



Microbiology I.
Practical training



2. LÉKAŘSKÁ FAKULTA
UNIVERZITA KARLOVA



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KARLOVA

Bacterial culture and procedures for bacterial identification

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Motol University Hospital

Methods of direct proof

What to choose?

Sample

Microscopy

Cultures

PCR / Antigen test

MALDI TOF MS

Biochemistry tests

Serotyping

Identification methods

Cultures

```
graph TD; A[Cultures] --- B[What is it?]; A --- C[How is it done?]; A --- D[What are we proving by that?]; A --- E[What agar plates do we use?]; A --- F[What are the benefits?];
```

What is it?

How is it done?

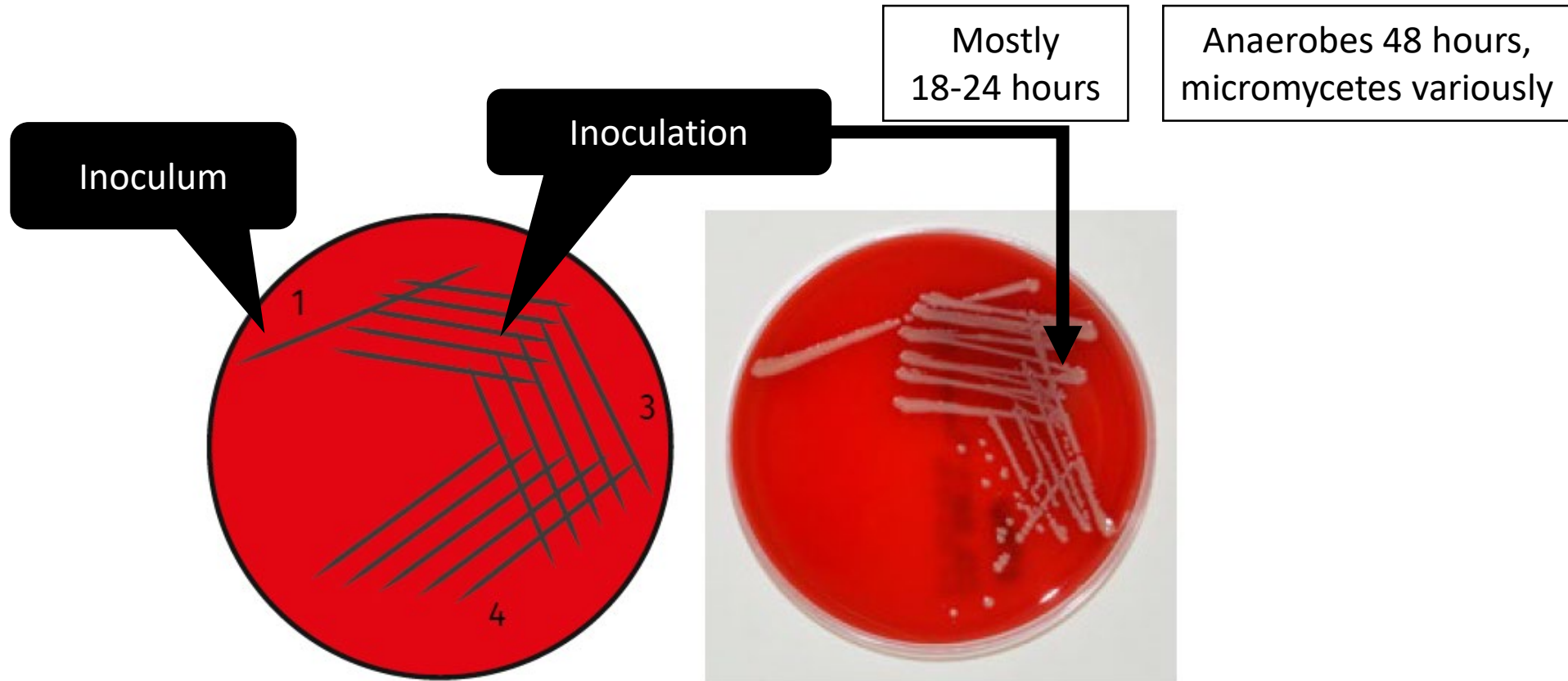
What are we proving by that?

What agar plates do we use?

What are the benefits?

Bacterial culture

= Targeted and controlled bacterial multiplication for their detection



Apart from bacteria: fungi, less parasites and rarely viruses

Cultures

```
graph TD; A[Cultures] --- B[What is it?]; A --- C[How is it done?]; A --- D[What are we proving by that?]; A --- E[What agar plates do we use?]; A --- F[What are the benefits?]; subgraph Box [ ]; E; F; end
```

What is it?

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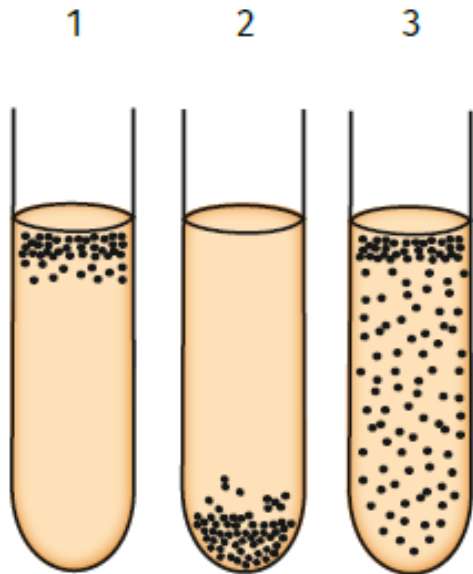
What are the benefits?

Culture media

They provide an energy substrate for the cultivation of the microbe

Two basic types:

1) Liquid



- 1 – Gathering at top
- 2 - Gathering at bottom
- 3- Turbid growth

Thioglycolate broth

Conditions	Possibilities
Energy source	In the plate / medium
Atmosphere	O ₂ vs. CO ₂ vs. anaerostat
Temperature	25°C vs. 37°C
Another	Humidity, pH, pressure

2) Solid



Composition of Thioglycollate Broth

Ingredients	Gms/liter
L-cystine	0.50
Sodium chloride	2.50
Glucose	5.50
Yeast extract	5.00
Pancreatic digest of casein	15.0
Sodium thioglycollate	0.5

Final pH (at 25°C): 7.1 ± 0.2

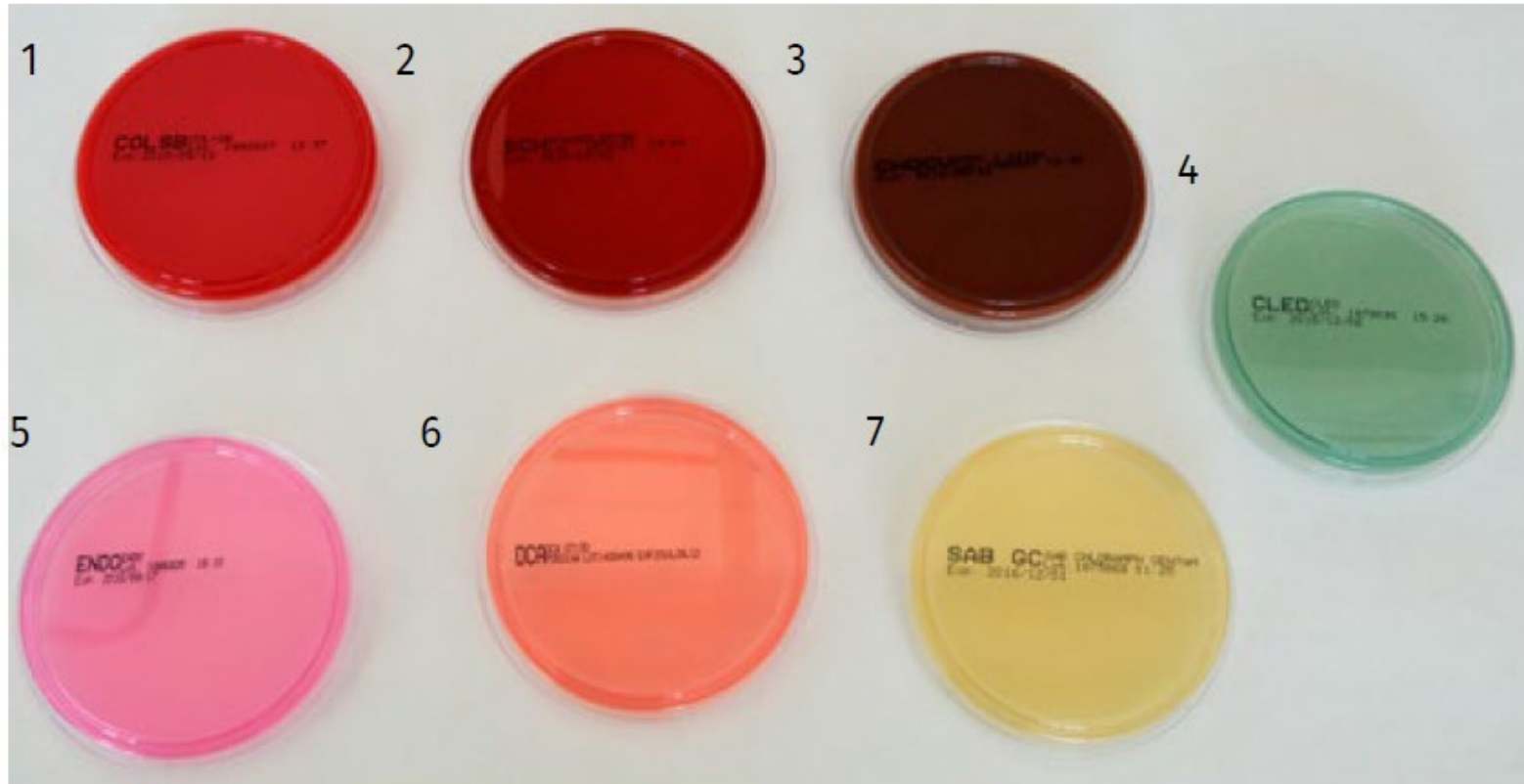
<https://microbenotes.com/thioglycollate-broth/>

