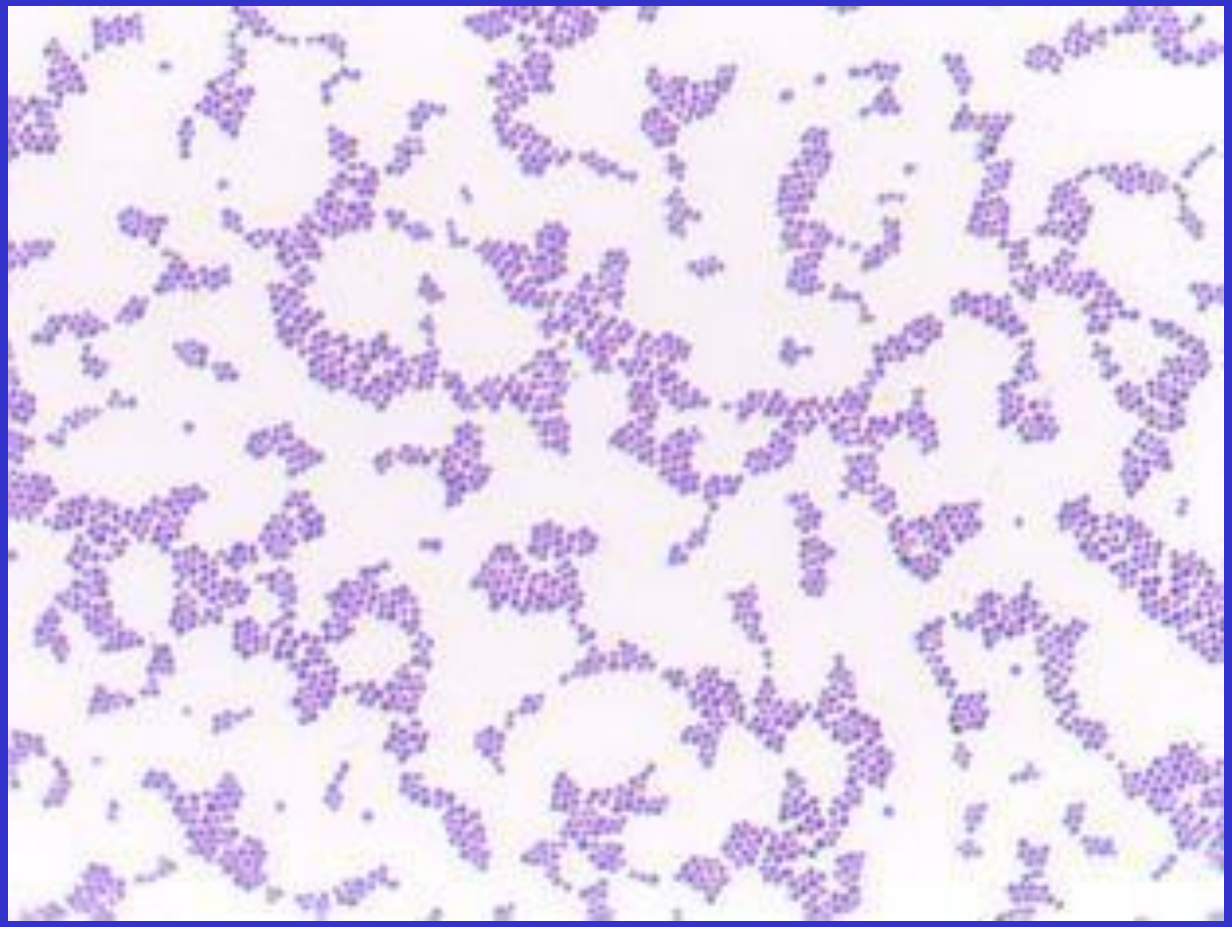
- Carbol-fuchsin (on filter paper!)
  - heating 3x (until steaming)
- Differentiation: acidic alcohol
  - 3% HCl, 96% ethanol
  - "acid fast"
- thorough water washing
- counter staining 1-2'
  - methylene blue or malachite green
- careful rinsing, drying with filter paper

Antibiotic susceptibility test with disc diffusion ("antibiogram")

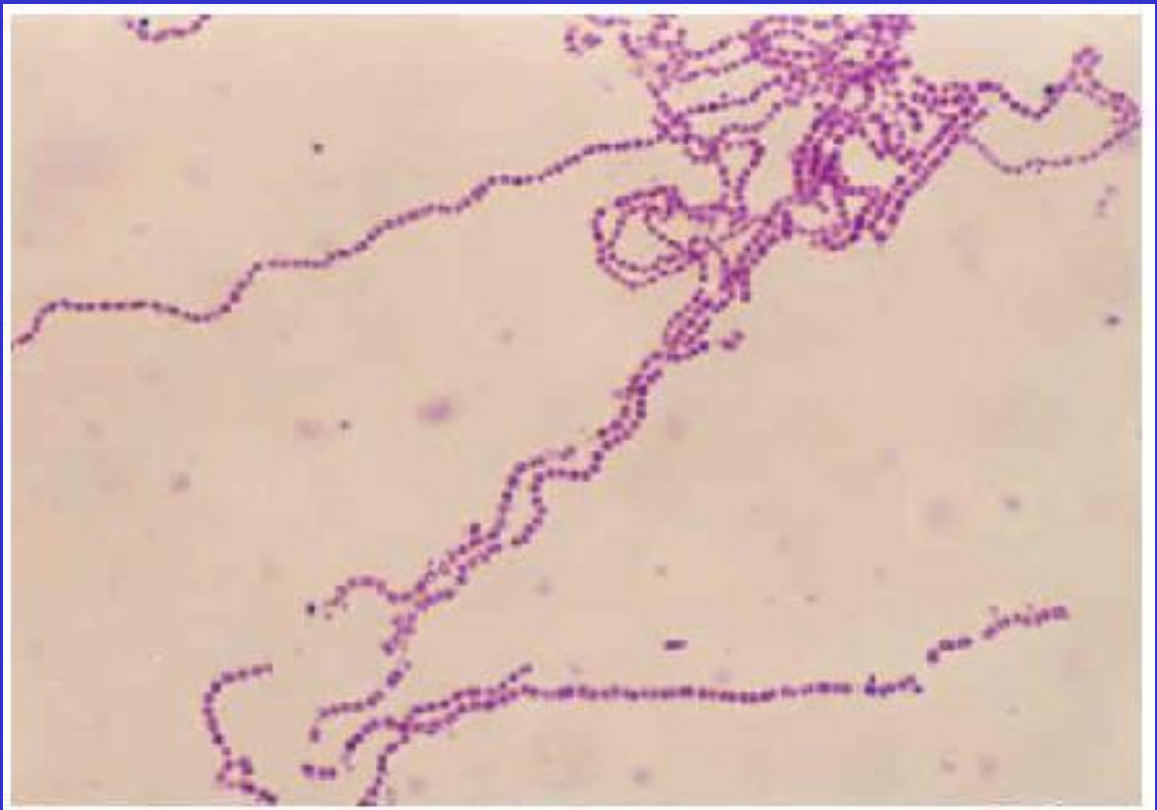


IV. Microscopic preparations

1.a *Staphylococcus* (Gram +)



