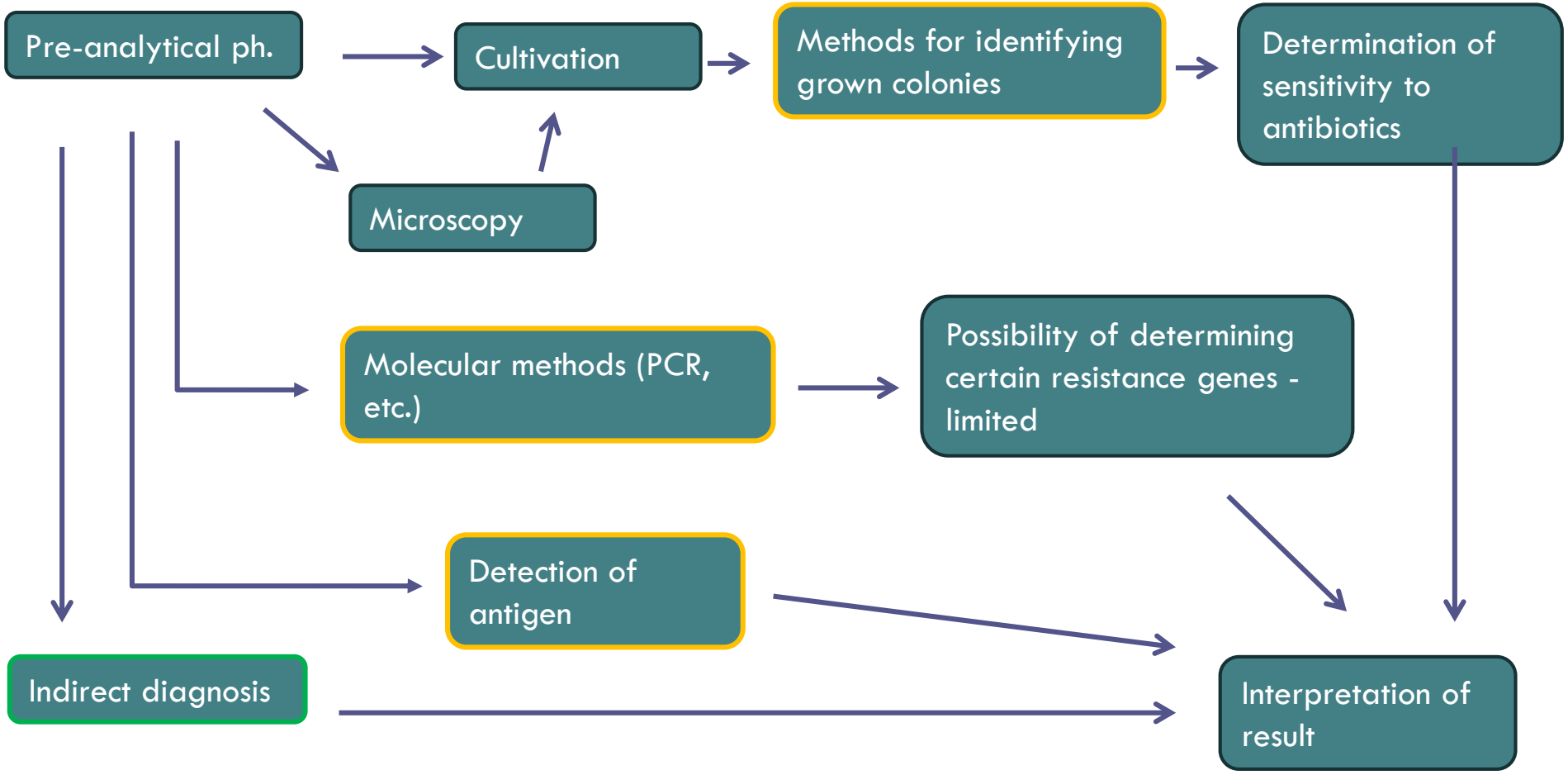
Microbiological testing in bacteriology



Previous practical lesson

- Indications for light microscopy
- The main advantage of microscopy in bacteriology
- The two most common staining methods

BACTERIAL CULTURE AND PROCEDURES LEADING TO THE IDENTIFICATION OF BACTERIA

MUDr. Anežka Gryndlerová

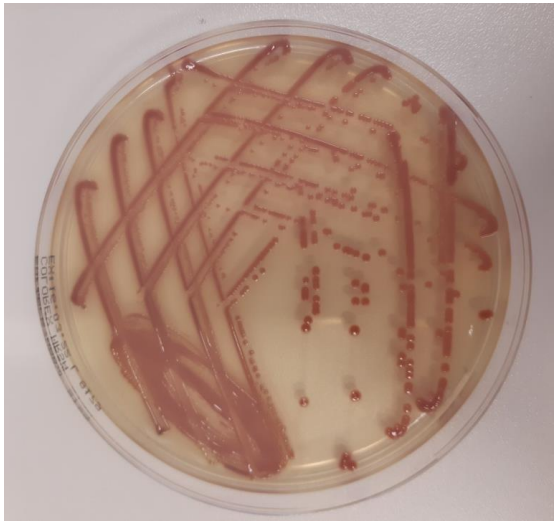
Content

- Bacterial culture
- Practical part – inoculation on agar
- Identification methods
- Blood culture
- Laboratory tour – cultivation, "BACTEC", "MALDI-TOF"

Bacterial culture

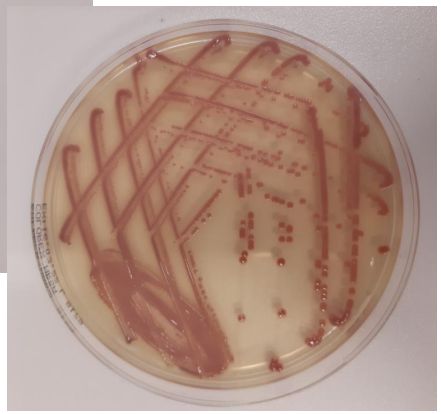
Culture

- Microbial proliferation; objective – detection and subsequent identification of bacteria
- Growth conditions – ?
  - Nutrients
  - Temperature
  - Atmosphere
  - Humidity, pH, osmotic pressure
  - Protection against external damage
- Culture media, incubators (thermostats)



Cultivation media - classification

- Liquid
  - Advantage over solid media?
    - Easy access to nutrients – bacterial growth
  - Disadvantage
    - Cannot be identified using standard methods
- Solid
  - Colonies
- Transport vs. culture media



Cultivation media – classification

- General
  - Nutrient broth, nutrient agar
- Enriched
  - Blood agar, chocolate agar
- Selective
- Diagnostic
- Selective-diagnostic