Common ingredients of culture media

- **Peptone**- source of carbon and nitrogen.
- **Beef extract**- source of amino acid, vitamins, minerals.
- **Yeast extract**- source of vitamin, carbon, nitrogen.
- **Distilled water**
- **Agar**- solidifying agent.

<https://microbenotes.com/types-of-culture-media/>

Solid media



1 – blood agar, 2 – Schaedler agar, 3 – chocolate agar, 4 – CLED agar,
5 – MacConkaey agar, 6 – deoxycholate-citrate agar, 7 – Sabouraud agar

Solid media

Including both basic and selective ones



Medium	Usage
Blood agar	Almost everything, especially G+ bacteria (hemolysis)
Schaedler's agar	Anaerobes
Chocolate agar	Fastidious bacteria
CLED agar	Urine
MacConkey agar	G- bacteria
Deoxycholate agar	<i>Salmonella</i>
Sabourad agar	Yeasts
Karmali agar	<i>Campylobacter</i>
CIN agar	<i>Yersinia</i>
Müller-Hinton agar	AST

Cultures

```
graph TD; A[Cultures] --- B[What is it?]; A --- C[How is it done?]; A --- D[What are we proving by that?]; A --- E[What agar plates do we use?]; A --- F[What are the benefits?]; style F stroke:#f00,stroke-width:2px
```

What is it?

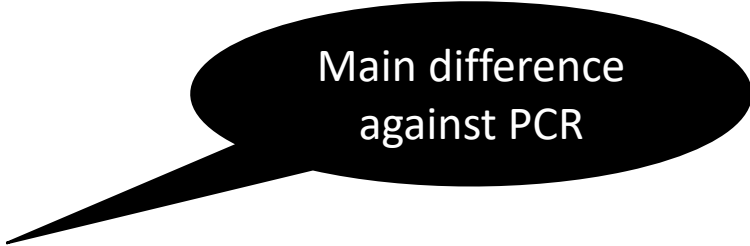
How is it done?

What are we proving by that?

What agar plates do we use?

What are the benefits?

Přednosti kultivace

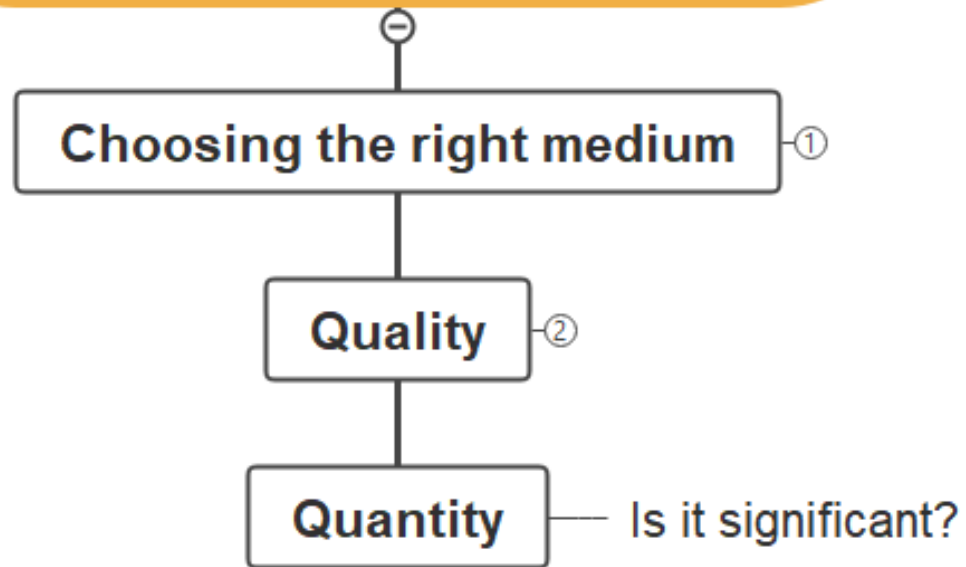


Main difference
against PCR

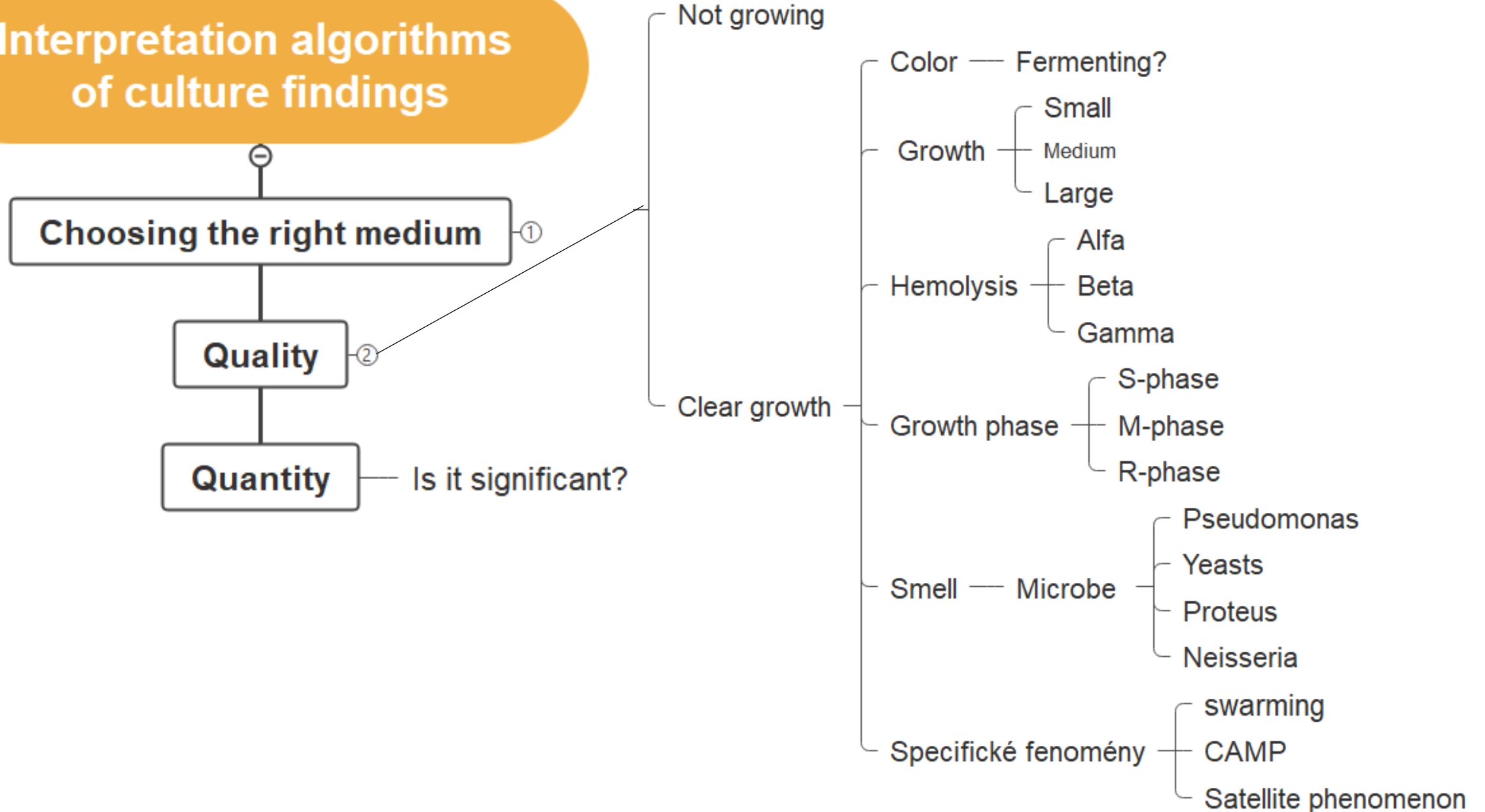
- Everything depends on the acquisition of live culture
- Detection of properties and phenotypes
 - Hemolysis – clarification with the destruction of erythrocytes
 - Phase S, M, R
- Determination of AST (Antibiotic Susceptibility Testing)
- Possible serotyping - if pathogenetically or epidemiologically important