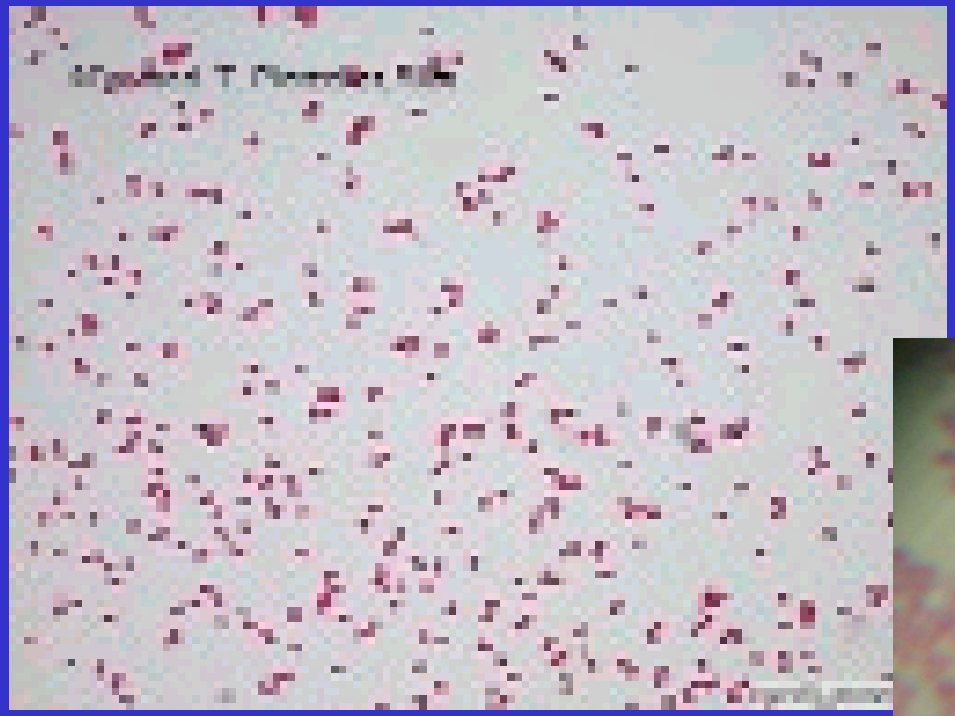
1.b *Streptococcus pyogenes* (Gram +)

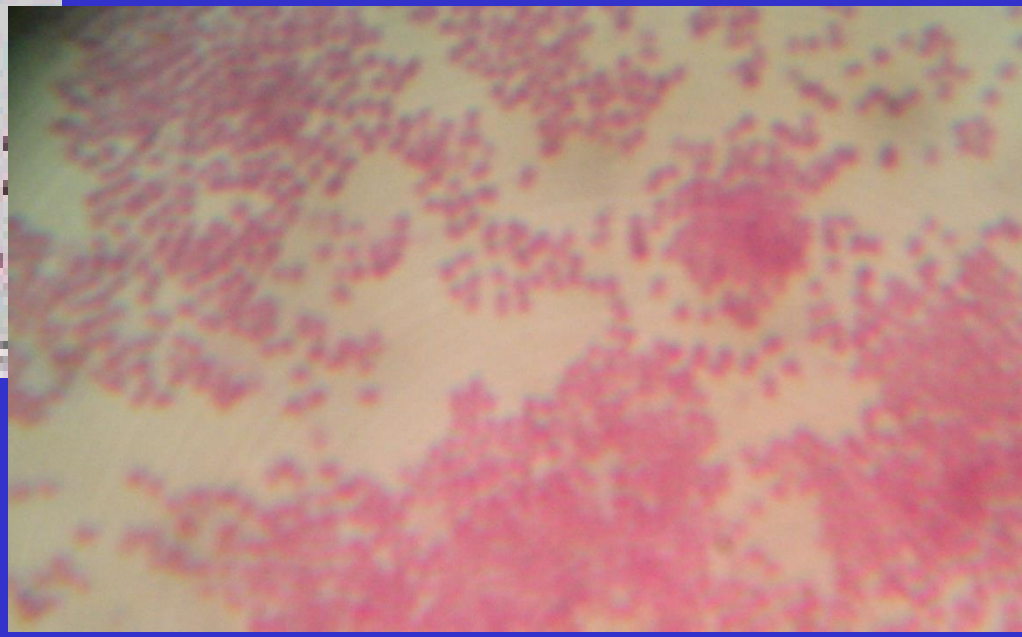


Gram pozitiv coccus (*Streptococcus pyogenes*)

