

Searching for microbes Part IV.

Biochemical identification

Ondřej Zahradníček

To practical for VLLM0421c

Contacts to me:

777 031 969

zahradnicek@fnusa.cz

ICQ 242-234-100

Survey of parts of this slide show

Identification methods – global principles

Identification methods – problems & solutions

Identification methods – practical approaches (details inside)

Identification methods – not based on substrates

Tale

- Once a sick student told to his sister: I have borrowed some things from my school mates, and now I have to give them back. But I am sick. Would you be so kind and go and give the things to them?
- **Microbiological textbook** belongs to **Peter**. He has **blue eyes** and **blonde hair**.
- **Coloured pencils** belong to **Ahmed**. He has **brown eyes** and **black hair**.
- **The photograph of Britney Spears** belongs to **John**. He has **brown eyes** and **blonde hair**.

The sister knew now:

- It is not possible to use eye colour only to identify her brother mates.
- It is also not possible to use hair colour only to identify them.
- It is necessary to use combination of both.

	Peter	Ahmed	John
Eyes	blue	brown	brown
Hair	blonde	black	blonde

Remember:

- If the sister would meet a monster, half-John, half-Ahmed, she would not be able to identify it.
- We require **pure strains of bacteria to be able to identify them**. It is not possible to work with mixtures of bacteria!

Identification
methods –
global
principles

Survey of direct methods

Method	Specimen examination	Identification
Microscopy	yes	yes
Cultivation	yes	yes
Biochemical identificat.	no	yes
Antigen detection	yes	yes
Animal experiment	yes	usually not
Molecular methods	yes	usually not*

*but in molecular epidemiology – detection of similarity of strains - yes

General principle I

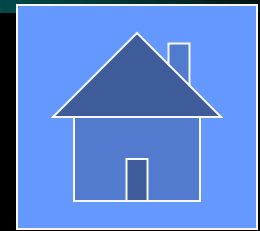
- Bacteria have their **specific metabolism**
- **Industrial microbiology** exploits bacterial metabolism (mostly fermentative catabolism) to production various stuff, including a lot of food
- **Medical microbiology** exploits differences in metabolism between various bacteria
- **Inter-species differences interest us here. Differences between strains are rather a complication**

General principle II

- *Even between mammals there are differences. Human body is not able to produce vitamin C, body of some mammals is.*
- We offer certain **substrate** to a bacterium, and we search, whether bacteria change it into a **product** using an enzyme. A product has to be different from substrate **by physical phase** or **colour**. If it is not different, we use an **indicator**
- **There are a lot of ways technical form of this test type.**

Of course...

- it is big difference, whether bacteria perform **fermentation** or **aerobic respiration**
- it is a difference, whether bacteria breakdown rather **proteins and aminoacids** (e. g. genus *Proteus*) or rather **sugars** (e. g. genus *Klebsiella*)
- breakdown of a certain substrate is often a sign of **adaptation to a certain environment** (well adapted enterobacteria download lactose, that they find in our intestine)



To review...

Do you know, that you have already met such a biochemical test? No? But yes, yes, at cultivation! It is ENDO AGAR

There is a biochemical test in it: it diferenciates bacteria into lactose positive, and lactose negative species.



Photo O. Z.

Identification
methods –
problems &
solutions

Problems

- There are also differences **between strains, not only between species**
- Rarely we can see, that 100 % or 0 % strains of a certain species produce a given enzyme
- More often is 90 %, 10 %, 70 %, 30 %...
- How it can be in practice:

Avinella produces joanellase in 90 % cases

Saantella produces joanellase in 10 % cases

Joanase positive microbe can be:

a typical Avinella, but also

an atypical Saantella

Problems - solution

- If we search for one attribute only, is big probability, that we will meet an atypical strain and identification will be false
- Nevertheless, it is very small probability, that a strain would behave atypically e. g. in ten various tests in the same time
- Therefore the more tests, so bigger probability, that we are not mistaken