Culture interpretation

Interpretation algorithms of culture findings



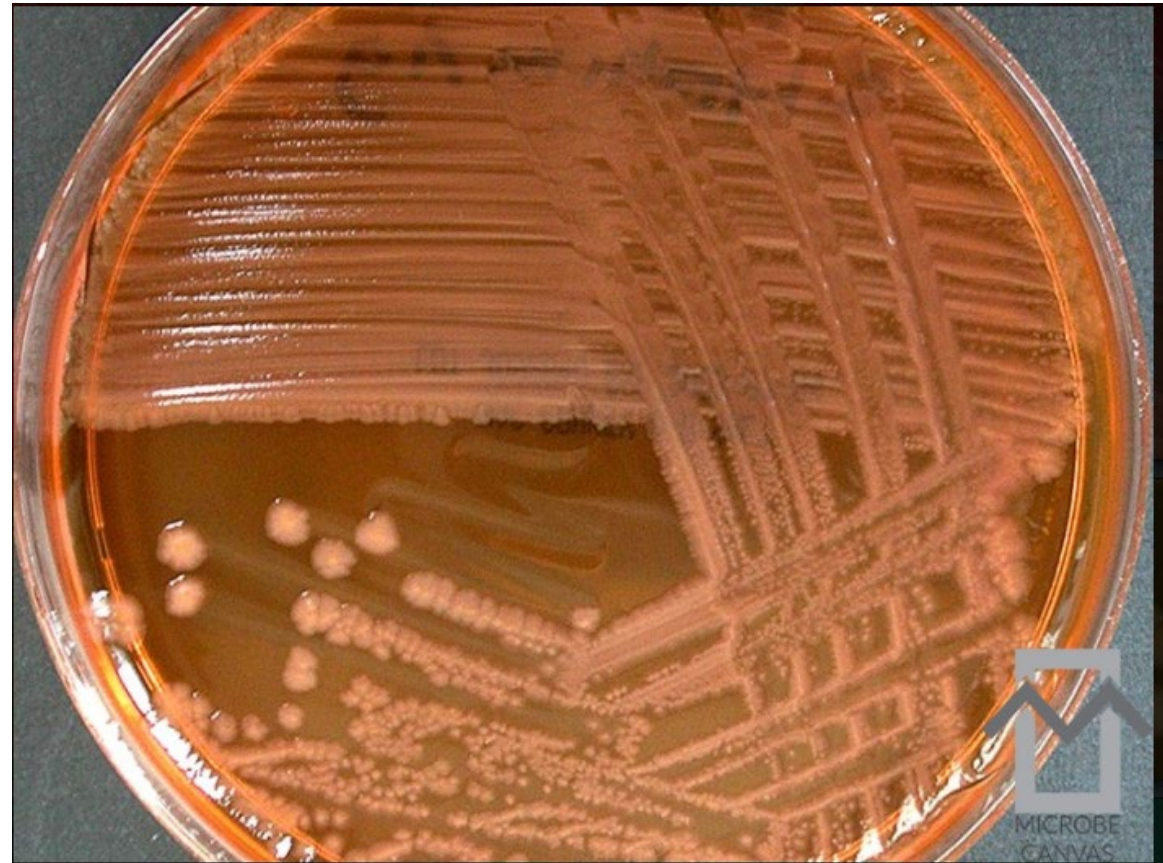
Interpretation algorithms of culture findings



Color – lactose fermentation



Lac +: *Escherichia*, *Citrobacter*,
Enterobacter and *Klebsiella*



Lac - : *Shigella*, *Salmonella*, *Serratia* or non-
fermenting rods

Color – lactose fermentation, what to do next



Lac+: *Escherichia*, *Citrobacter*,
Enterobacter and *Klebsiella*

- When is that not enough?
- Serotyping/agglutination

Type	Note
O157:H7	Word
O26	CZE
O104:H4	Epidemics
O103	Often cause of HUS
O111	Often cause of HUS
O1415	Often cause of HUS

Children under 2
years of age due
to HUS

Haemolytic uremic syndrome (HUS)

= thrombocytopenia, haemolytic anaemia and acute renal failure
Typical for children under 2 years old, in epidemics (summer) - beef, unpasteurized dairy products.

Bakterie EHEC už usmrtila 35 lidí, z toho 34 v Německu

SVĚT 12. června 2011 20:24

BERLÍN - Počet úmrtí na infekci střevní bakterie EHEC v Německu vzrostl o další dvě osoby na 34 a úřady se obávají, že to ještě není konečná bilance už ustupující epidemie.



1 příspěvek



I se Švédkou, která zemřela koncem května, si epidemie vyžádala už 35 mrtvých. Němečtí odborníci se navíc obávají těžkých a možná doživotních zdravotních následků u osob, u nichž se nákaza enterohemoragické E. coli (EHEC) projevila. Světová zdravotnická organizace (WHO) uvádí, že nákaza nebo podezření na ni se objevila dohromady u 3255 osob.

Reklama

První rok vlády

**Covid bez
lockdownů?**

**Zvládli jsme
to společně**

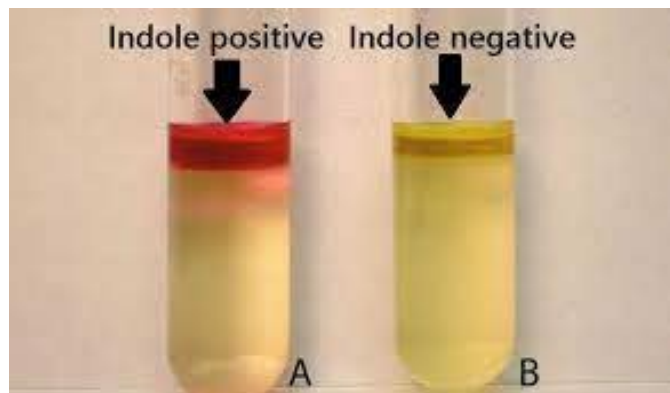


VLÁDA
ČESKÉ REPUBLIKY

Color – lactose fermentation, what to do next

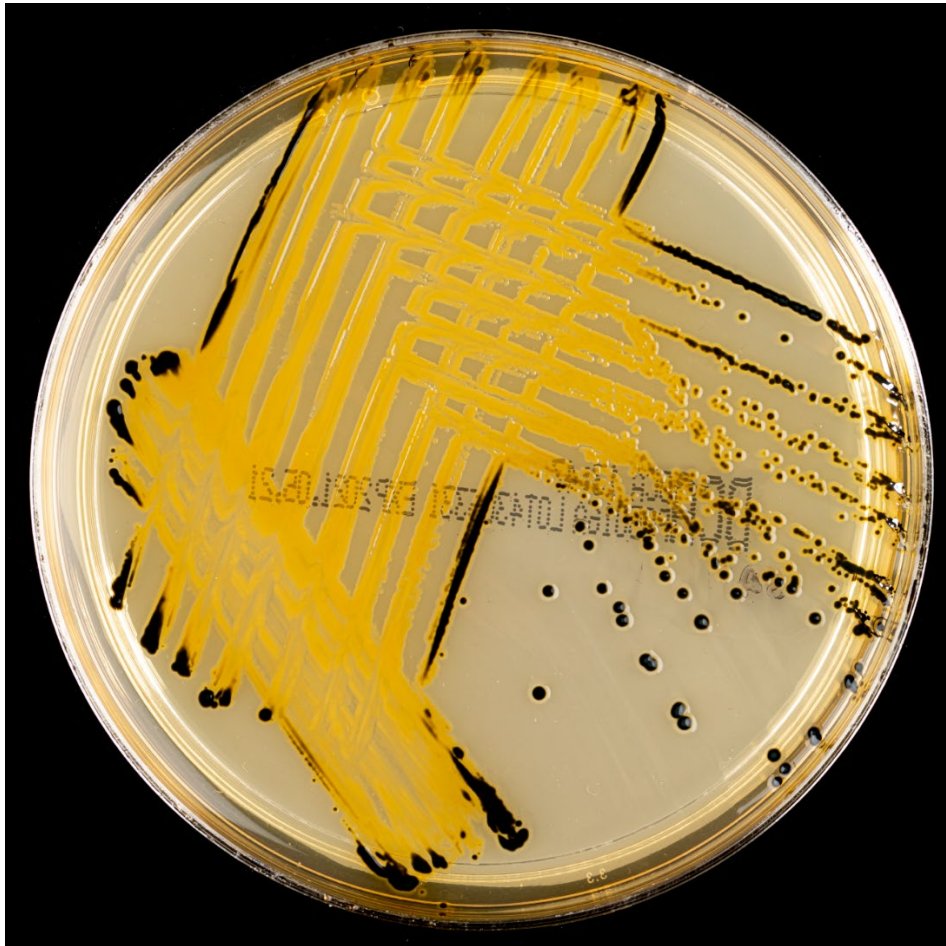
- How do I know it's *Shigella* and not *E.coli*?
 - MALDI-TOF MS will not help