WAGENINGEN
UNIVERSITY & RESEARCH

https://shop.wur.nl/wbvr/bm272.html
https://en.wikipedia.org/wiki/Chocolate_agar

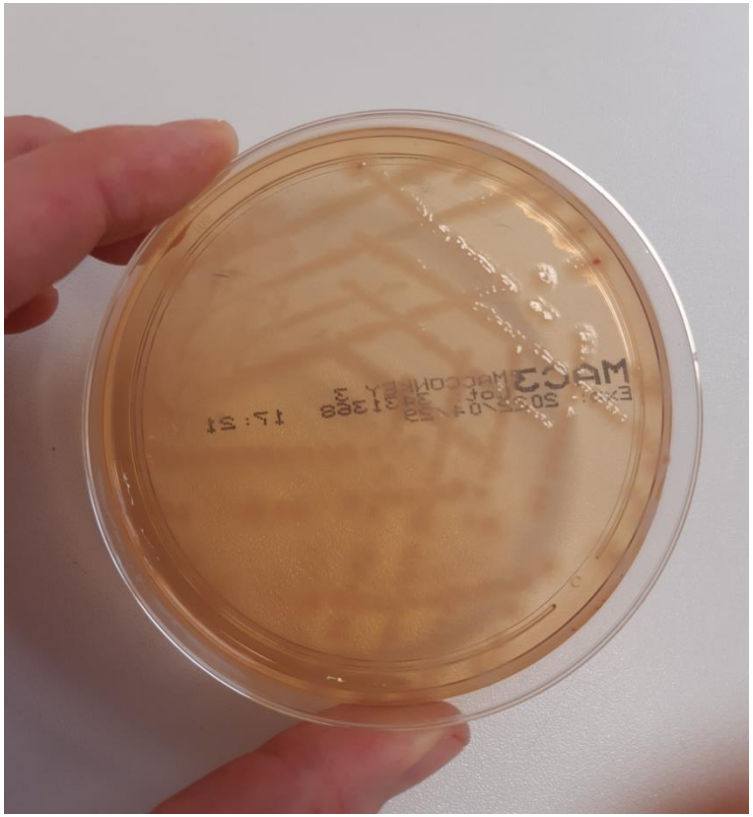
Selective media

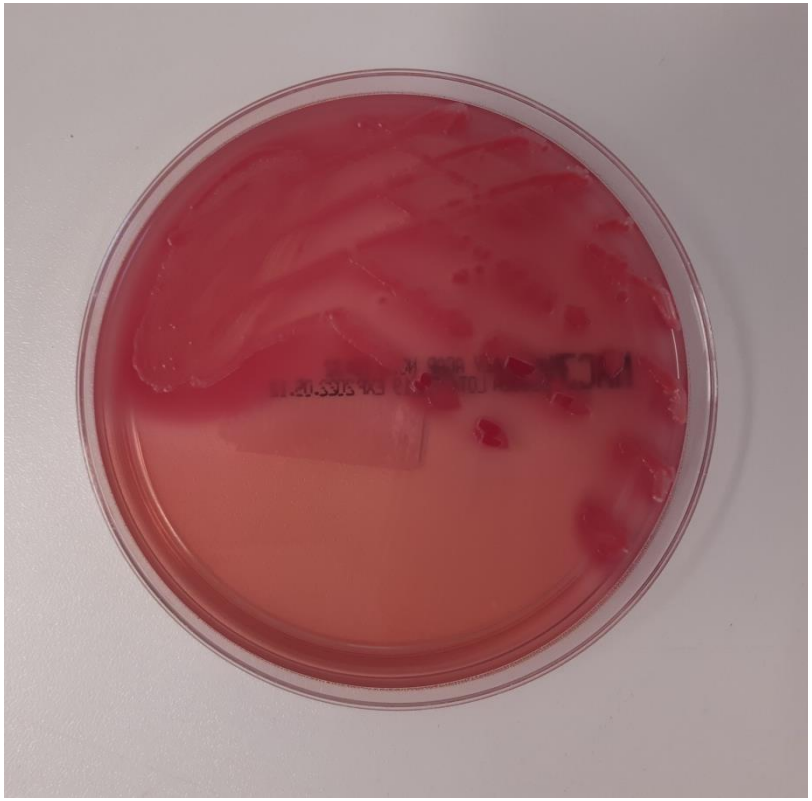
- Sabouraud agar - yeast
  - Components?
- Karmali agar – Campylobacter spp.
- (KA + 10% NaCl – staphylococci)

Diagnostic media

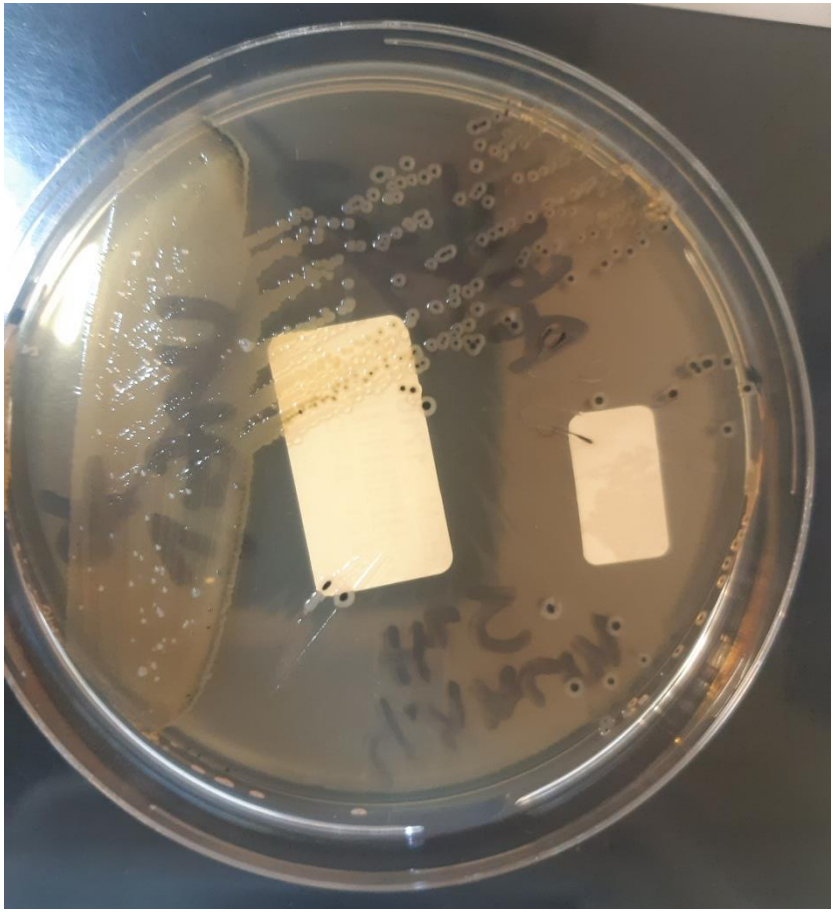
- Liquid media sets for biochemical analysis

Selective diagnostic media - MacConkey agar





Selective diagnostic media – DC (deoxycholate-citrate) agar



Ferrous sulphide, Ondřej Mangl – https://commons.wikimedia.org/w/index.php?curid=2894868