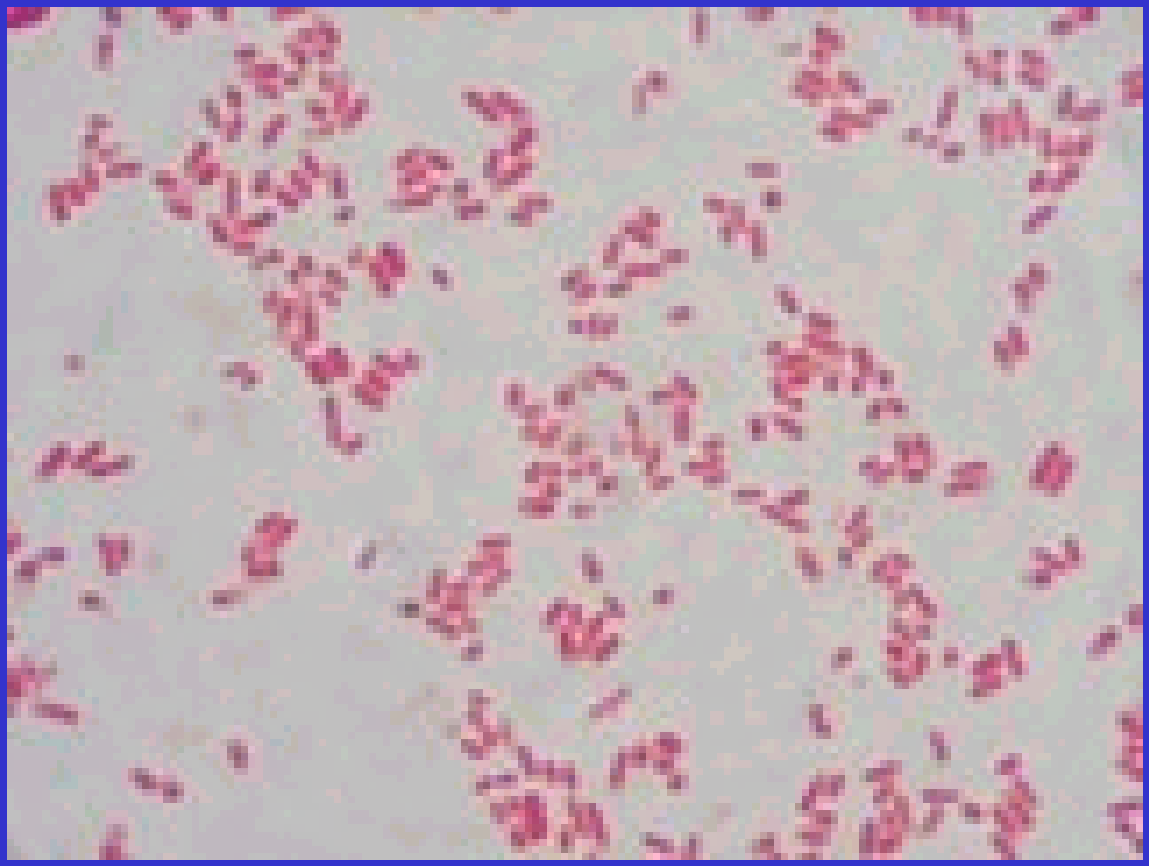
1.c *Streptococcus pneumoniae* (G +)

1.d *Neisseria* (Gram -)

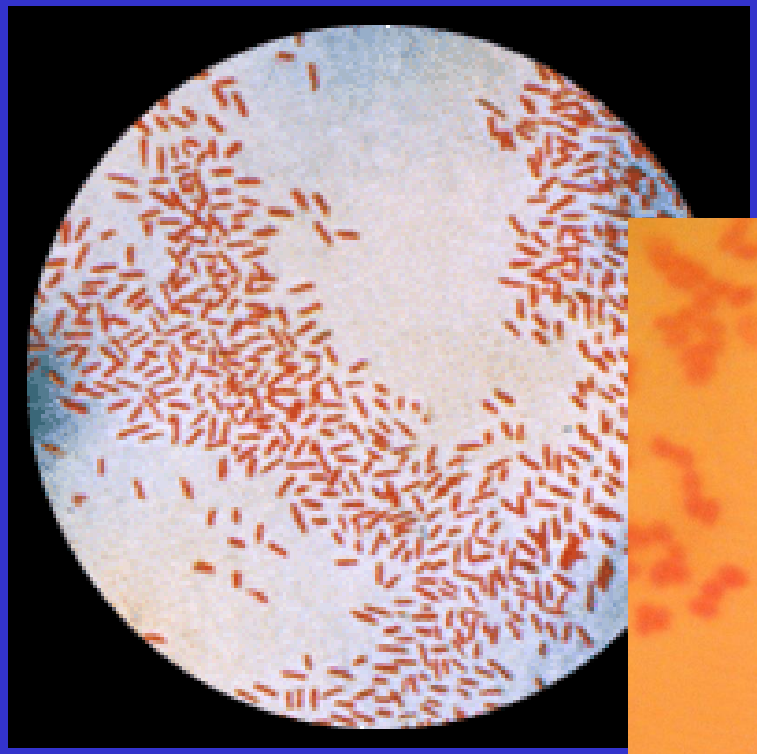




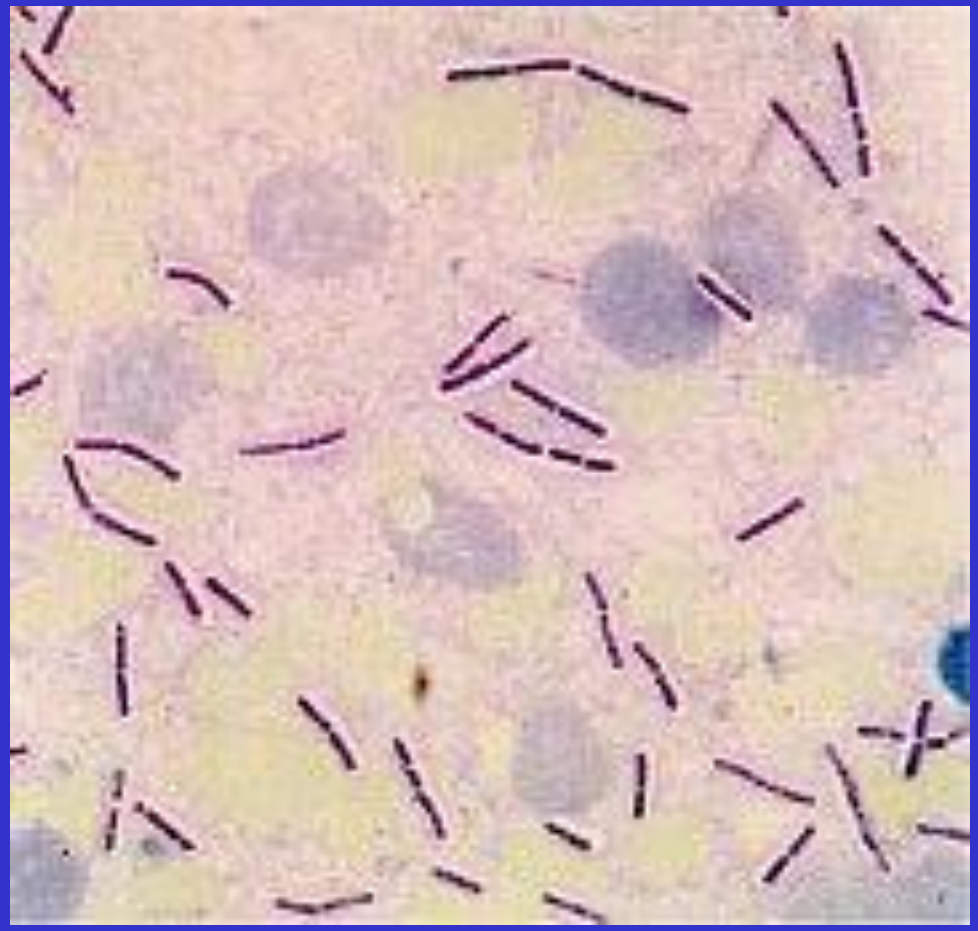
1.e *E. coli* (Gram -)