Test	E.coli	Shigella
Gas	+ (bubbles)	- (no)
Indol	+ (pink)	- (yellow)



Lac - : *Shigella*, *Salmonella*, *Serratia* or non-fermenting rods and 10% of *E.coli* strains

Color – H₂S utilization

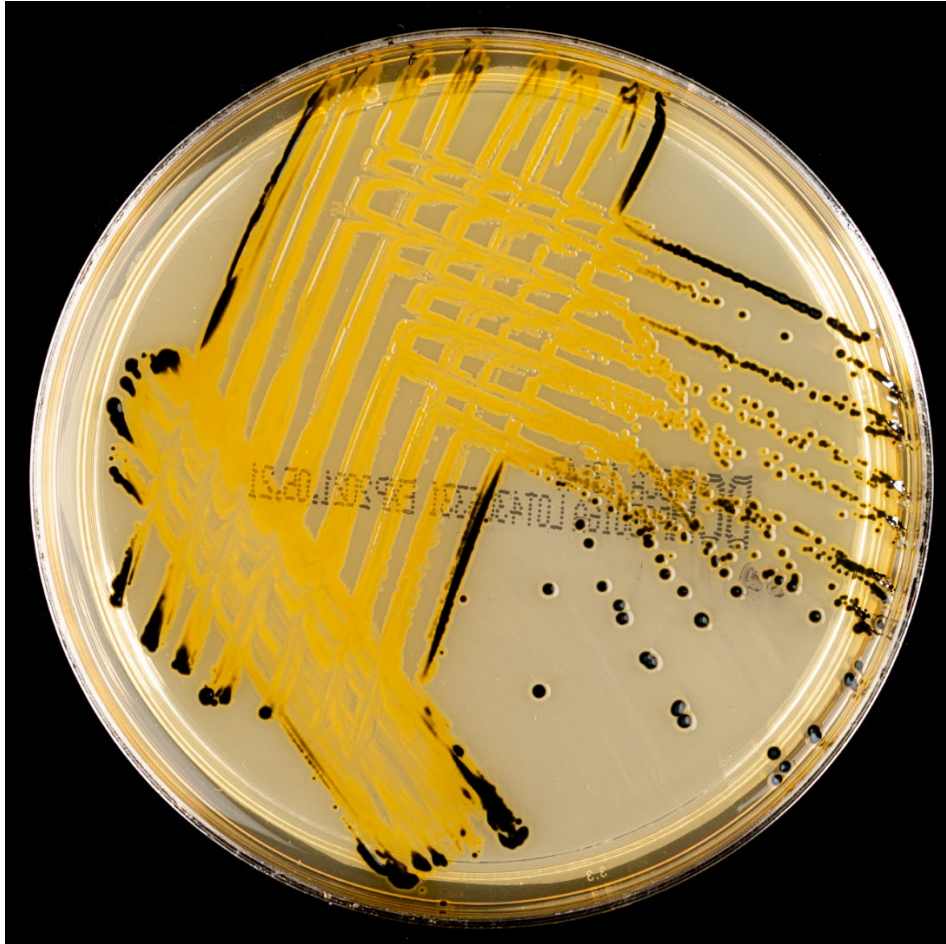


Salmonella and *Citrobacter*

Serotyping

Determinants of O-antigen	Determinants of H-antigen	
1,9,12	g,m	Enteritidis
1,4,12	i	Typhimurium
6, 7, 14	R	Infantis
9,12	d	Typhi (+ capsular antigen Vi)

Color – H₂S utilization



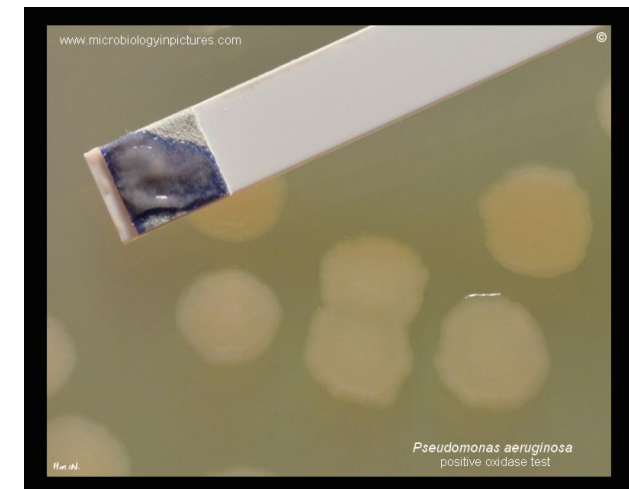
Salmonella and *Citrobacter*

Serotype	O Antigens	H Antigens Phase 1	H Antigens Phase 2
Agona	1, 4, [5], 12	f, g, s	[1, 2]
Anatum	3, {10} {15} {15, 34}	e, h	1, 6
Bareilly	6, 7, 14	y	1, 5
Blockley	6, 8	k	1, 5
Bovis Morbificans	6, 8, 20	r, [i]	1, 5
Brandenburg	1, 4, 12	e, h	e, n, z15
Bredeney	1, 4, 12, 27	l, v	1, 7
Chester	1, 4, [5], 12	e, h	e, n, x
Derby	1, 4, [5], 12	f, g	[1, 2]
Dublin	1, 9, 12, [VI]	g, p	-
Enteritidis	1, 9, 12	[f], g, m, [p]	[1, 7]
Gallinarium	1, 9, 12	-	-
Gloucester	1, 4, 12, 27	i	l, w
Hadar	6, 8	z10	e, n, x
Heidelberg	1, 4, [5], 12	R	1, 2
Indiana	1, 4, 12	z	1, 7
Infantis	6, 7, 14	R	1, 5
Javiana	1, 9, 12	l, z28	1, 5
Kentucky	8, 20	i	z6
Kottbus	6, 8	e, h	1, 5
Lagos	1, 4, [5], 12	i	1, 5
Lille	6, 7, 14	z38	-
Livingstone	6, 7, 14	d	l, w
Mbandaka	6, 7, 14	z10	e, n, z15
Meleagridis	3, {10} {15} {15, 34}	e, h	l, w
Montevideo	6, 7, 14	g, m, [p], s	[1, 2, 7]
Muenchen	6, 8	d	1, 2
Newport	6, 8, 20	e, h	1, 2
Orion	3, {10} {15} {15, 34}	y	1, 5
Paratyphi B	1, 4, [5], 12	b	1, 2
Saintpaul	1, 4, [5], 12	e, h	1, 2
Senftenberg	1, 3, 19	g, [s], t	-
Stanley	1, 4, [5], 12, [27]	d	1, 2
Thomson	6, 7, 14	k	1, 5
Typhimurium	1, 4, [5], 12	i	1, 2
Virchow	6, 7	r	1, 2
Weltevreden	3, {10} {15}	r	z6