Probability of the result

- As we have said, the more tests we use, the better chance that we are not mistaken we have
- Nevertheless, that chance is never entire 100 %
- It is possible to say e. g., that our hypothetical strain is
 - for 99.3 % *Avinella elegans*
 - for 0.5 % *Saantella pulcherima*
 - for 0.2 % something different
- It is on decision of identifier, whether such a probability ratio is enough, or other discrimination tests are necessary

Not only percent of probability, but also typicality index of a strain

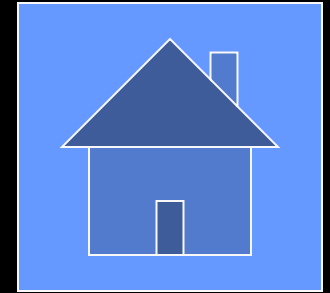
- In reality biochemical identification result is usually characterized by two numbers, not just one:
 - **% probability:** e. g. there is 90% probability, that strain really is *Avinella elegans* and not something other
 - **Typicity Index:** ratio of identity with an „ideal strain“ of *Avinella elegans*. When a strain is ideal, $T_{in} = 1.00$; when strain e. g. does not produce joannase, although 90 % of *Avinella* strains produce it, T_{in} will be less than 1.00



Examples

- A strain has identification 99 %, typicity index 0.95. Ideal situation, probably „is to that“.
- A strain has identification 99 %, but typicity index only 0.63. It might be an atypical strain (is good to know, what is the „test against the identification“), but also a diagnostic mistake
- Two strains have typicity index both 1.00, percent of probability each 49.5 % (one percent of remains to „other“). It means, that it is certainly one of them, but without discrimination tests we do not find, which one is it.

Identification
methods –
practical
approaches



Practical ways of doing it

- Quick tests (seconds to minutes)

Catalase test

Test with diagnostic strips

- Tests with incubation (hours to days)

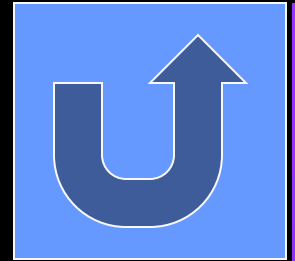
Simple test tube tests

Complex test tube tests

Sests of simple/complex test tube tests

Test in microtitration plates

– Other tests (e. g. Švejcar's plate)



Catalase test

- **Catalase test:** very simple: we mix bacteria with substrate (H_2O_2 solution). Bubbles = positivity. **Principle:** $2 \text{H}_2\text{O}_2 \rightarrow 2 \text{H}_2\text{O} + \text{O}_2$
- Some examples of practical use:




Catalase +



Catalase -

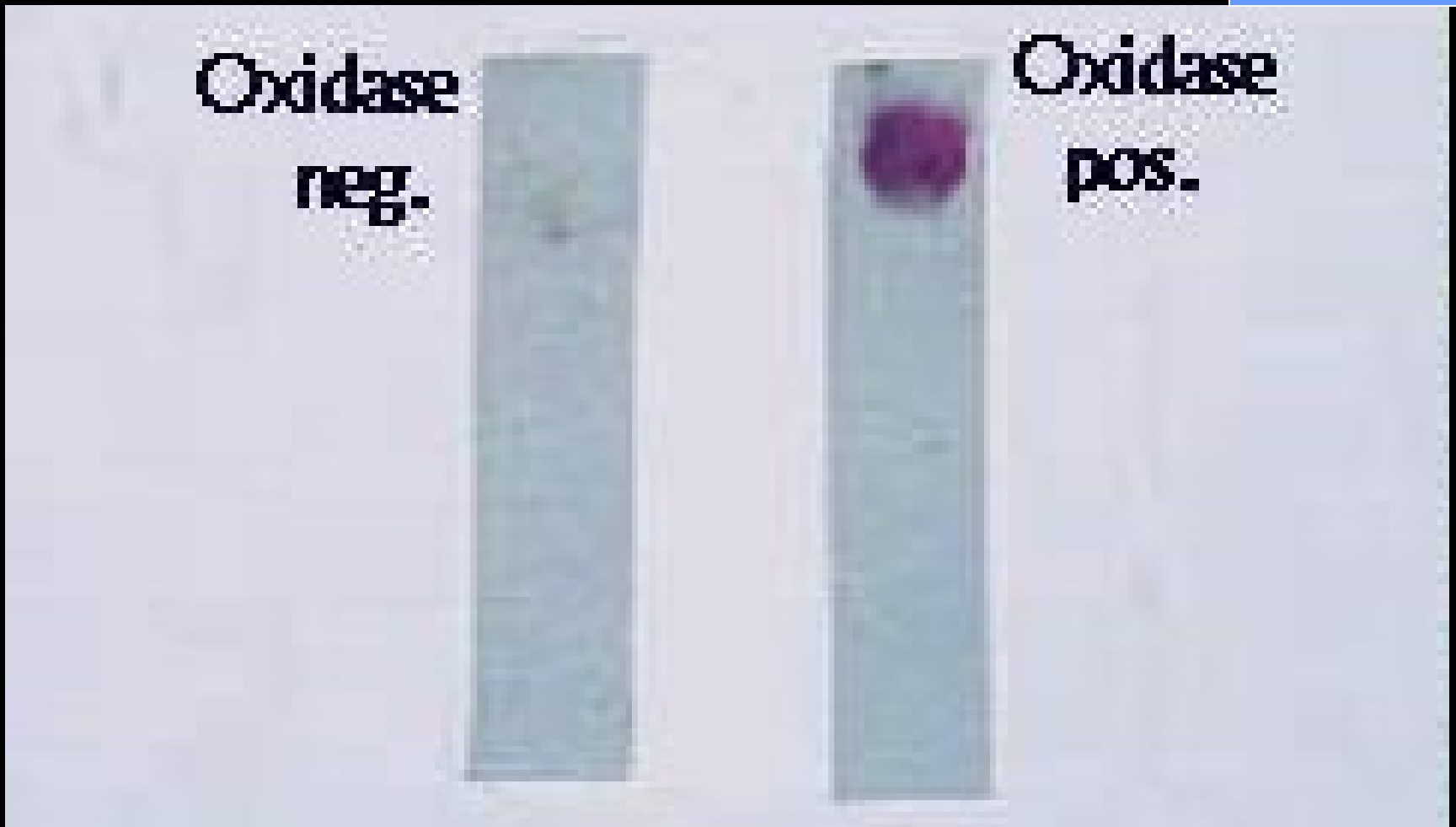


Tests with diagnostic strips

- **Tests with dg. strips** – We touch colonies by reaction area. If positive, the area changes its colour. The more common are:
 - **oxidase** – strip **becomes blue** (examples of use: )
 - **INAC** – strip after minutes **becomes blue-green**
 - **PYR** – strip after minutes , addition of a reagent and one more minute of waiting **becomes red**
 - **betalactamase strip** – testing of some resistance factors (see in two weeks)

If positive, the area changes its colour. Sometimes it requires several minutes to wait (INAC in *Moraxella* dg.), sometimes to wait and than to add a reagent (PYR-test).