1.f *Haemophilus influenzae* (Gram -)

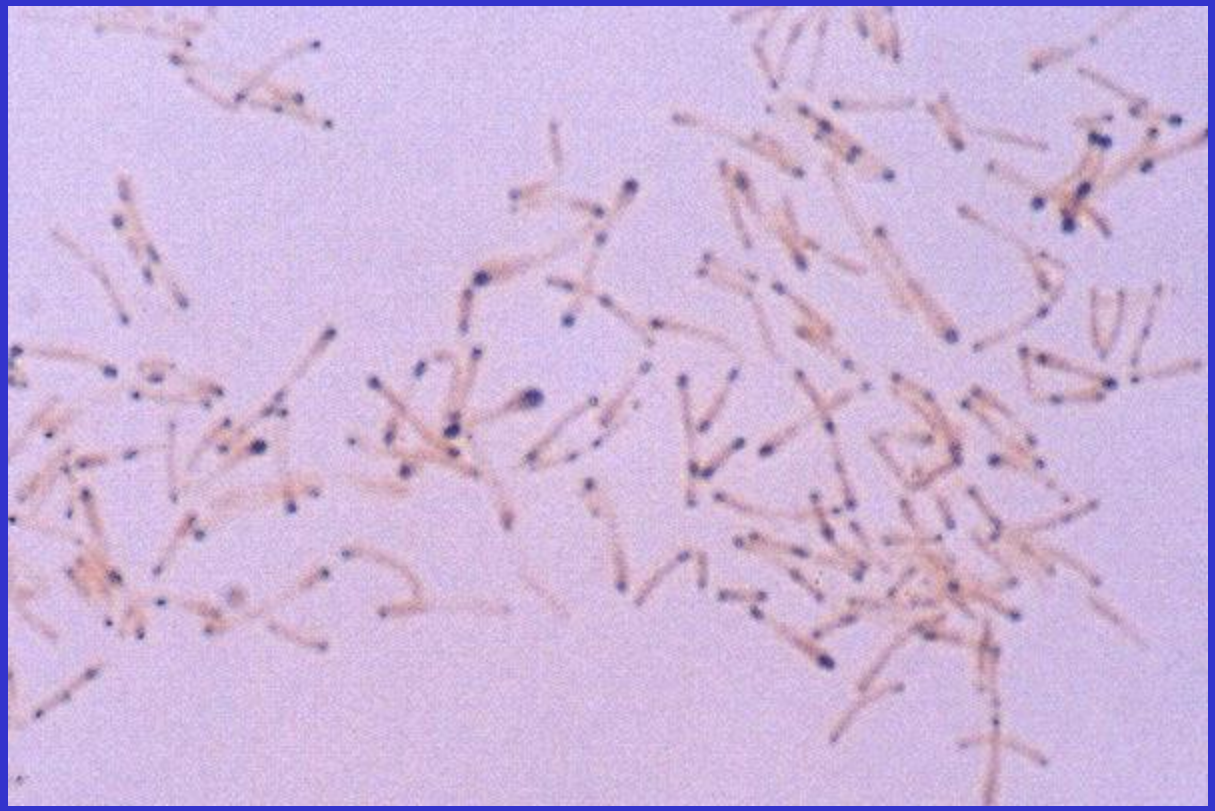


1.g *Bacillus* (Gram + spore forming)

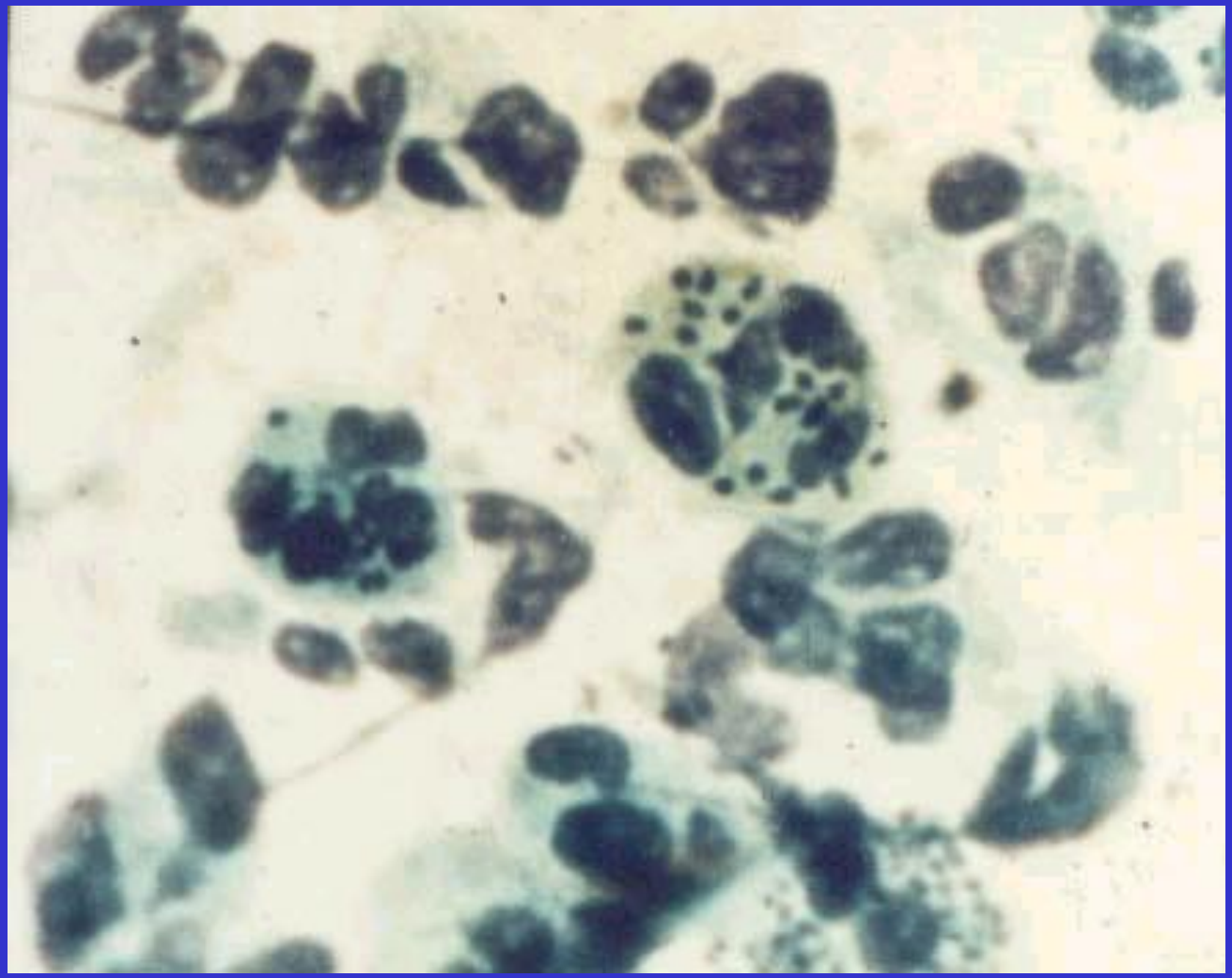


1.h *Clostridium tetani* and *perfringens* (Gram + spore forming)