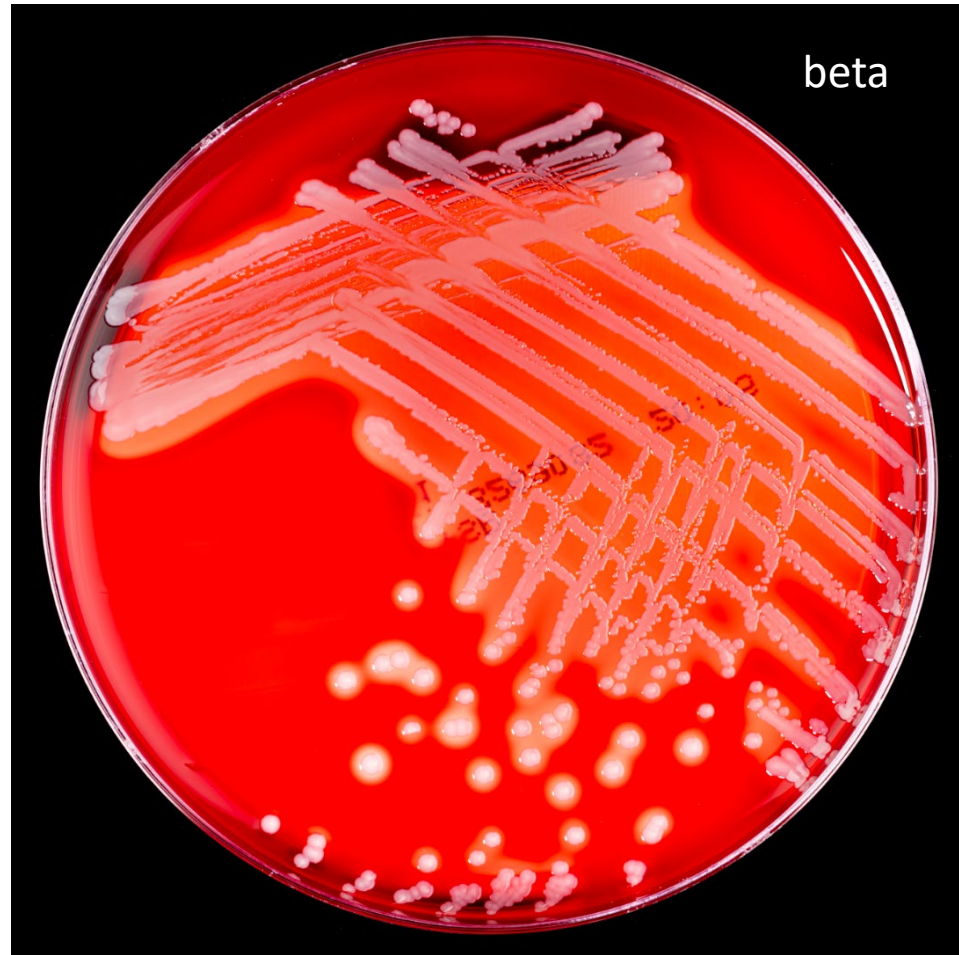
Color – oxidase production



P. aeruginosa



Hemolysis and colony sizes

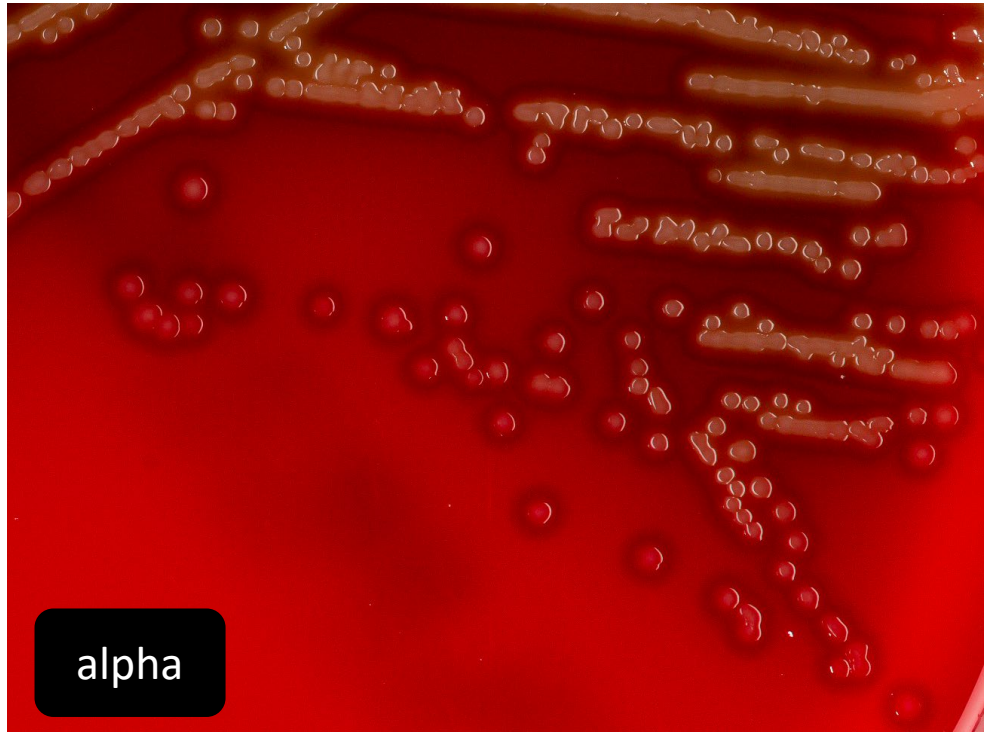


S. aureus

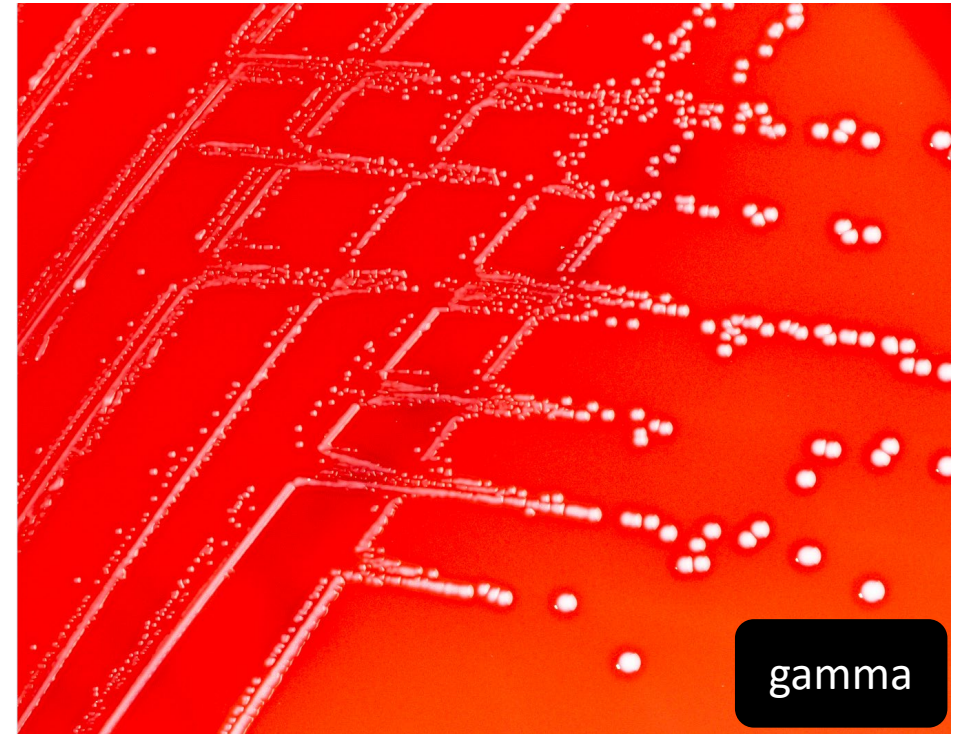


S. pyogenes

Hemolysis and colony sizes



S. pneumoniae



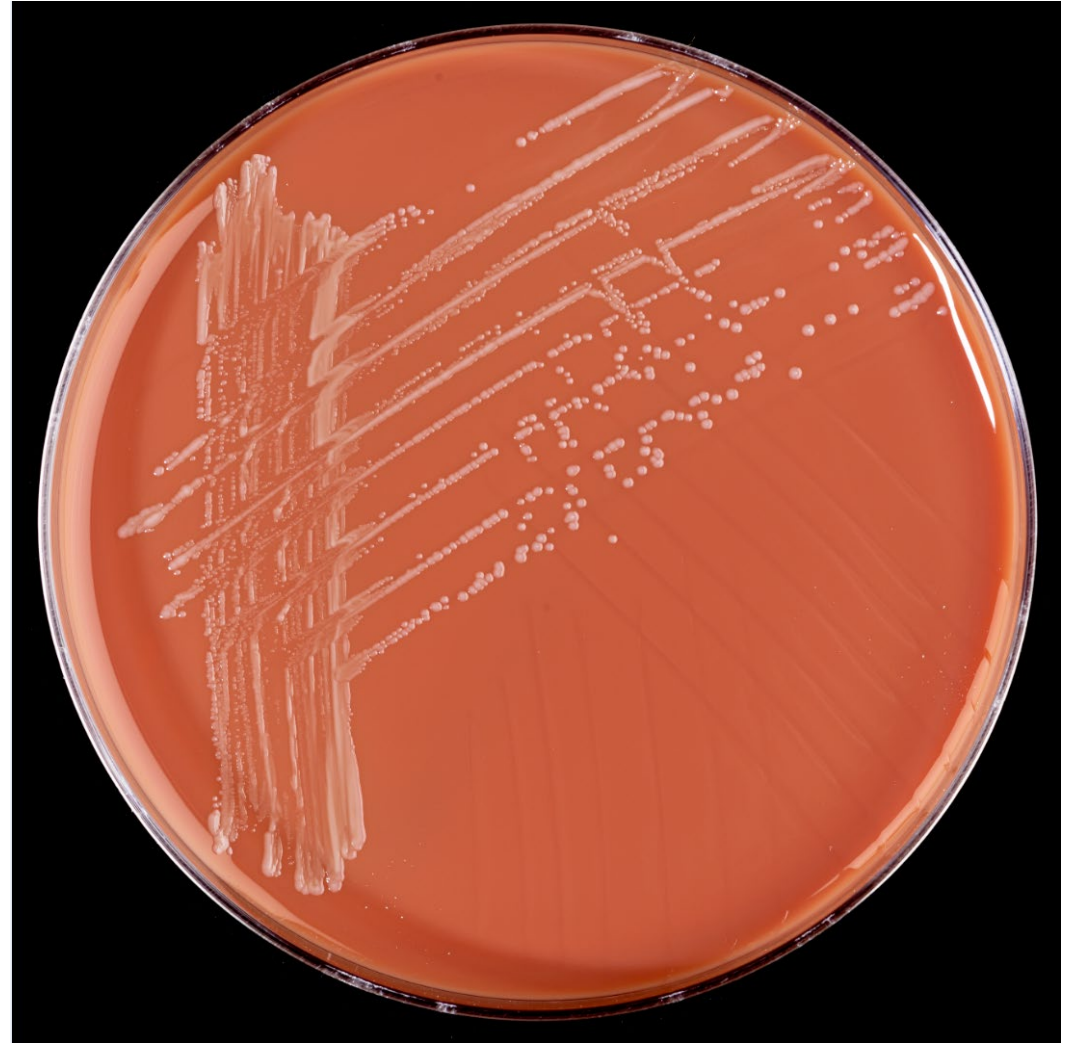
E. faecium

Fastidious bacteria

- Haemophilus
- Neisseria



Satellite phenomenon



Growth on media

- S-phase – normal, smooth
- M-phase – mucosal, mucus
- R-phase – dry, in the absence of r