Oxidase test

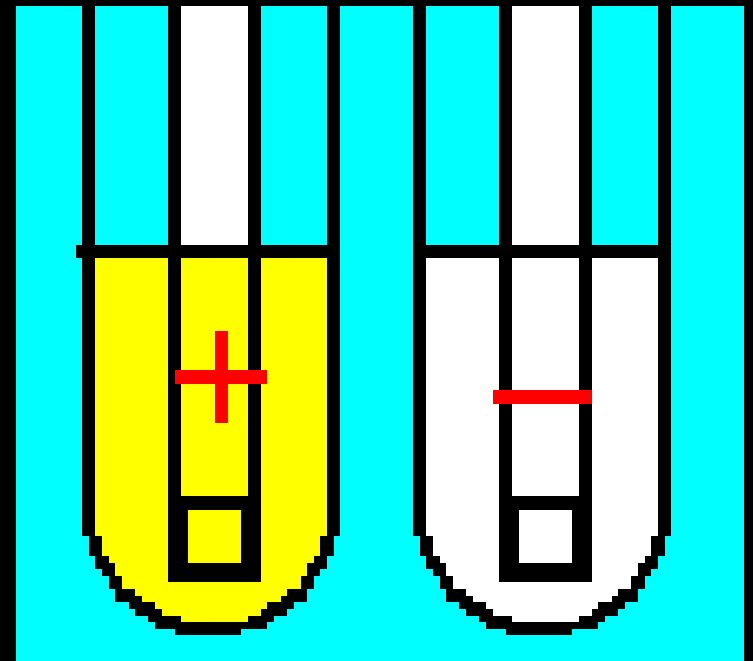


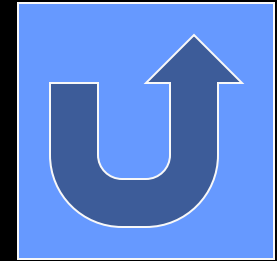
Simple test-tube tests

- They may be in liquid phase, or in agar.
- In both cases, substrate is in a test tube, eventually together with an indicator.
Substrate may be also added in form of a strip with reaction area with it (ONPG-test).
- Test positivity = colour change (in whole volume, or as a ring at the surface)

ONPG test

- **ONPG:** An example of a **simple test-tube test**. The substrate is poured to a test-tube and a strip is added. When the fluid turns yellow, it means test positivity.





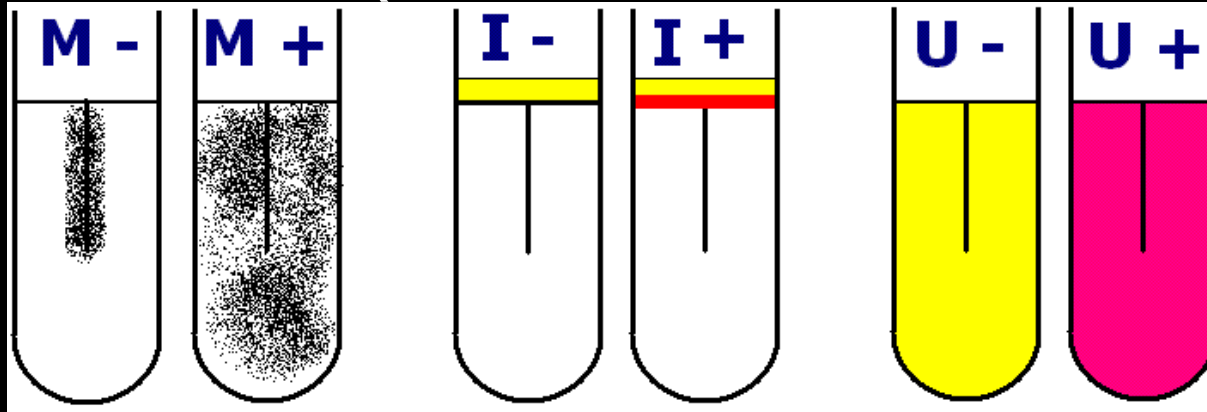
Examples of simple test-tube tests

- **Arabinose** – liquid. Turning yellow = positive, remains green = negative (for enterococci)
- **Simmons citrate** – agar. Turning blue = positive, green = negative →
- **ONPG and VPT** – with addition of a strip. In ONPG, the liquid turns yellow; in VPT a red ring at the surface is produced



Complex test-tube tests

- In one test-tube we have more reactions
- For example **MIU test**:
 - **M = motility** (turbidity is spread through a half-liquid agar, not only in site of inoculation)
 - **I = indol** (positivity = red ring)
 - **U = urea** (breakdown of urea is indicated by the whole