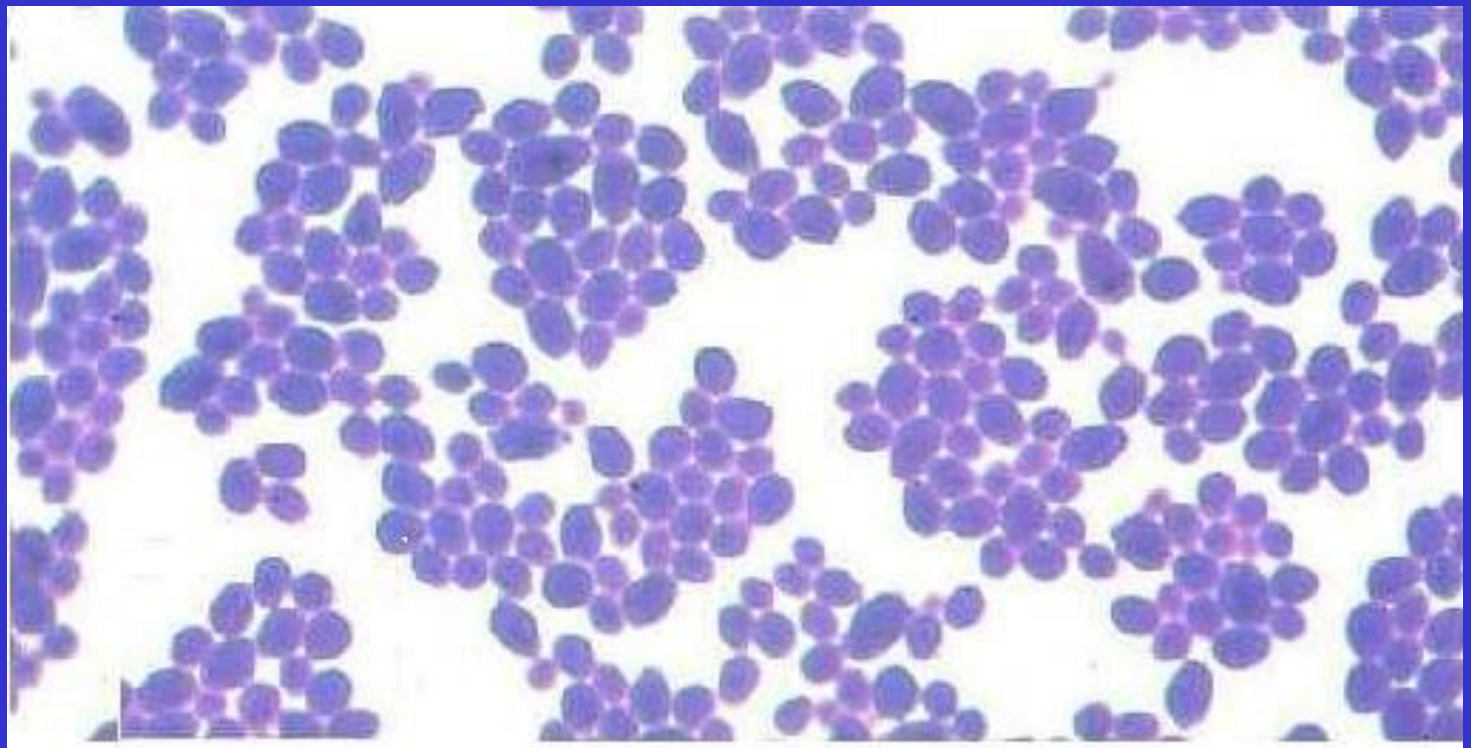
1.i *Corynebacterium* - Neisser staining



2.a *Neisseria gonorrheae* in pus
(methylene blue staining)