Proteus spp. – swarming motility

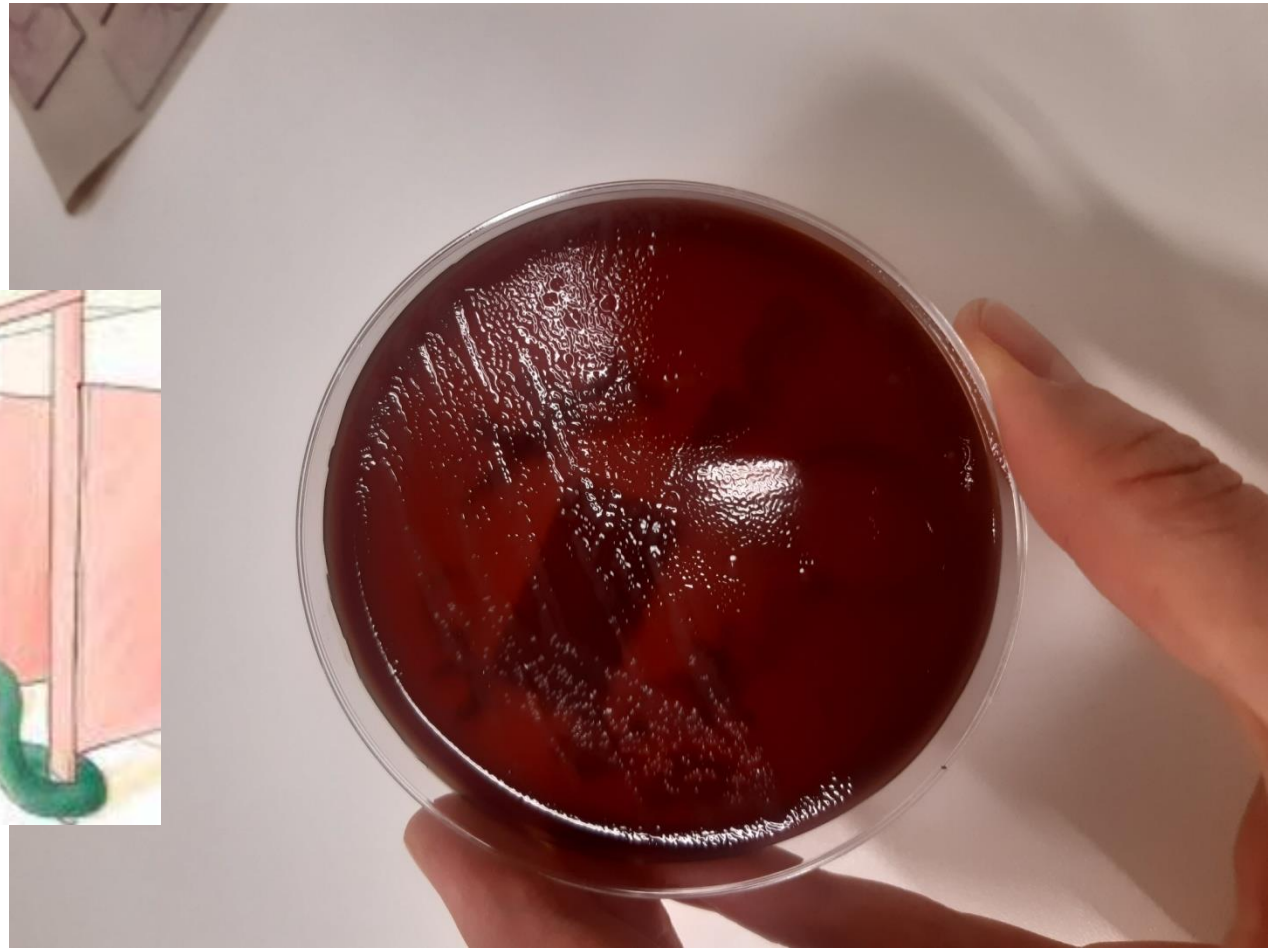
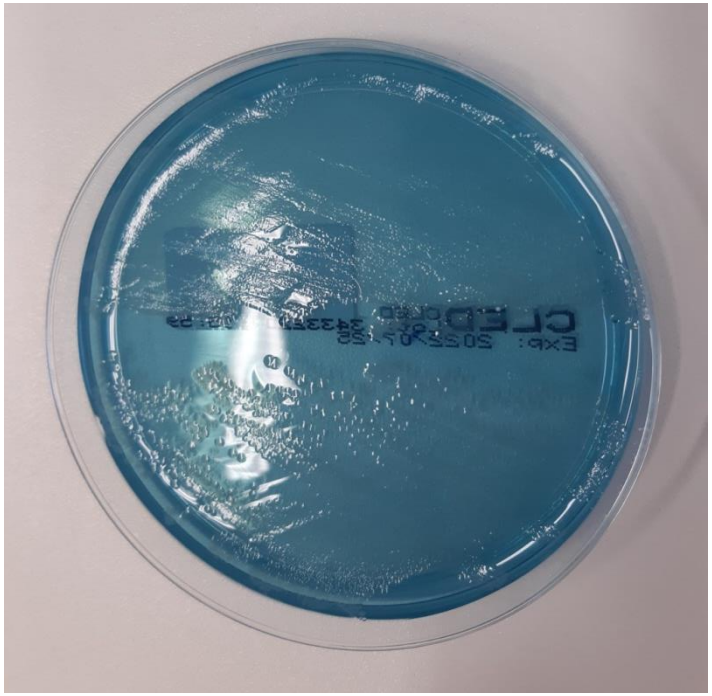
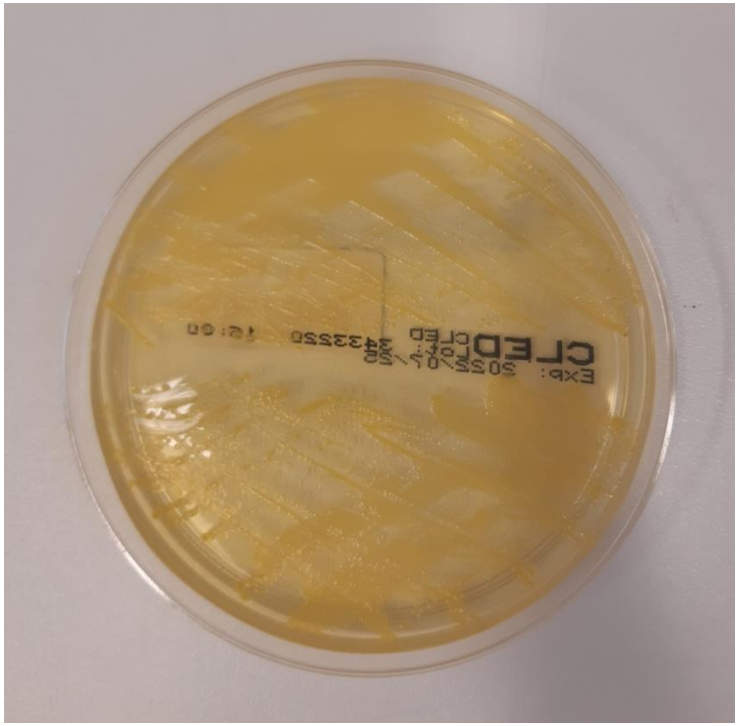


Image from SketchyMicro

Selective diagnostic media - CLED

- cystine–lactose–electrolyte-deficient agar





Temperature - ?