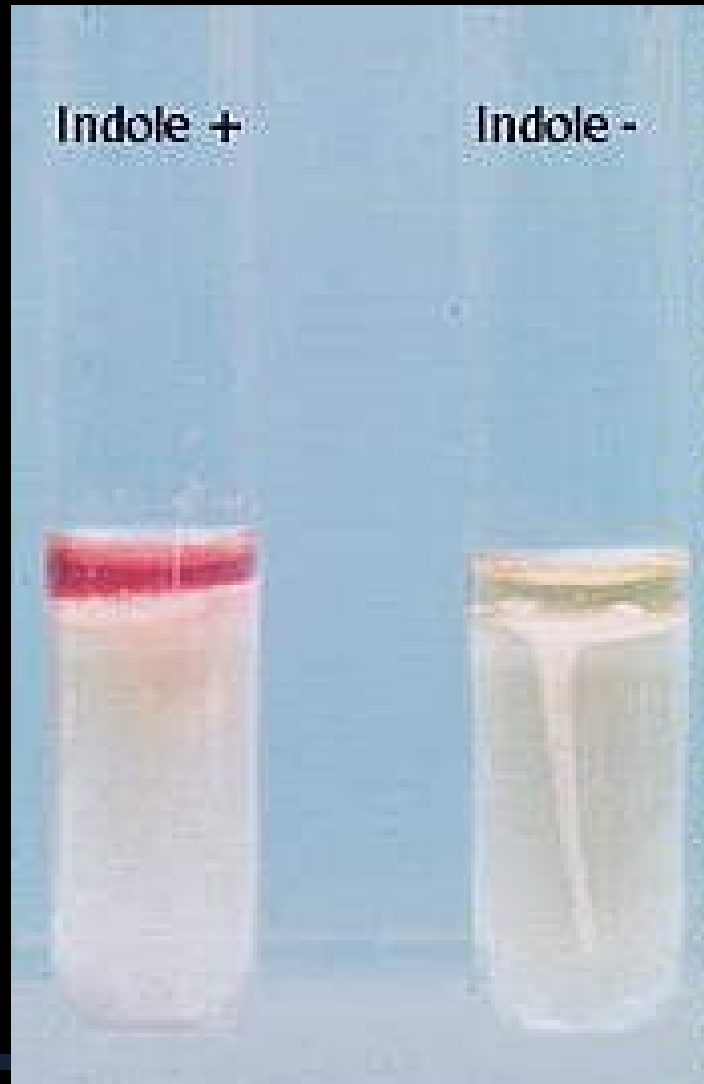
- Or **Hajna medium**

MIU could be also done as three individual tests: motility...



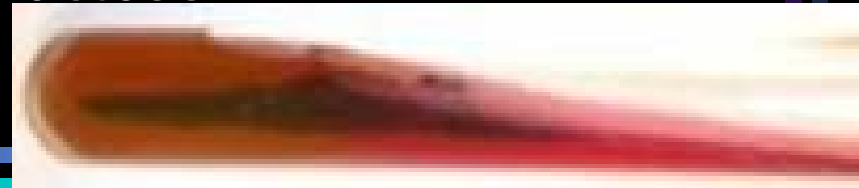
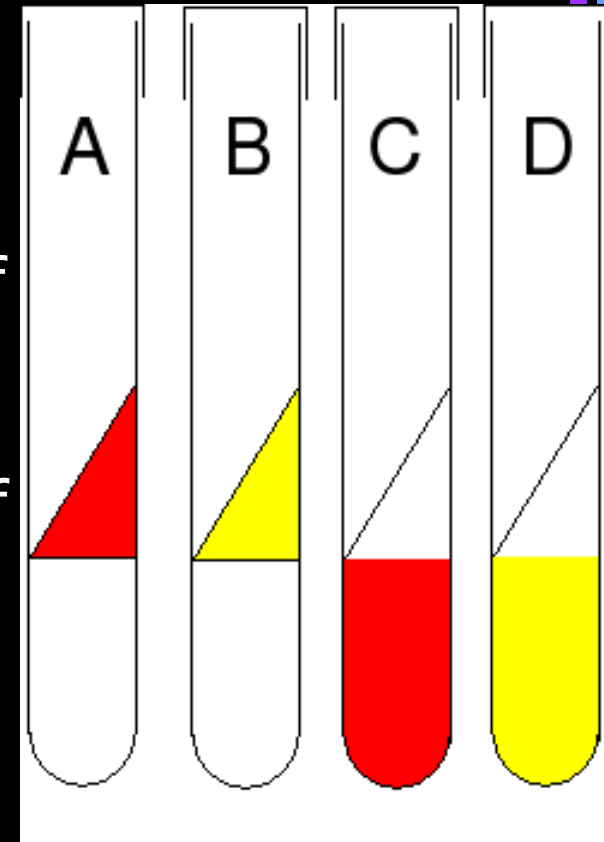
...indol and urea