Streptococcus pneumoniae
(M-phase)



**Encapsulated =
more virulent**

ASM MicrobeLibrary.org © Buxton

Smells of the bacteria

- Proteus
- Pseudomonas
- Neisseria
- Yeasts

Neděle 5. 3. 2023, svátek má Kazimír

TÝDEN.cz

HLAVNÍ STRANA | DOMÁCÍ | ZAHRANIČÍ ▼ | SPORT ▼ | BYZNYS | ZDRAVÍ | KULTURA ▼ | LIDÉ | T

FOTBAL | **HOKEJ** | TENIS | MOTORISMUS | ZIMNÍ SPORTY | OSTATNÍ SPORTY |

[Hlavní strana](#) > [Sport](#) > [Hokej](#) > [NHL](#)

Tady něco smrdí

Lék na únavu i kocovinu. Proč hvězdy NHL šňupají čpavek?



Smells of the bacteria

- **Proteus**
- **Pseudomonas**
- Neisseria
- Yeasts



Smells of the bacteria

- Proteus
- Pseudomonas
- **Neisseria**
- Yeasts



Smells of the bacteria

- Proteus
- Pseudomonas
- Neisseria
- **Yeasts**



Smell**Microorganism**

Dirt, earthy, rain, [geosmin](#)

soil microbes like *Streptomyces*

Fake grape, rotting fruit

Pseudomonas aeruginosa

Corn chips, corn tortillas

Pseudomonas aeruginosa

Butterscotch

Streptococcus anginosus

Yogurt

Lactobacillus

Poop

Escherichia coli Nissile

Poop and vomit

Clostridium

Cream corn or cooked broccoli

Campylobacter

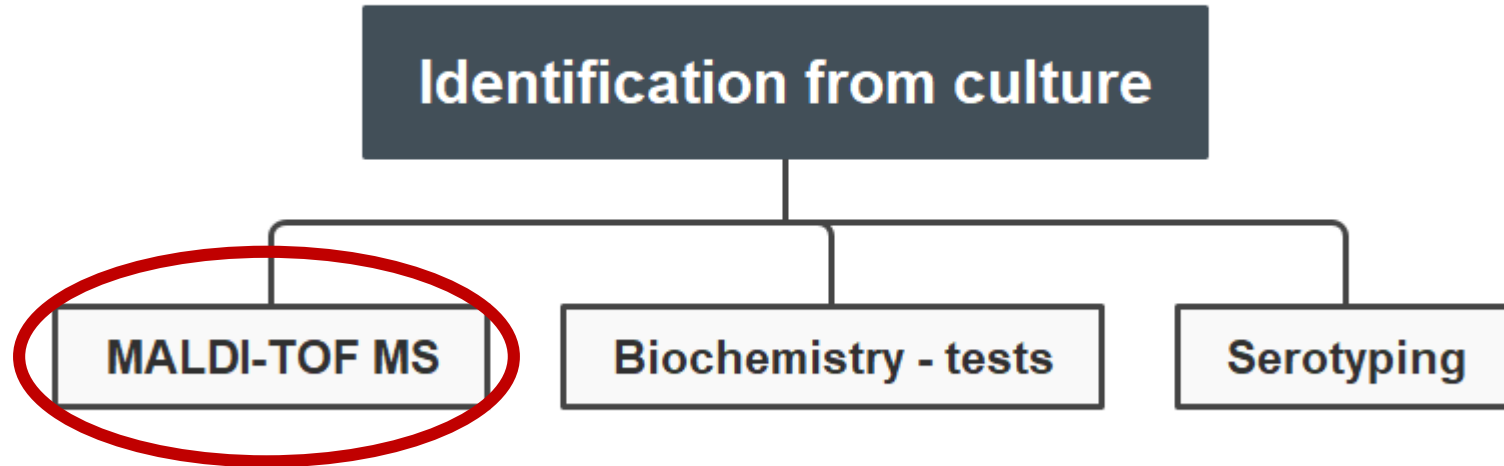
Ammonia and alcohol-ey bread

Exophiala dermatitidis (Black yeast)

New shoe, rubber

Pseudomonas chlororaphis

ID from cultures



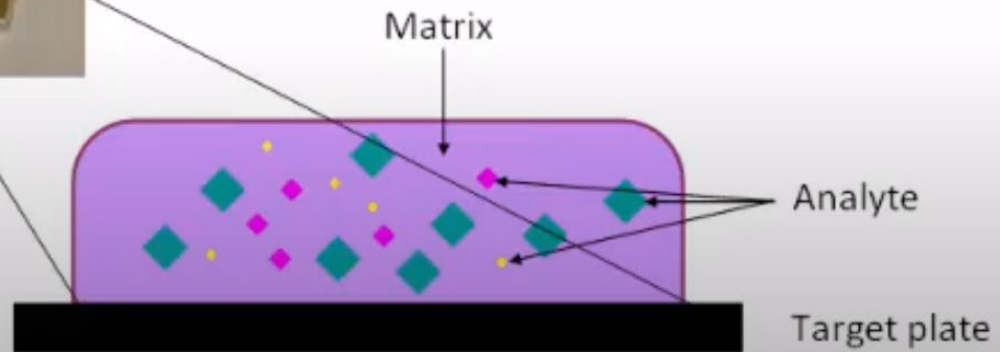
Matrix-Assisted Laser Desorption Ionization Time Of Flight Mass Spectrometry