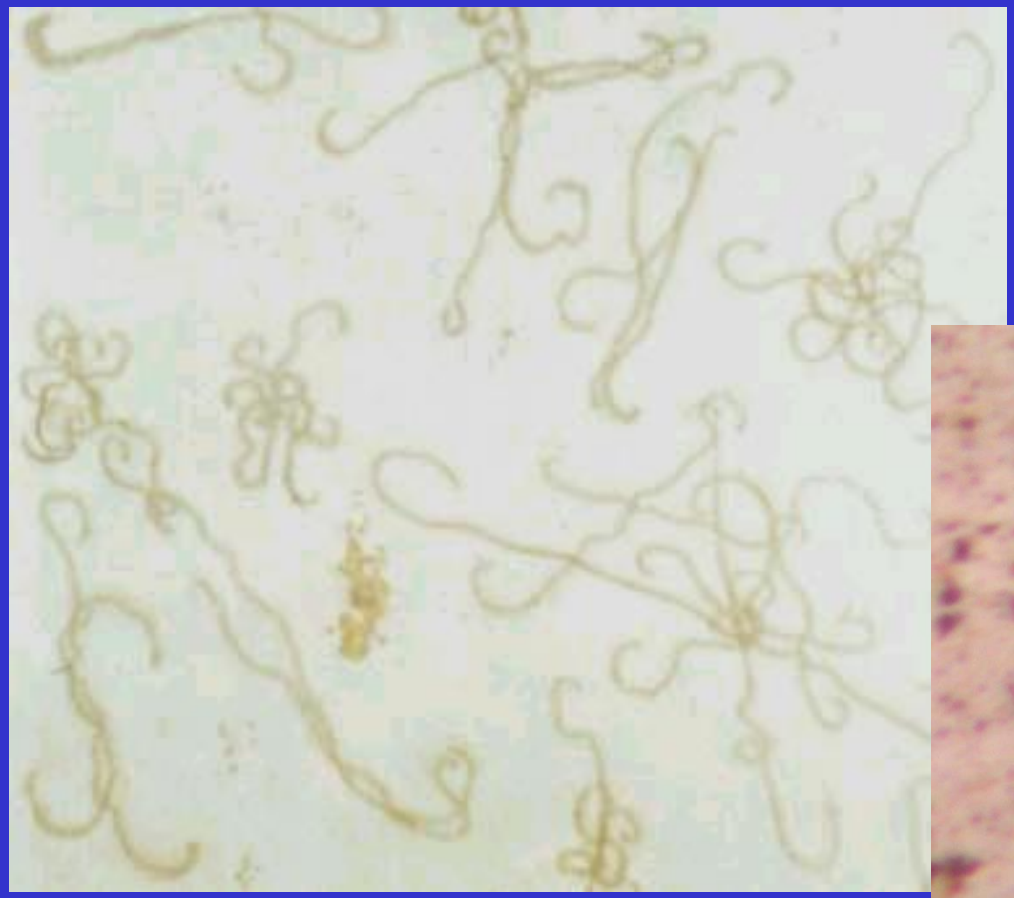


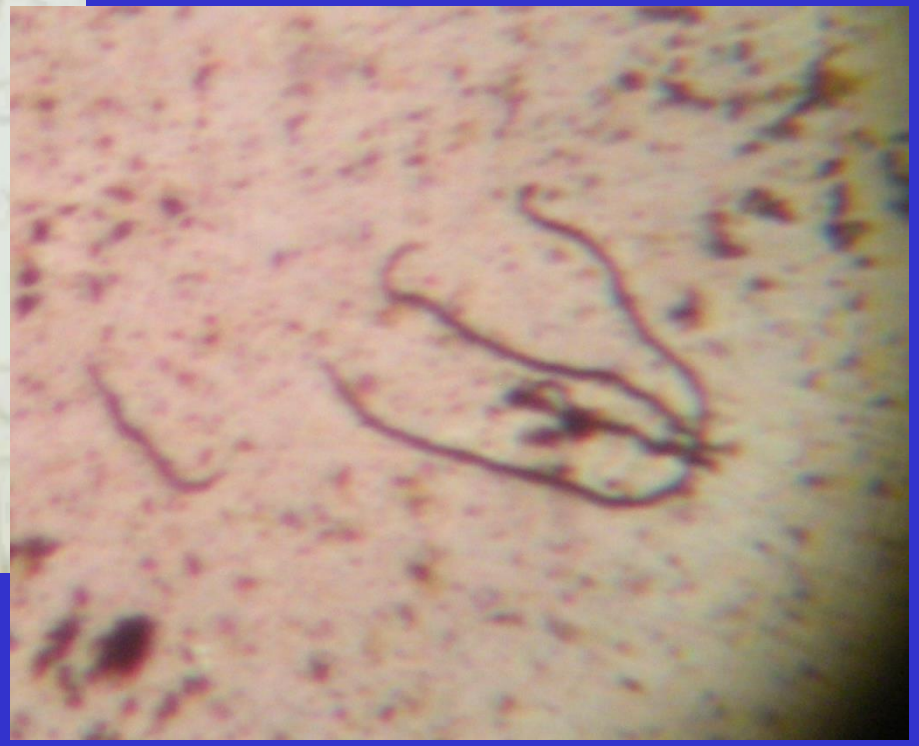
2.b *Candida albicans* from culture



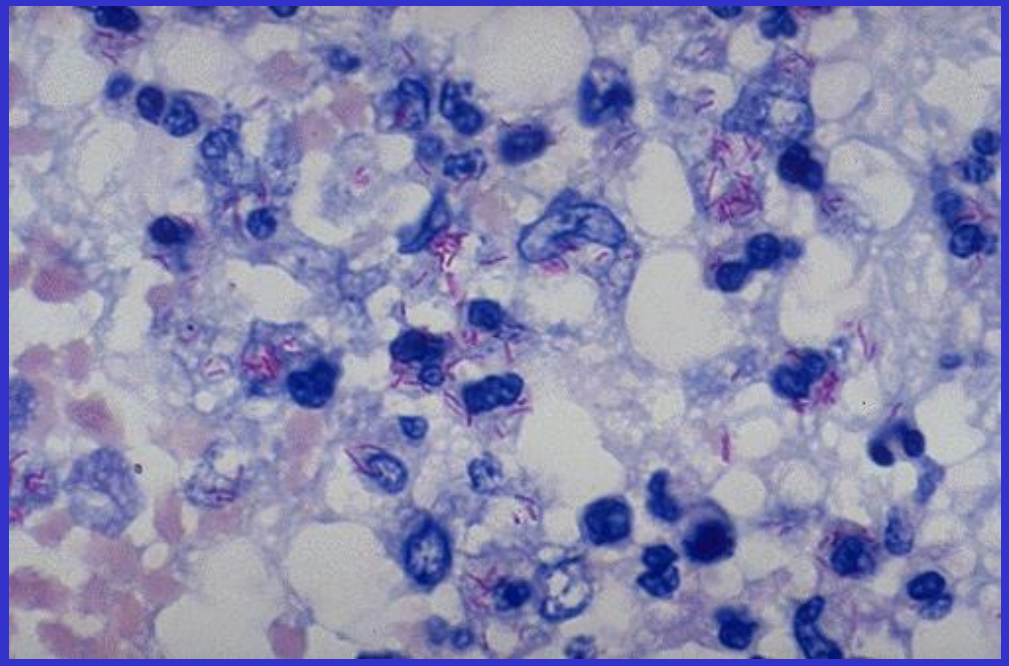
Sarjadzó gomba *(Candida albicans)* egyszerű festéssel (metilénkék) készített kenete