medic.med.uth.tmc.edu

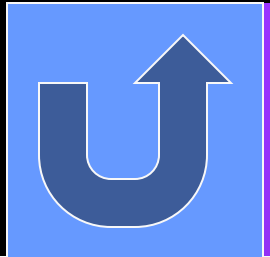


Hajna medium

- Colour of the bottom part of the medium unchanged: bacterium does not ferment glucose (differentiation of so named G- non-fermenters × enterobacteria)
- Bottom part turns black – formation of H_2S
- Broken medium, with bubbles – gas formed from glucose
- Bottom part yellow, upper part red – bacterium is a glucose fermenter, lactose non fermenter
- The whole medium is yellow – lactose fermented, too



Example of conclusion of Hajna + MIU



Test	Hajna			MIU		
	Glc	Lac	H ₂ S	Mot	Ind	Ure
<i>Pseudomonas aeruginosa</i>	-	-	-	-	-	+
<i>Escherichia coli</i>	+	+	-	+	+	-
<i>Proteus mirabilis</i>	+	-	+	+	-	+
<i>Salmonella enterica</i>	+	-	+	+	-	-
<i>Citrobacter freundii</i>	+	+	(+)	+	-	-



Sests of test-tubes

- Complex test-tube tests have some problems. Often positivity of one test disables to see another one. **It is difficult to authomatize them** and they require experienced personel.
- More simple, although sometimes more expensive solution, is **a set of several simple test-tube tests**
- *It is, of course, also possible to combine both simple and complex tests (e. g. Hajna + MIU + Simmons citrate + ornithin dekarboxylase – in our laboratory)*