- Principles of **MALDI-TOF MS**
- Pros and cos

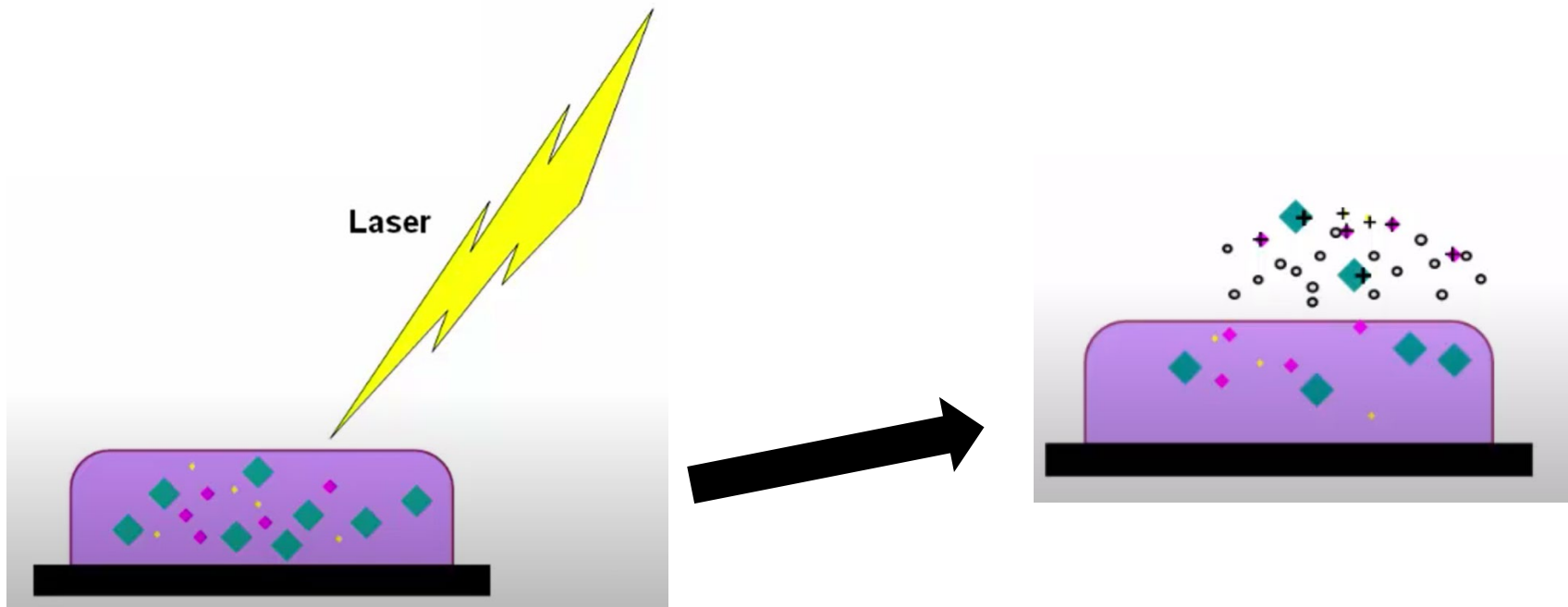


Matrix-Assisted Laser Desorption Ionization Time Of Flight Mass Spectrometry

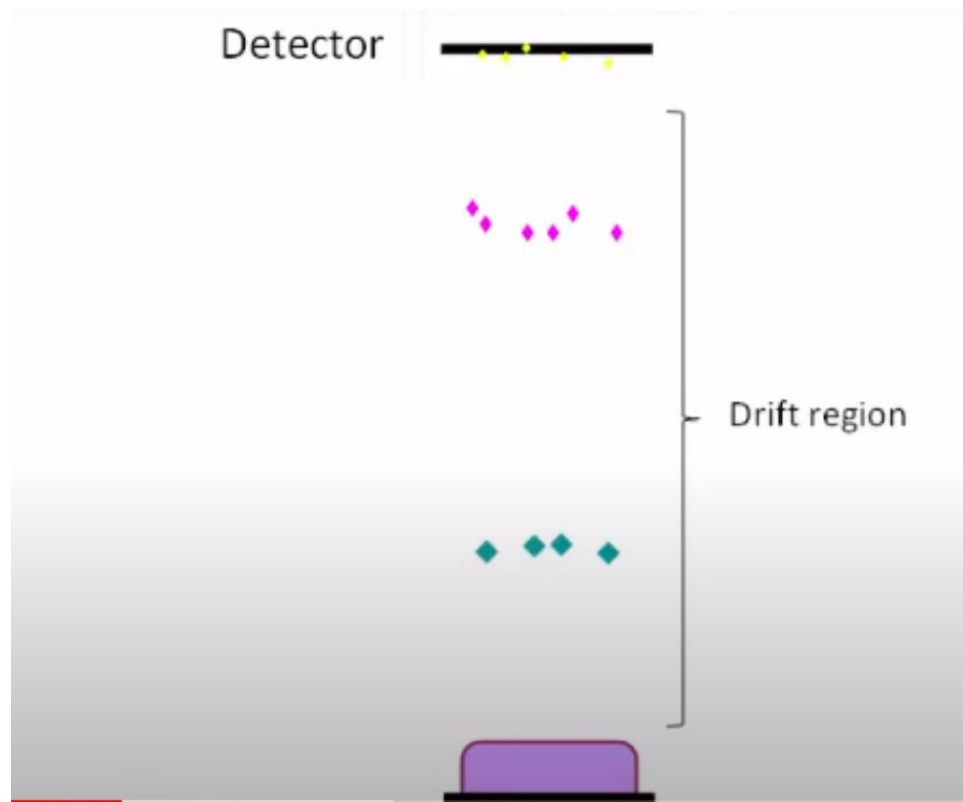
[Video YT](#)



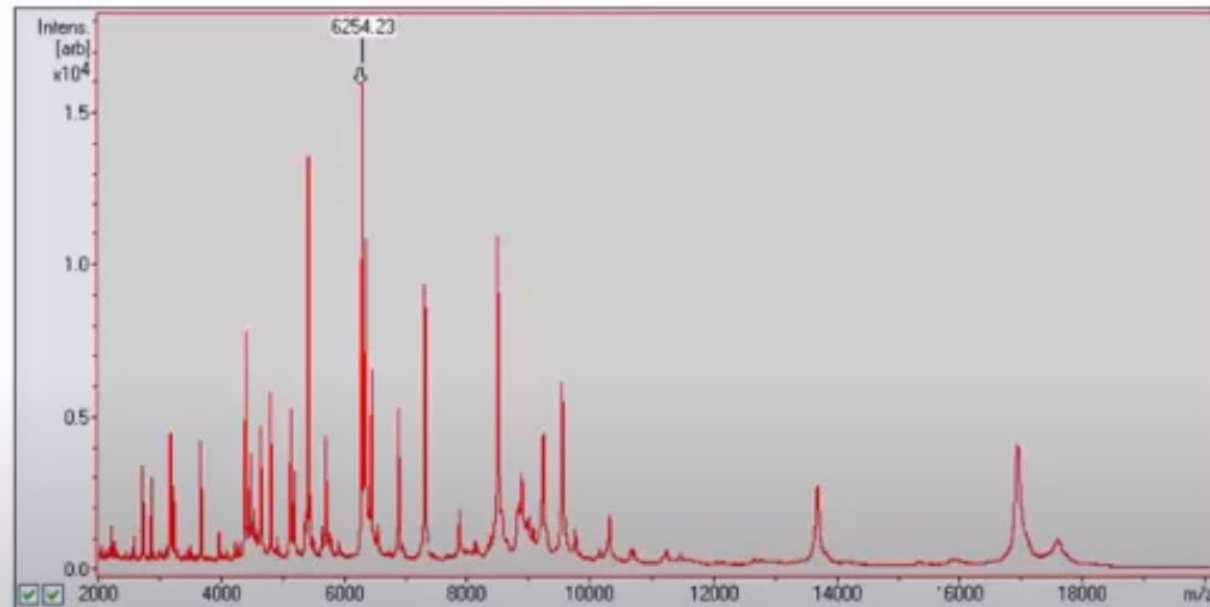
Matrix-Assisted Laser Desorption Ionization Time Of Flight Mass Spectrometry



Matrix-Assisted Laser Desorption Ionization Time Of Flight Mass Spectrometry



Matrix-Assisted Laser Desorption Ionization Time Of Flight Mass Spectrometry



Platforms

- MALDI-ToF MS = gold standard for identification of bacteria
- Four platforms on the european market



BIOTYPER Smart/Sirius
(Bruker, Germany)



VITEK MS
(Biomérieux, France)



AXIMA Performance
(Shimadzu, Japan)



EXS 2600
(Zybio, China)

Result:

Score	Interpretation
≥ 2.00	Reliable species ID
1.70 - 1.99	Reliable genus only ID
≤ 1.69	Not reliable

Hodnoty	✓ Vložit
04.03.2024-08:53:00	
2.13 ++ Clostridioides difficile	
2.04 ++ Clostridioides difficile	
04.03.2024-08:58:00	
2.13 ++ Clostridioides difficile	
2.04 ++ Clostridioides difficile	

Hodnoty	✓ Vložit
10.03.2023-10:28:00	
2.22 ++ Streptococcus pyogenes	
2.21 ++ Streptococcus pyogenes	
10.03.2023-10:33:00	
2.22 ++ Streptococcus pyogenes	
2.21 ++ Streptococcus pyogenes	

Hodnoty	✓ Vložit
04.03.2024-08:53:00	
Nelze změřit	
04.03.2024-08:58:00	
1.76 + Eggerthella lenta	
1.76 + Eggerthella lenta	

Hodnoty	✓ Vložit
04.03.2024-08:53:00	
1.79 + Anaerococcus murdochii	
1.76 + Anaerococcus murdochii	
04.03.2024-08:58:00	
1.79 + Anaerococcus murdochii	
1.76 + Anaerococcus murdochii	

Hodnoty	✓ Vložit
04.03.2024-08:53:00	
Nelze změřit	

How does it work - summary

MALDI-ToF MS (Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry) is an analytical technique used for the identification of microorganisms and other biomolecules. Here is a simplified description of how it works:

- **Sample preparation:** Microorganisms are first isolated from a culture and placed on the surface of a matrix, which is a substance that helps to create ions from the sample.
- **Laser irradiation:** The sample in the matrix is bombarded with a pulsed laser, causing molecules to evaporate from the surface of the matrix along with microorganisms. This process generates ions from molecules in the sample.
- **Ion flight:** The generated ions are accelerated by an electric field towards the detector. In this step, time-of-flight plays a crucial role, which depends on the mass of the ions. Heavier ions have a longer flight time than lighter ones.
- **Detection and analysis:** Ions are detected based on their time-of-flight and used to create a mass spectrum. This spectrum contains peaks corresponding to different masses of ions in the sample.

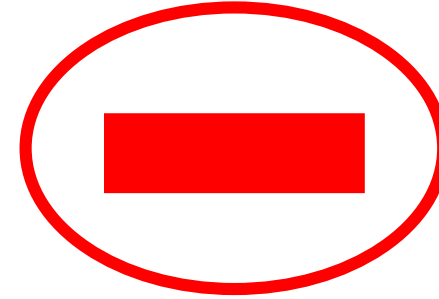
What does it show? The mass-to-charge ratio (m/z) ultimately indicates the mass number (mass) of the ion divided by its charge. This value is crucial for the identification of molecules and microorganisms because each type of molecule has its characteristic mass-to-charge ratio. By comparing the mass spectra of samples with reference spectra, it is possible to identify the composition of the sample.