- 35-37°C
- Lower temperatures: *Yersinia spp., Listeria spp.*
- Higher temperatures: *Campylobacter spp.*

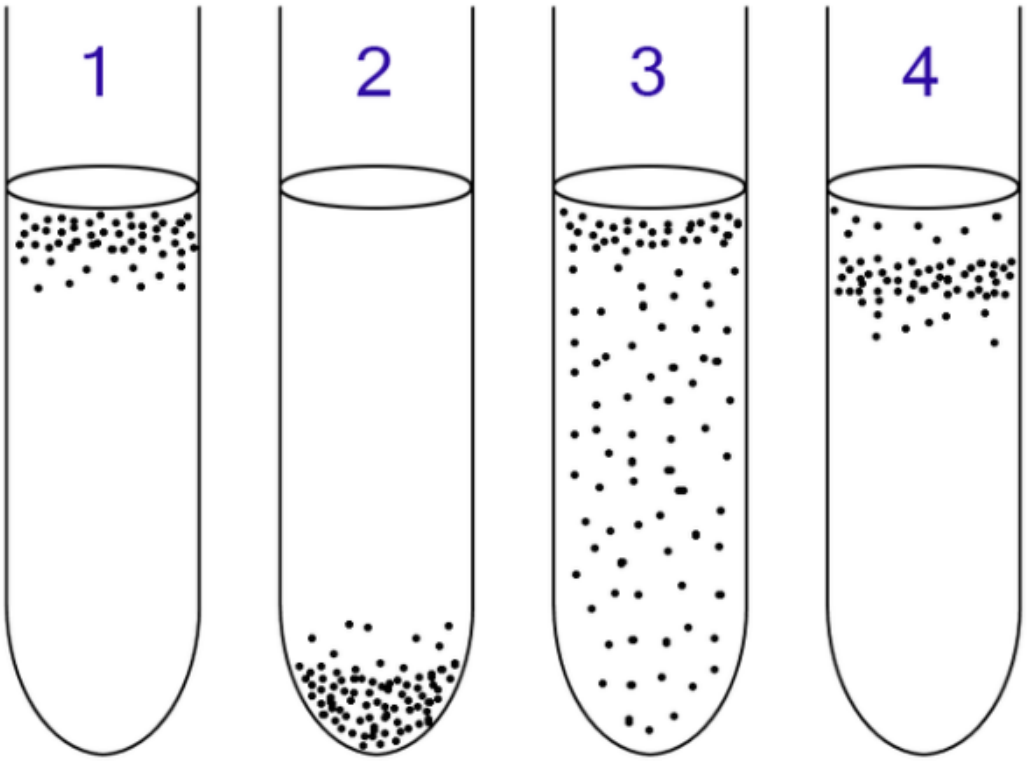
Atmosphere

- Obligate aerobic bacteria
- Obligate anaerobic bacteria
- Facultative anaerobic bacteria

- Microaerophilic bacteria
- Capnophilic bacteria

- Anaerobic chamber, jars with atmosphere generator

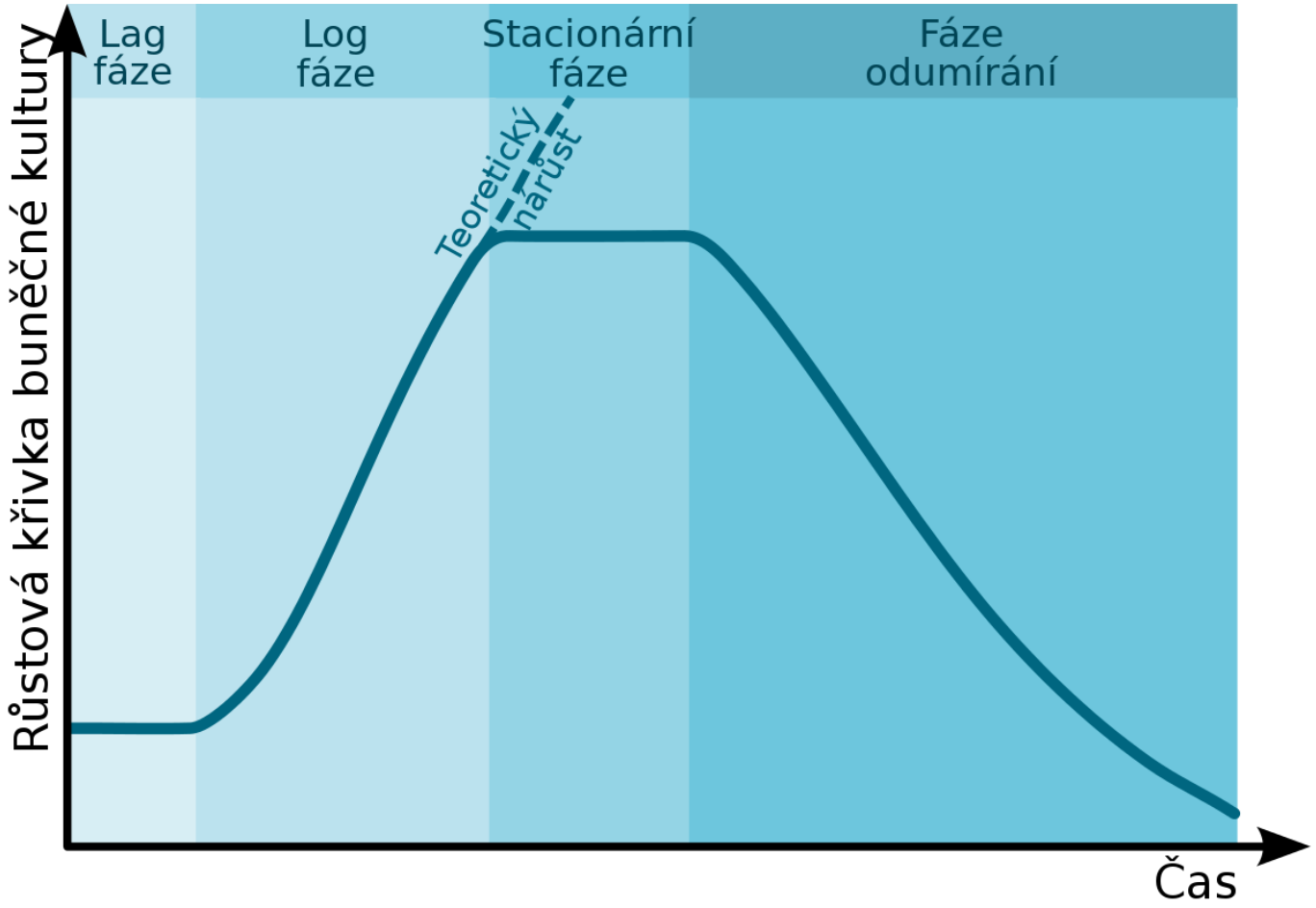
Growth in liquid medium



Cultivation time

- 24 hours
- Extended cultivation
  - *Campylobacter jejuni, Yersinia spp.*
  - *Neisseria gonorrhoeae, Neisseria meningitidis*
  - *Legionella pneumophila, Bordetella spp.*
  - *Mycobacterium tuberculosis*
  - …
  - Additional special culture conditions often required (medium, atmosphere, etc.)
    → must be specified on the request form
  - Micromycetes – min. 48 h
- Mix of colonies of different bacterial species
  → isolation of a subculture