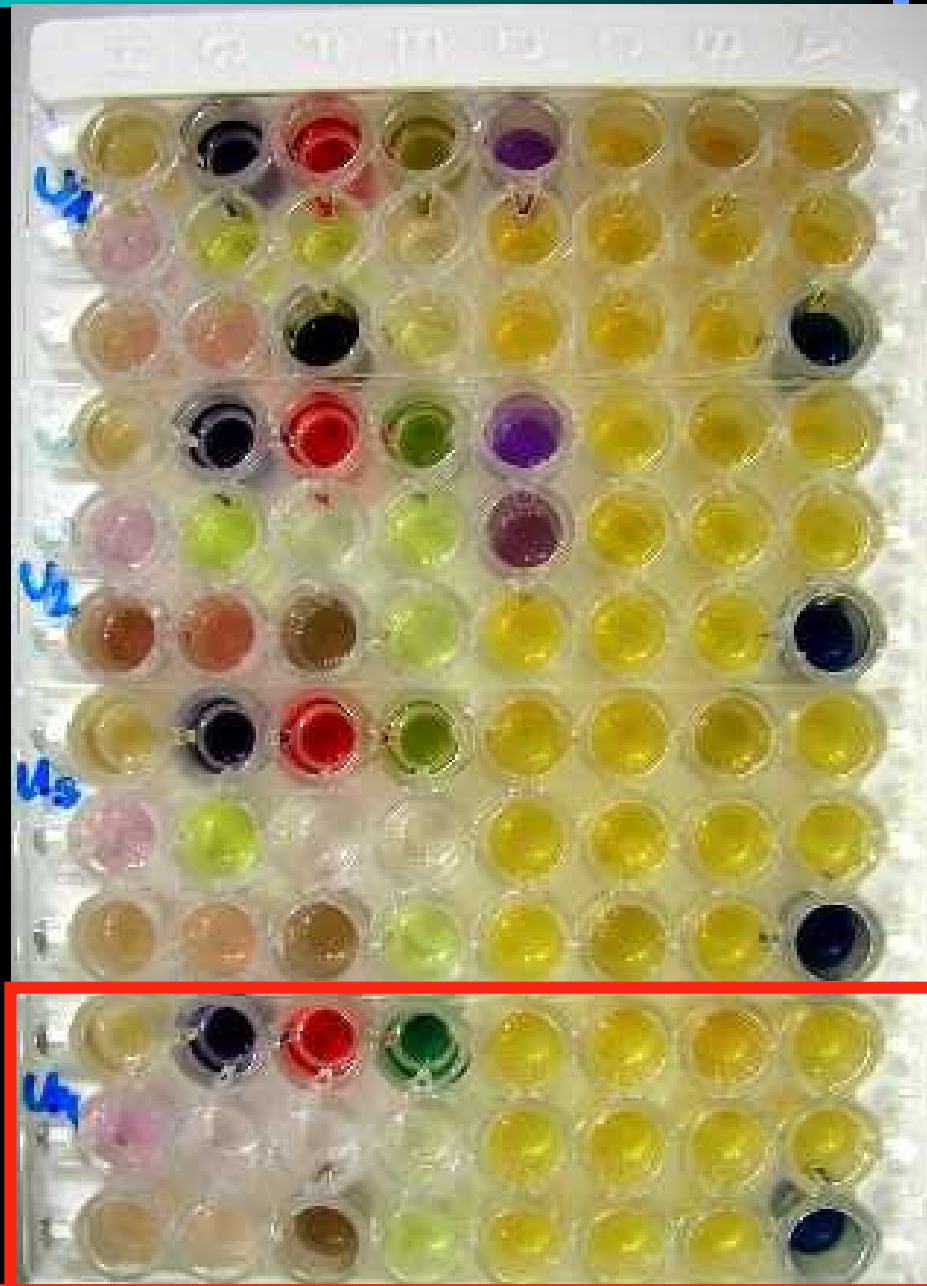
Miniaturisation: tests in microtitration plates

- Miniaturisation of a simple test-tube tests set → tests in microtitration plate wells. Each test-tube is replaced by a well.
- Number of tests in sets is variable from seven (Neisseria Test) to more than fifty
- Technical detail are various. Nevertheless, always the substrate is dried, bacteria are mixed with saline of suspension medium and then it is mixed with the dried substrate

Tests by Pliva Lachema (most common in Czechia)

- The factory produces plates with **dried substrates**, placed in the bottom of the wells
- We prepare a **bacterial suspension in saline or suspension medium**
- To each well we add **one or two suspension drops**
- The remainder of suspension is often used as test tube test with a diagnostic strip (ONPG, VPT)
- Both the plate and the test tube is **incubated in a thermostat**

NEFERMtest 24
Pliva Lachema: one
frame enables testing of
four triple-strips (four
tests, determination of
four various strains)



Other producers

(the same principle, small differences in practical form)

www.ilexmedical.com/products_engl/api.htm



Foto: O. Z.



www.ilexmedical.com/products_engl/api.htm

<http://www.oxid.com/bluePress/uk/en/images/PR020505.jpg>





Evaluation of plate tests

- Such a test gives us **a row of results** – usually in form of „+“ (test is positive, substrate changed) or „-“ (test negative, substrate unchanged, original colour).
- **An example of a:** + - + + + - - - - -
+ + + +
- There are several ways, how to convert such a row into a „legible result“

Ways of evaluation

- **Comparison with a table** is possible for simple tests and clear results only.
- Conversion into **octal codes** plus searching result in the code list. Common.
- Result input directly into **a computer**, which gives us the result. Not always practical

Computer evaluation is often used when the reading itself is automatic, e. g. on a spectrophotometer.