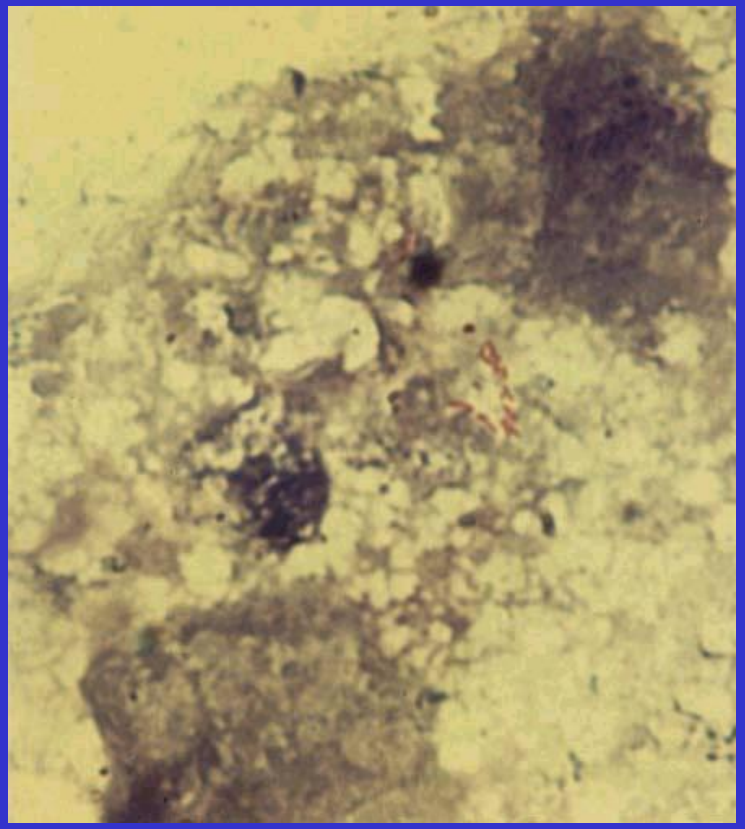
3. Silver impregnation: *Leptospira*



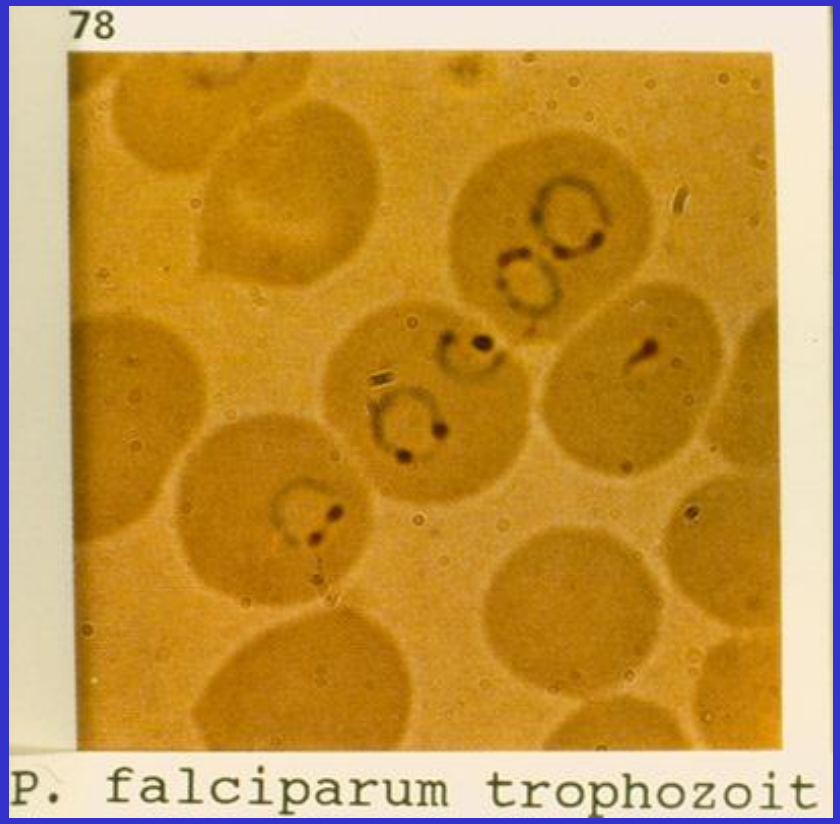


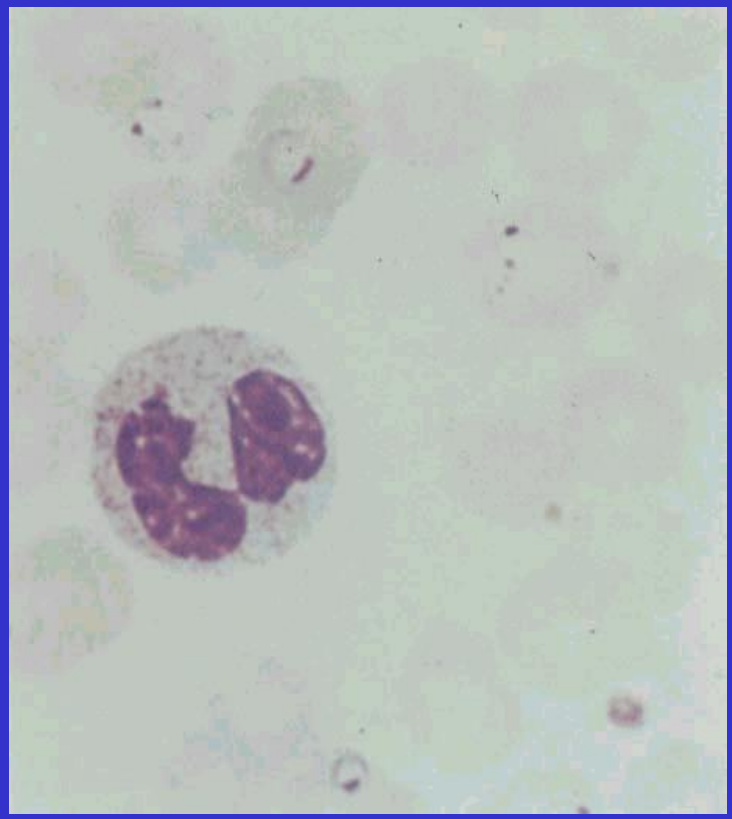
4. Ziehl-Neelsen staining: *Mycobacterium tuberculosis*