Bacterial growth curve



Appearance of colonies

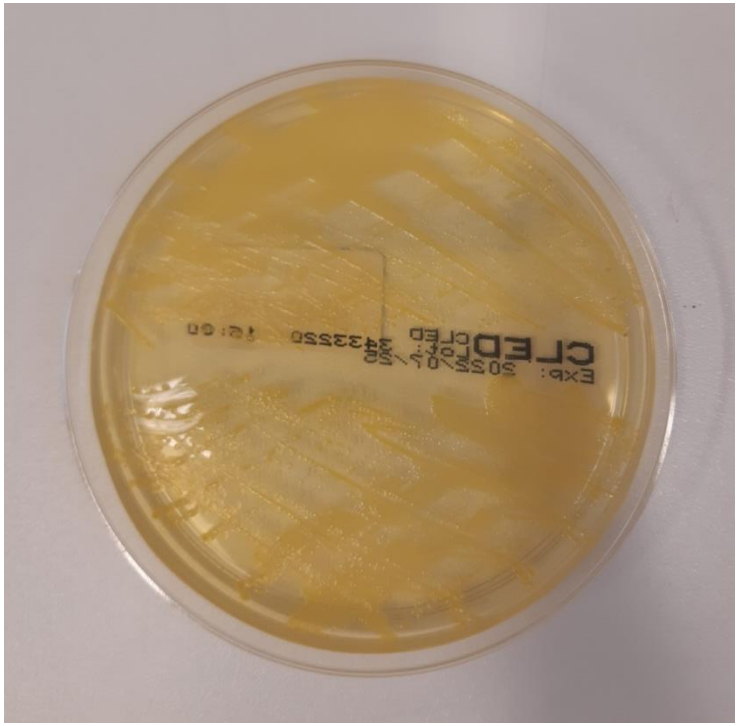
- Influenced by the type of culture medium used and culture conditions
- Size, shape, edges, profile, odour, colour
- Surface
  - Smooth – S phase
  - Mucous – M phase
  - Rough – R phase
  - …

Colony appearance - haemolysis on blood agar

- β haemolysis
- α haemolysis = viridation

Semi-quantification of grown colonies

- Significant bacteriuria
  - Always ≥ 10^5 CFU/mL; details in clinical microbiology



DG: N23 / Neurčená renální kolika
Odd: **UROU** / Urologie - urgentní příjem
Odběr: 28.02.2024

Poj: 111 / VZP
Lékař: MUDr. Pokorný Daniel
Příjem: 29.02.2024-08:48

Číslo vzorku: CH-24-3108
IČZ: 05002079 Odb: 706
Ukončení: 02.03.2024-14:14

Materiál: **Moč** střední proud
Vyšetření: moč - kultivace
Neshody na příjmu:
Chybějící údaje na žádance:
datum a čas odběru neuveden, elektronická žádanka neodeslána. Nelze posoudit validitu vzorku pro interpretaci výsledku vyšetření.

PRIMOKULTIVACE

Nález 1: **Escherichia coli** kvant 10^6

ANTIBIOGRAM (disková difusní metoda)

ampicilin	R	amoxicilin /klavulanát	C
fosfomycin	C	ceftazidim	C
kotrimoxazol	R	amikacin	C
nitrofurantoin	C	piperacilin /tazobactam	C
ciprofloxacin	C	cefepim	C
mecillinam	C	ertapenem	C
cefuroxim	C	imipenem	C
gentamicin	C	meropenem	C
cefotaxim	C		

Zkratky: C = citlivý, R = rezistentní, I = intermediální, * = výsledek k dispozici po konzultaci s ATB střediskem

Kódy pro pojišťovnu: 82015(1), 82058(1), 82063(3)

Central venous catheter culture

- Maki´s method (rolling)
- Sonification
- Semi-quantification

Cultivation of a central venous catheter

DG: D481 / Novotvar NNCH - pojivová a jin	Poj: 111 / VZP	Číslo vzorku: CH-24-3056
Odd: **CH34** / 3.chir.klinika-JIP asept.	Lékař: MUDr. Martínková Vendula, Ph.	IČZ: 05002241 Odb: 5T1
Odběr: 28.02.2024-09:51	Příjem: 28.02.2024-10:56	Ukončení: 01.03.2024-12:51

Materiál: **Katetr cévní** centrální žilní
Vyšetření: cévní katetr - vyšetření

MAKI

Nález 1: **Staphylococcus epidermidis** > 15 CFU

ANTIBIOGRAM (disková difusní metoda)

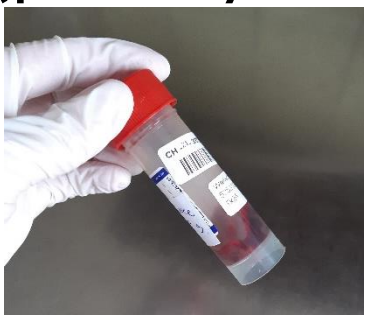
oxacilin.....	R	rifampicin.....	C
kotrimoxazol.....	R	ofloxacin.....	R
erythromycin.....	C	vankomycin.....	C
klindamycin.....	C	teikoplanin.....	C
tetracyklin.....	C	gentamicin.....	R

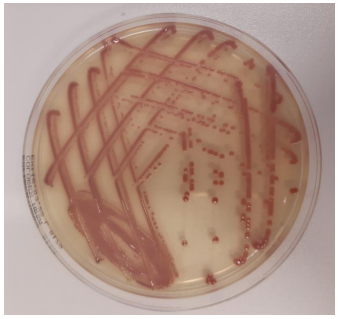
SONO

Nález 2: **Staphylococcus epidermidis** kvant 10^2

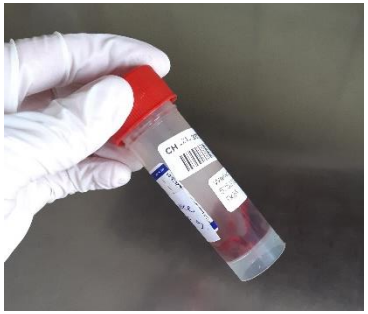
Zkratky: C = citlivý, R = rezistentní, I = intermediální, * = výsledek k dispozici po konzultaci s ATB střediskem

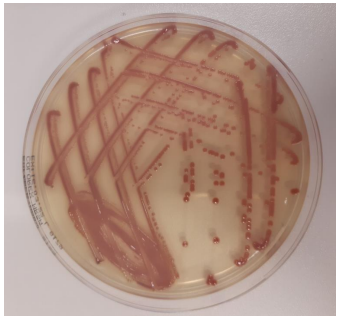
- Primokultivace (primary culture)





- Pomnožení (liquid medium)





Žadatel: Pneumologie ambulance běžná (05002206) Příjem: 13.02.2024-11:59
Lékař: MUDr. Ngo Viet Nghia Uzavřeno: 17.02.2024-09:33
Diagnóza: R05 Kašel Plátce: 111 VZP

Protokol: IN-24-2595 --- Konečný výsledek ---
Vzorek: **Aspirát DCD** fibroaspirát
Vyšetření: DCD - kultivace

MIKROSKOPICKY

Preparát z klinického materiálu: buněčná drť, řasinkové epitelie, leukocyty ojediněle, bez mikrobů

PRIMOKULTIVACE

Nález: viridující streptokoky, neisserie

POMNOŽENÍ

Nález 1: **Klebsiella pneumoniae**

ANTIBIOGRAM (disková difuzní metoda)

ampicilin.....	R	amoxicilin /klavulanát...	C
cefuroxim.....	C	ceftazidim.....	C
kotrimoxazol.....	C	cefepim.....	C
ciprofloxacin.....	C	piperacilin /tazobactam..	C
tetracyklin.....	C	ertapenem.....	C
gentamicin.....	C	imipenem.....	C
amikacin.....	C	meropenem.....	C
cefotaxim.....	C	tigecyklin.....	C

Mikroaerofilní kultivace

Nález: dtto

Screening na kvasinky a plísně

Nález: negativní

Výsledek prodloužené mykologické kultivace sdělíme v případě pozitivity dodatečně - celková délka kultivace je 5 dní.