Advantages




- Cost-effective
- Short processing time, high sample turnover
- Wide range of detected microorganisms
- Interlaboratory reproducibility and adaptability (when having the same supplier)

Limitace



- Does not have a sensitivity test to antibiotics
- Problem detection of some microorganisms (Bruker)
 - **Misidentification: *Shigella* as *E.coli***
 - They do not distinguish related species
 - Klebsiella
 - Enterobacter
 - Listeria
 - Streptococcus mitis group
- Different protocols and databases – may not allow data transfer between laboratories

How does the result look like?

 ÚSTAV LÉKAŘSKÉ MIKROBIOLOGIE UK 2.LF a FN v MOTOLE V Úvalu 84, 15006 Praha 5 - Motol, tel: 224435370 Přednosta: prof. MUDr. Pavel Dřevínek, Ph.D.			
K-23-2382 předběžný výsledek			
Materiál: BAL - bronchoalveolární laváž Vyšetření: DCD - kultivace			
MIKROSKOPICKY			
Preparát z klinického materiálu: řasinkové epitelie, leukocyty ojedíněle, g+koky ojedíněle			
PRIMOKULTIVACE			
Nález 1: viridující streptokoky ojedíněle			
Nález 2: Streptococcus pneumoniae mukozní ojedíněle			
<u>ANTIBIOGRAM (disková difuzní metoda)</u>			
penicilin.....	C	norfloxacin.....	C
ampicilin.....	C	cefotaxim.....	C
erythromycin.....	C	rifampicin.....	C
klindamycin.....	C	vankomycin.....	C
kotrimoxazol.....	C	linezolid.....	C
Nález 3: Neisseria sp			
POMNOŽENÍ			
Nález 4: Streptococcus pyogenes Beta-hemolytický streptokok, skupina A			
Mikroaerofilní kultivace			
Nález 5: Haemophilus influenzae			
<u>ANTIBIOGRAM (disková difuzní metoda)</u>			
ampicilin.....	C	tetracyklin.....	C
amoxicilin /klavulanát.....	C	nalidixová kyselina.....	C
cefuroxim.....	C	chloramfenikol.....	C
cefotaxim.....	C	kotrimoxazol.....	C
Vazby mezi antibiotiky: Kys. nalidixová - screening: Výsledek „citlivý“ znamená, že testovaný kmen je citlivý k ciprofloxacinu, leofloxacinu, moxifloxacinu a ofloxacinu.			
Screening na kvasinky a plísňe			
Nález: negativní Výsledek prodloužené mykologické kultivace sdělíme v případě positivity dodatečně - celková délka kultivace je 5 dní.			
Zkratky: C = citlivý, R = rezistentní, I = intermediální, * = výsledek k dispozici po konzultaci s ATB střediskem			
Výsledek předběžně uvolnil: 09.03.2023-12:09 MUDr. Kabelíková Petra			

Let's take a look!

But before that – sometimes it's unfair, but one game is not the whole [career](#)

Identification from culture

```
graph TD; A[Identification from culture] --> B[MALDI-TOF MS]; A --> C[Biochemistry - tests]; A --> D[Serotyping];
```

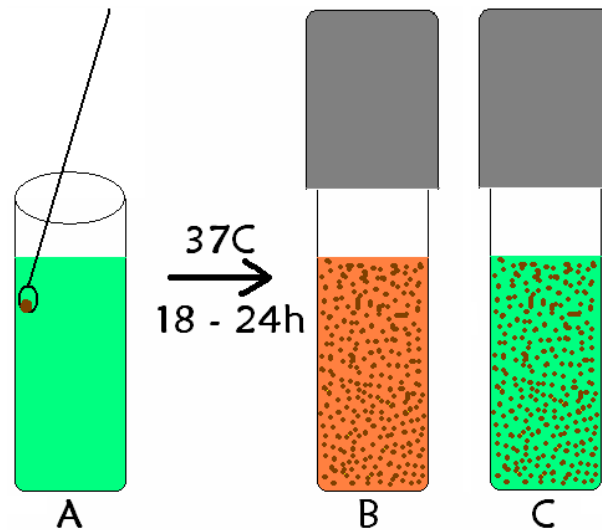
MALDI-TOF MS

Biochemistry - tests

Serotyping

Biochemical tests

Use of enzyme and gas production, fermentation of sugars, etc.
individual tests in a test tube, diagnostic strips
Commercially manufactured kits



Biochemical identification

A. Inoculation of pure microbial culture into liquid soil with substrate (e.g. sugar), pH indicator and cultivation

B POSITIVE REACTION: use of the substrate (e.g. fermentation or oxidation) by microbial culture and colour change of indicator

C. NEGATIVE REACTIONS

Biochemical tests in G-bacteria

DON'T TAKE THE EXAM
– IT'S JUST FOR AN
OVERVIEW

	Glukóza	Laktóza	Sacharóza	Manitol	Urea	Sirovodík	Indol
Salmonella Typhi	+	-	-	+	-	+	-
Většina ost.salmonel	+	-	-	+	-	+	+
Escherichia coli	+	+	+/-	+	-	-	+
Klebsiela pneumoniae	+	+	+	+	+	-	+/-
Enterobakter cloacae	+	+	+	+	+/-	-	-
Proteus mirabilis	+	-	?	-	+	+	-
Morganella morgani	+	-	-	-	+	-	+

Identification from culture

```
graph TD; A[Identification from culture] --> B[MALDI-TOF MS]; A --> C[Biochemistry - tests]; A --> D[Serotyping];
```

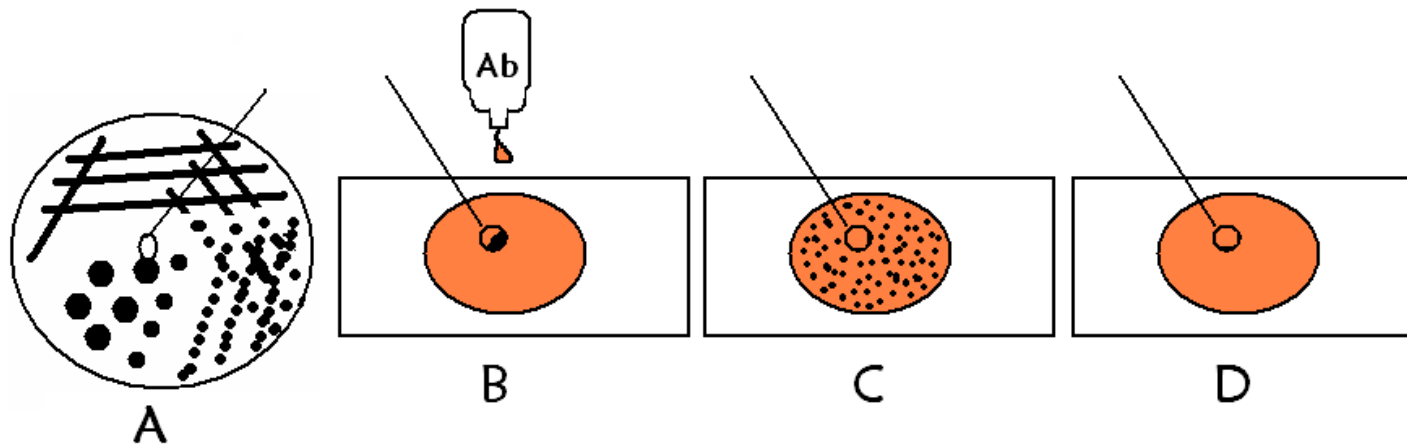
MALDI-TOF MS

Biochemistry - tests

Serotyping

Performing serotyping

- Use the loop to scoop up one colony of the grown culture (A) and mix with the antibody (B) on a slide
- By swinging the slide we mix the suspension and observe the formation of agglutinates visible to the eye (up to about 30 s)(C),
negative reaction (D)



When is it useful?

- Especially for E. coli/shigella and salmonella
- Suitable for the exam



Color – lactose fermentation, what to do next



Lac+: *Escherichia*, *Citrobacter*,
Enterobacter and *Klebsiella*

- When is that not enough?
- Serotyping/agglutination

Type	Note
O157:H7	Word
O26	CZE
O104:H4	Epidemics
O103	Often cause of HUS
O111	Often cause of HUS
O1415	Often cause of HUS

Children under 2
years of age due
to HUS

Haemolytic uremic syndrome (HUS)

= thrombocytopenia, haemolytic anaemia and acute renal failure
Typical for children under 2 years old, in epidemics (summer) - beef, unpasteurized dairy products.

E. coli serotyping

Type	Note
O157:H7	World
O26	CZE
O104:H4	The german epidemics (2011)
O103	Belong to hout the most common EHEC
O111	
O145	

E. coli serotyping based on the detection of:

- somatic O-antigens (174 types),
- flagellum H-antigens (53 types), and
- capsular K-antigens (80 types).

The O-antigen identifies the serogroup, the H-antigen identifies the specific serotype.

More about EHEC on SZÚ website: [zde](#)

Salmonella serotyping

Determinants of O-antigen	Determinants of H-antigen	
1,9,12	g,m	Enteritidis
1,4,12	i	Typhimurium
6, 7, 14	R	Infantis
9,12	d	Typhi (+ capsular antigen Vi)