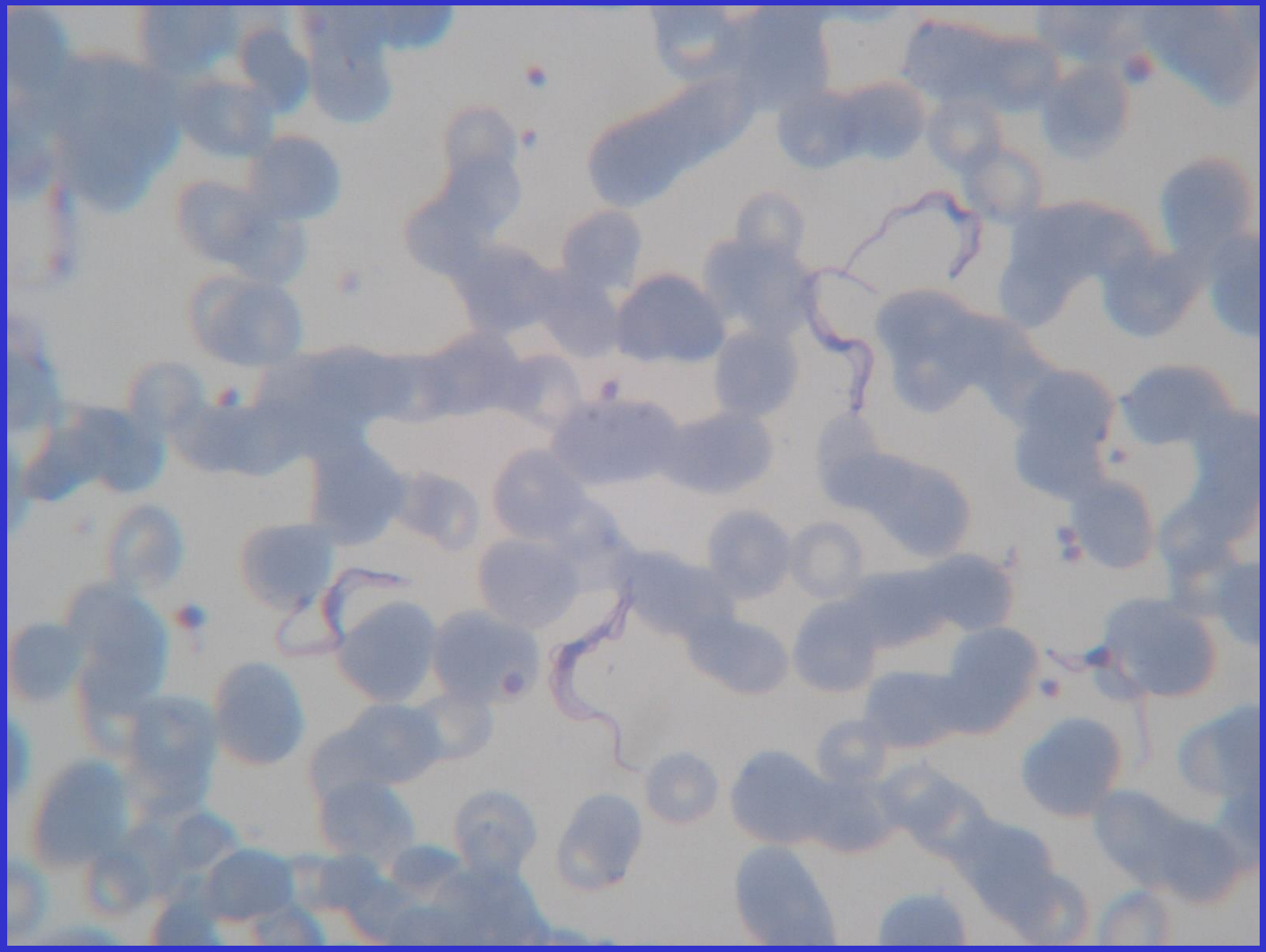


5.a *Plasmodium falciparum* in thin blood film (Giemsa staining)





5.b *Trypanosoma brucei* in thin blood film (Giemsa staining)



6. Anorectal cellulose-tape preparation (*Enterobius*)

Vizsgálati anyag vétele
féreg fertőzésben

VI. Tests performed by the students

Catalase test

<u>Catalase +</u>: *Staphylococci*

<u>Catalase -</u>: *Streptococci*

- performance: adding a few drops of H_2O_2 to the culture, strong bubbling if positive

- $H_2O_2 \rightarrow H_2O + O_2$

Clumping test - Coagulase test

Coagulase +: *Staphylococcus aureus*

Coagulase -: *all other Staphylococci (**"CNS"**)*

- "Staphaurex": latex agglutination

- performance: mixing bacteria with fibrinogen-bound latex particles on microscope slide → clumping

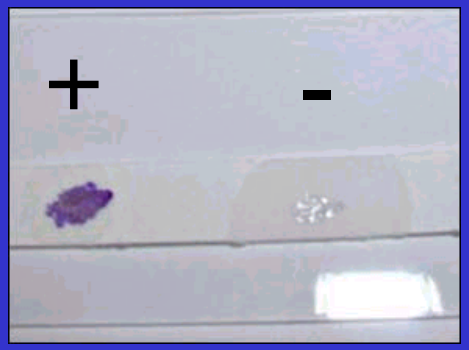
Oxidase test

Oxidase +: *Pseudomonas*, ***Neisseria***, *Vibrio, Campylobacter*

- purpose: detection of cytochrome-oxidase enzyme
- diagnostic use: differentiation of obligate aerobes

- performance:
  - filter paper on microscope slide
  - add oxidase reagent

    (= parephenylene-diamine derivative)
  - add bacteria
  - positivity: pink / purple colour



Indol test

- triptophane → indol (triptophanase enzyme)
- amylalcoholic (=organic) reagent → indol is dissolved here after mixing → intensive red ring on top (alcohol is lighter then water!)
- can be performed in ureum-indol tube

Indol +: *E. coli*

Indoltermelés kimutatása:
a peptonvízes baktériumtenyészetet amilalkoholban oldott sósavas paradimetil-amino-benzaldehiddel összerázva pozitív esetben az amilalkoholos fázis élénk vörös színű lesz

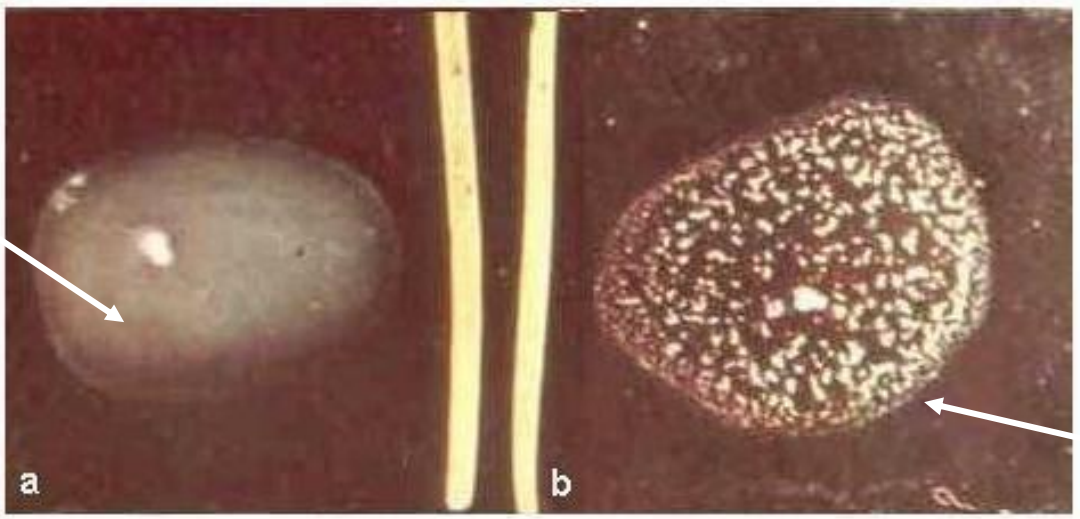
1. kontroll
2. negatív (Klebsiella)
3. pozitív (*E. coli*)

6. Slide agglutination with *E. coli*

NEGATIVE

POSITIVE

Bacterium suspension (control)



Tárgylemez agglutináció (szerotipizálás, qualitatív reakció, a: negatív, b: pozitív, rögös kicsapódás)

White particles, clear solution