Zkratky: C = citlivý, R = rezistentní, I = intermediální, * = výsledek k dispozici po konzultaci s ATB střediskem

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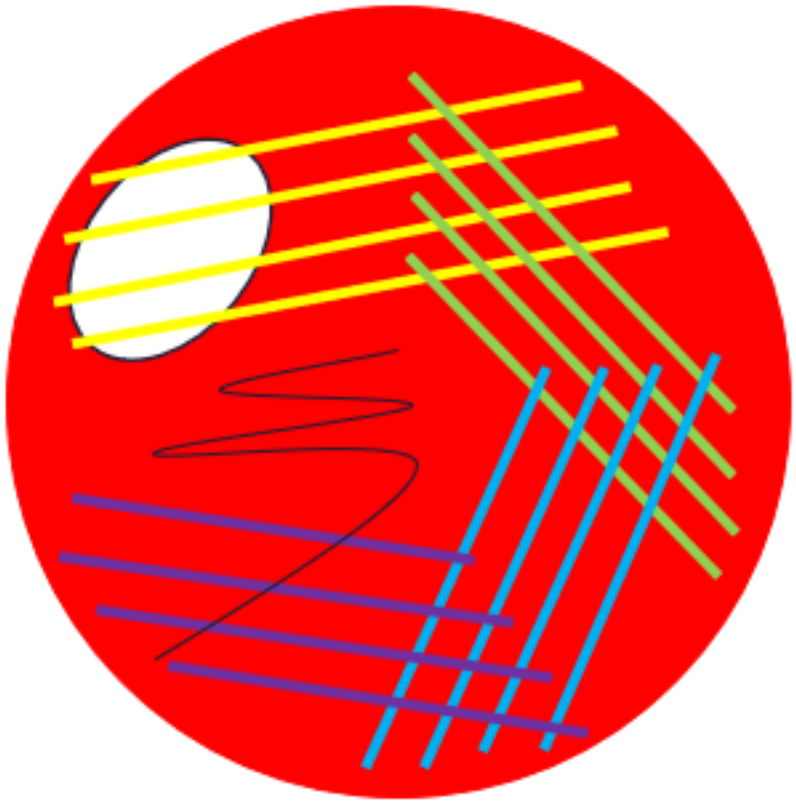
Kódy pro pojišťovnu: 82019(1), 82031(1), 82049(1), 82057(3), 82058(4), 82063(3), 98111(1)

LAB kontrola: Prudíková Olga VŠ kontrola: doc. MVDr. Melter Oto, Ph.D.

- Disadvantages of cultivation:
  - Time
  - Does not allow identification at the genus and species level
  - Not all pathogens can be (easily) cultured
- Advantages over PCR
  - Antibiotic susceptibility testing possible
  - Viability of microorganism
  - Detection of a wider range of agents (but 16S PCR and sequencing)
  - Time
  - Economic costs

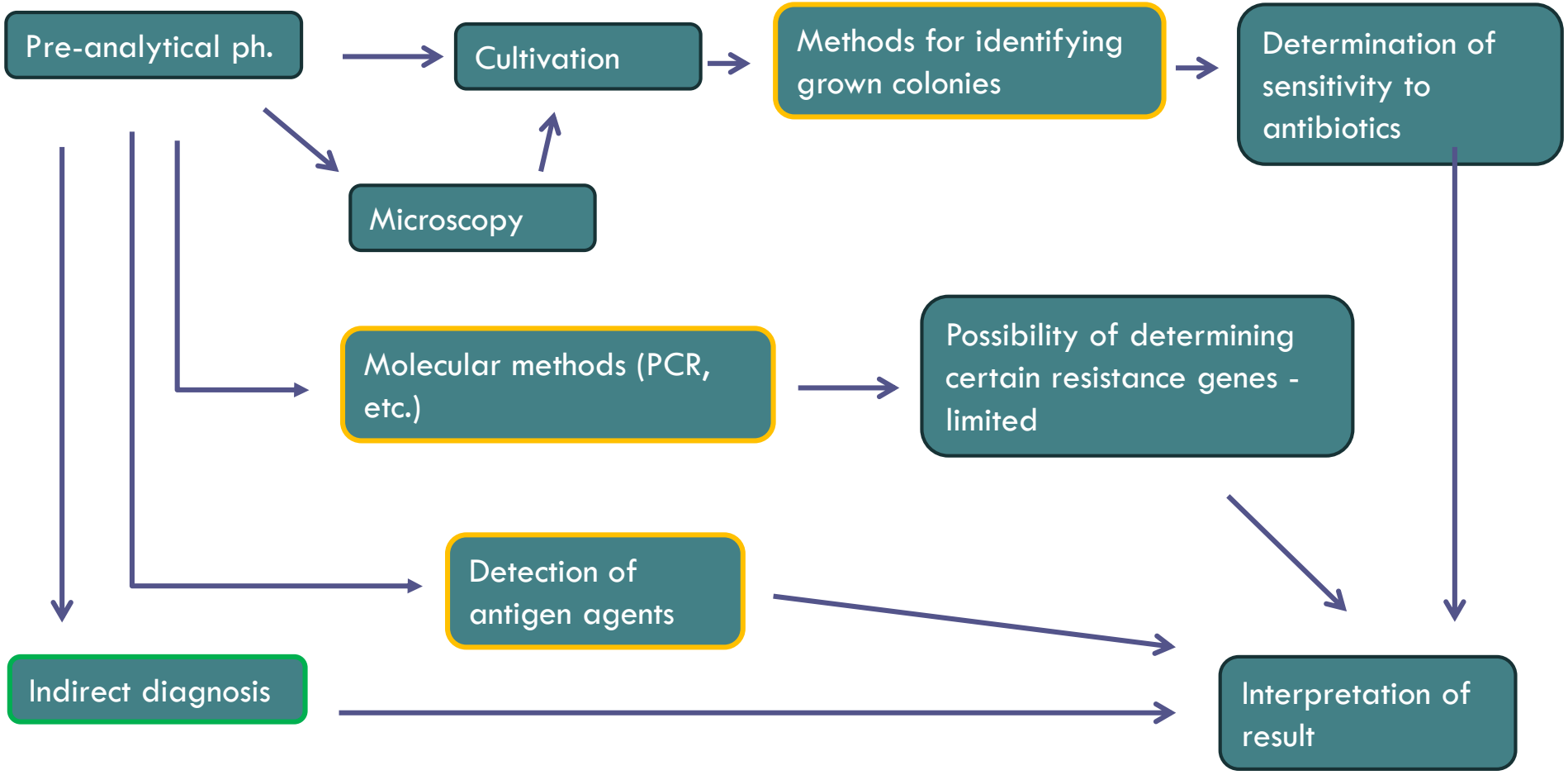
Practical part

- Inoculation of a strain to determine ATB sensitivity (for the next practical lesson)
- Collection of own samples – voluntary



Identification of bacteria

Microbiological testing in bacteriology



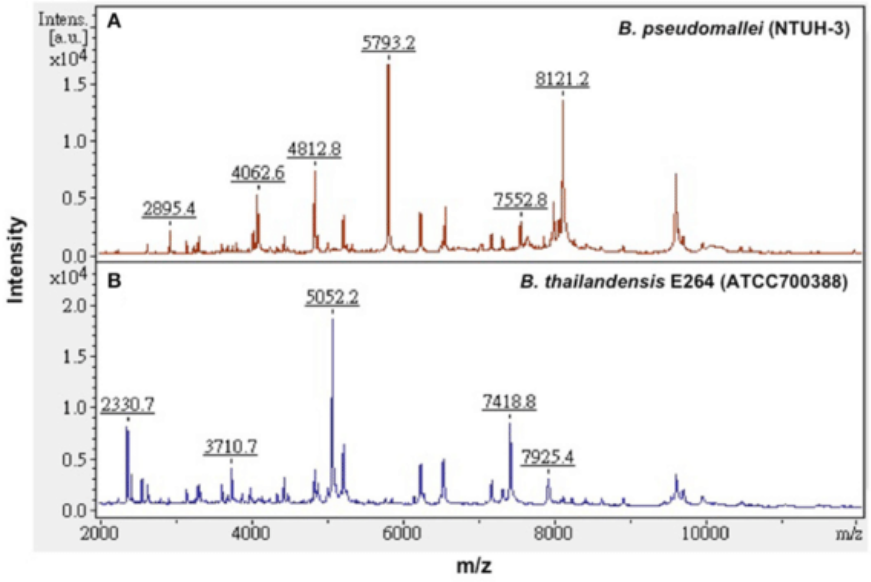
Identification of grown colonies

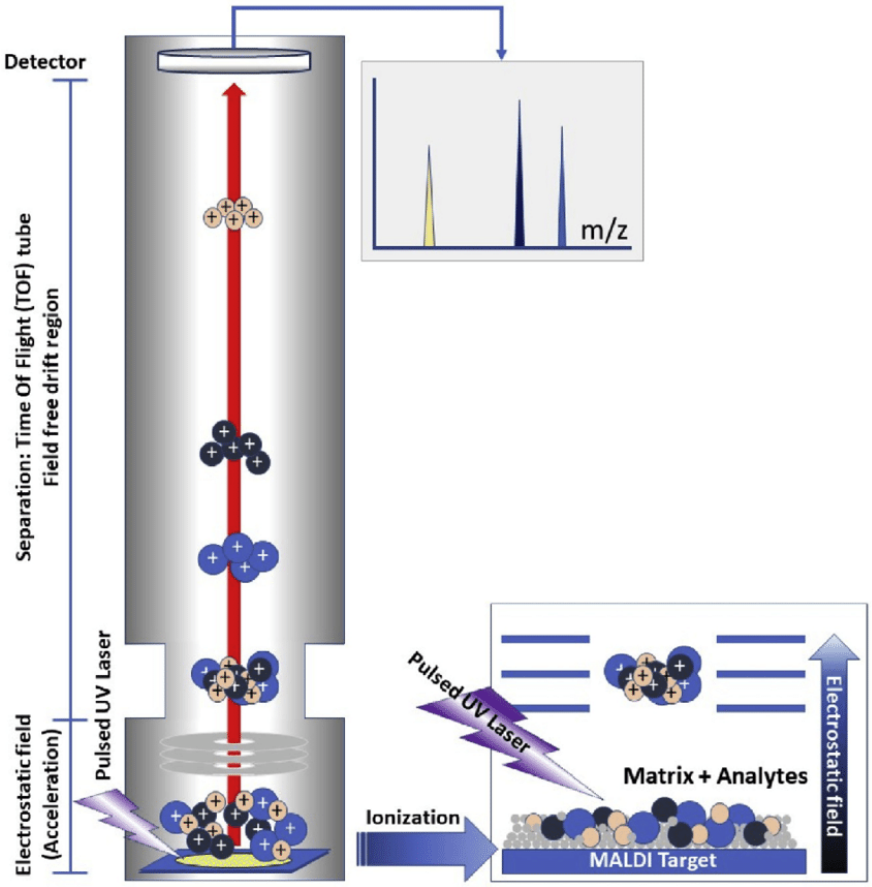
- MALDI-TOF
- Biochemical analysis
- Agglutination
  - Details in the practical course Serological methods

MALDI-TOF

(Matrix Assisted Laser Desorption Ionisation - Time Of Flight)

- Mass spectrometry
- The spectrum obtained is compared with the database
- video



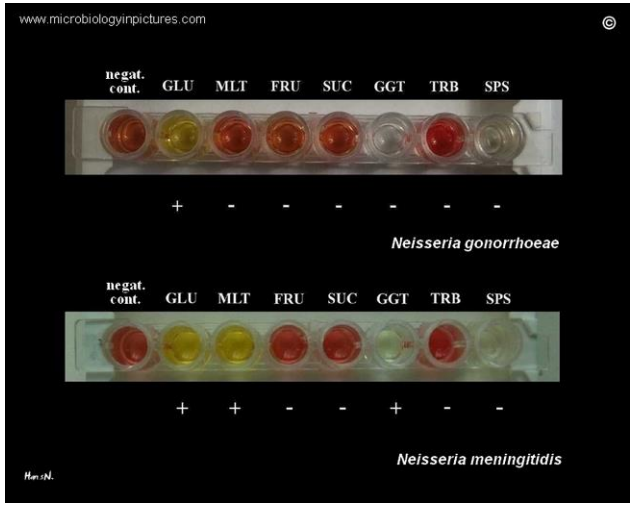


https://www.researchgate.net/figure/e-MALDI-TOF-MS-methodology-The-sample-is-co-crystallized-with-the-matrix-on-the-sample_fig1_330754933

https://www.researchgate.net/figure/Characteristic-MALDI-TOF-MS-spectra-Characteristic-spectra-of-A-B-pseudomallei_fig2_300087754

Biochemical analysis

- Test for the presence of specific enzymes, production of substances
  - Oxidase, catalase, coagulase, urease, PYR test, H_2S …
- Commercial kits
  - Enterotest, Neisseriatest



https://www.microbiologyinpictures.com/bacteria-photos/neisseria-gonorrhoeae-photos/neisseria-gonorrhoeae-biochemical-tests-for-identification.html

Further identification options

- Bile solubility test (*Streptococcus pneumoniae* vs. viridans streptococci)

Agglutination – detection of surface Ag

- Agglutination
- Differentiation of streptococci
- Detailed identification of certain enterobacterales (E. coli, Salmonella, etc.), ...

More in practical course Serological methods

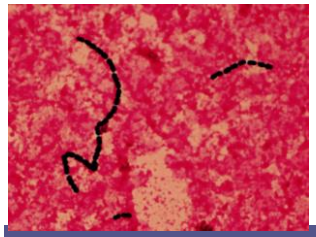
Blood culture

Blood culture

- Specific culture test detecting the presence of bacteria (or fungal organisms) in the bloodstream
- Sepsis, infective endocarditis, catheter infections, lobar pneumonia, etc.

Blood culture bottles

- 4-6 bottles, 8-10 ml each
- Blood culture – 1 sample (usually 4-6 bottles)
- BACTEC
- Duration of blood culture
  - Aerobic bottles – 5 days
  - Anaerobic bottles – 10 days
  - Paediatric, fungal bottles – 14 days



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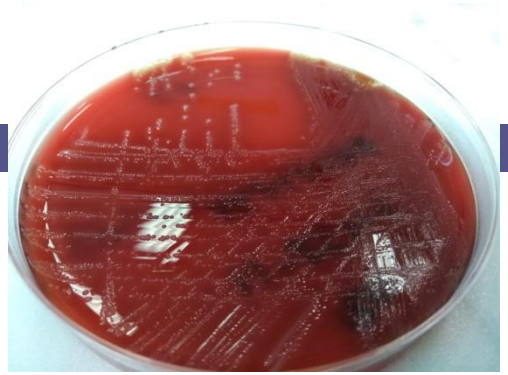
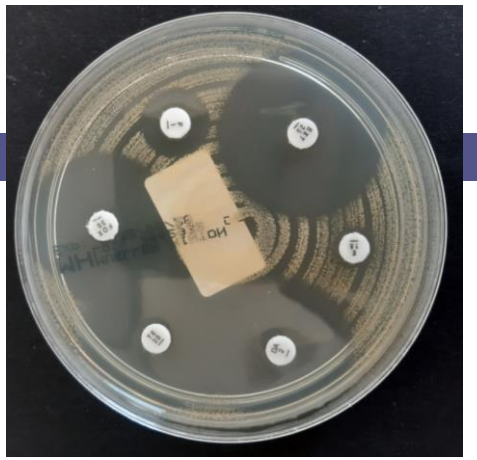




Blood culture positivity
- microscopy
- reporting to the department

Plating on culture agar and cultivation

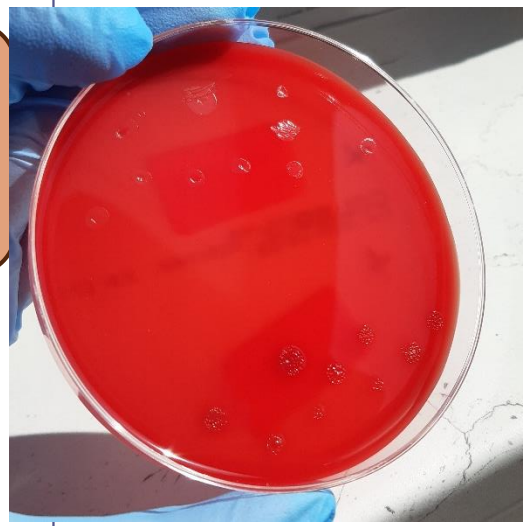
→ Identification of agents from grown colonies

→ Determination of antibiotic sensitivity

\+

Method for rapid identification of agents

→ Determination of antibiotic sensitivity
- possible "from the bottle"



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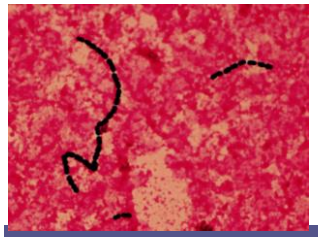
RAST – Rapid antibiotic susceptibility testing (preliminary, for some agents) "from the bottle", within 4-6 hours after positivity



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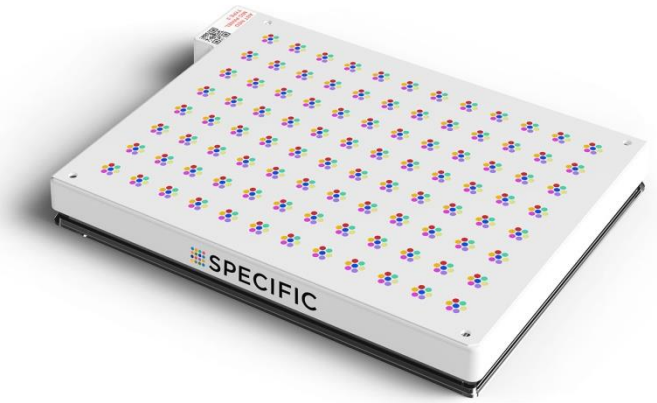
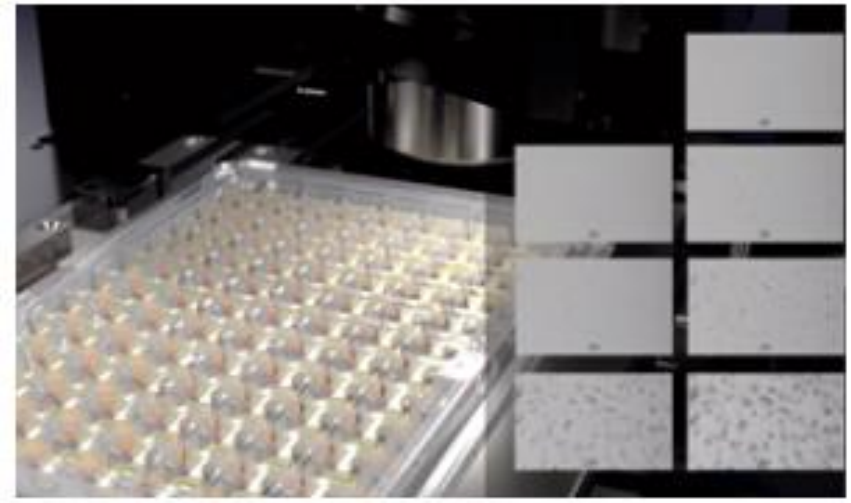
Blood culture positivity
- microscopy
- reporting to the department

Rapid identification of bacteria (PCR from positive bottle)



Automatic RAST systems (Rapid Antibiotic Susceptibility Testing)



BACTEC, MALDI-TOF

- Cultivation is a fundamental part of bacterial diagnostics.
- Cultivation alone is usually not sufficient to identify the bacterial species – identification methods such as MALDI-TOF or biochemical analysis are required.
- The standard cultivation time for bacteria is 24 hours, and in some cases even longer.
- Cultured microorganisms can be tested for their sensitivity to antibiotics (see next practical).
- If certain fastidious bacteria are suspected, it must be specified on the request form.
- Not all bacterial species are detected by cultivation in clinical practice (difficult to culture).