Octal codes – what is it, and why

- Mathematically it is conversion of binary system (+ + - - + + - - -, or 110011000) into octal system (written 630)
- For practical reasons, reading inside triplets is usually reverted – normally 1 1 0 converted into octal code should be six and 0 1 1 three, usually it is the opposite

Octal codes – II

- In practice, each triplet of result is converted into a number 0 to 7.
- When a test has e. g. 17 reaction, there is a dublet instead of triplet at the end, so the final number can be only 0, 1, 2, 3. When we have e. g. 16 (19, 22...) reactions, the final number should be zero or one only.

Practical example

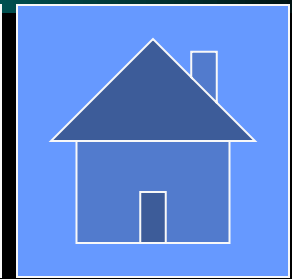
- We mark positive and negative reaction results
- Under each triplet we write 1 – 2 – 4
- For each triplet we read only „+“ numbers, not „-“ (these go out)

Test	JAN	LEN	MAG	TOM	PET	KAR	FRA	HAN
Result	+	-	+	+	+	-	-	-
	1	2	4	1	2	4	1	2
Code	5			3			0	

Re-counting the triplets

---	1 2 4		0
+ --	1 2 4	1	1
- +-	1 2 4	2	2
+ + -	1 2 4	1 + 2	3
-- +	1 2 4	4	4
+ - +	1 2 4	1 + 4	5
- + +	1 2 4	2 + 4	6
+ + +	1 2 4	1 + 2 + 4	7

For example, in ENTEROtest16 (17 tests)



	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	
	ONPG	H	G	F	E	D	C	B	A	H	G	F	E	D	C	B	A	
		First row of the plate							2 nd row of the plate									
+																		
-																		
?																		
?	+	-	+	+	+	-	-	-	-	-	-	-	-	+	+	+	+	
	1	2	4	1	2	4	1	2	4	1	2	4	1	2	4	1	2	
	5			3			0			0			6			3		

Identification
methods –
not based on
substrates

Other identification tests

- Besides tests based on substrate breakdown, we have also **other similar tests**, that find presence of some bacterial enzymes or virulence factors. For example:
 - Test of ability to coagulate rabbit plasma
 - Test of ability to agglutinate rabbit plasma
 - Test of ability to decapsulate an encapsulated strain (hyaluronidase test)
 - Motility testing – we have had it already

Plasmacoagulase and hyaluronidase (both tests are used in staphylococci)

www.hardydiagnostics.com

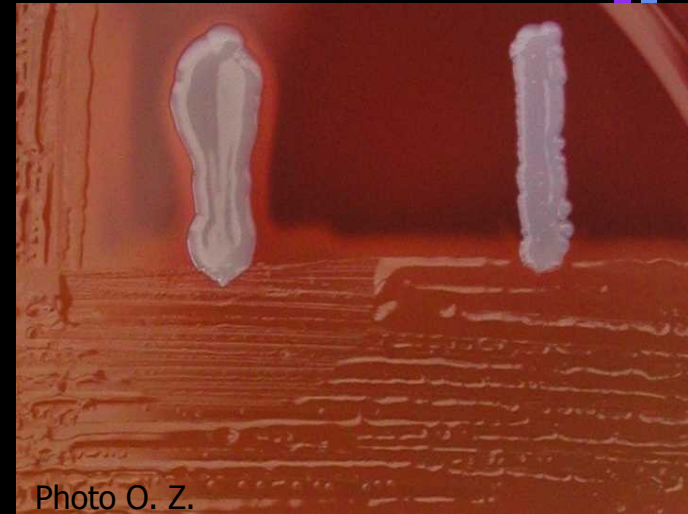
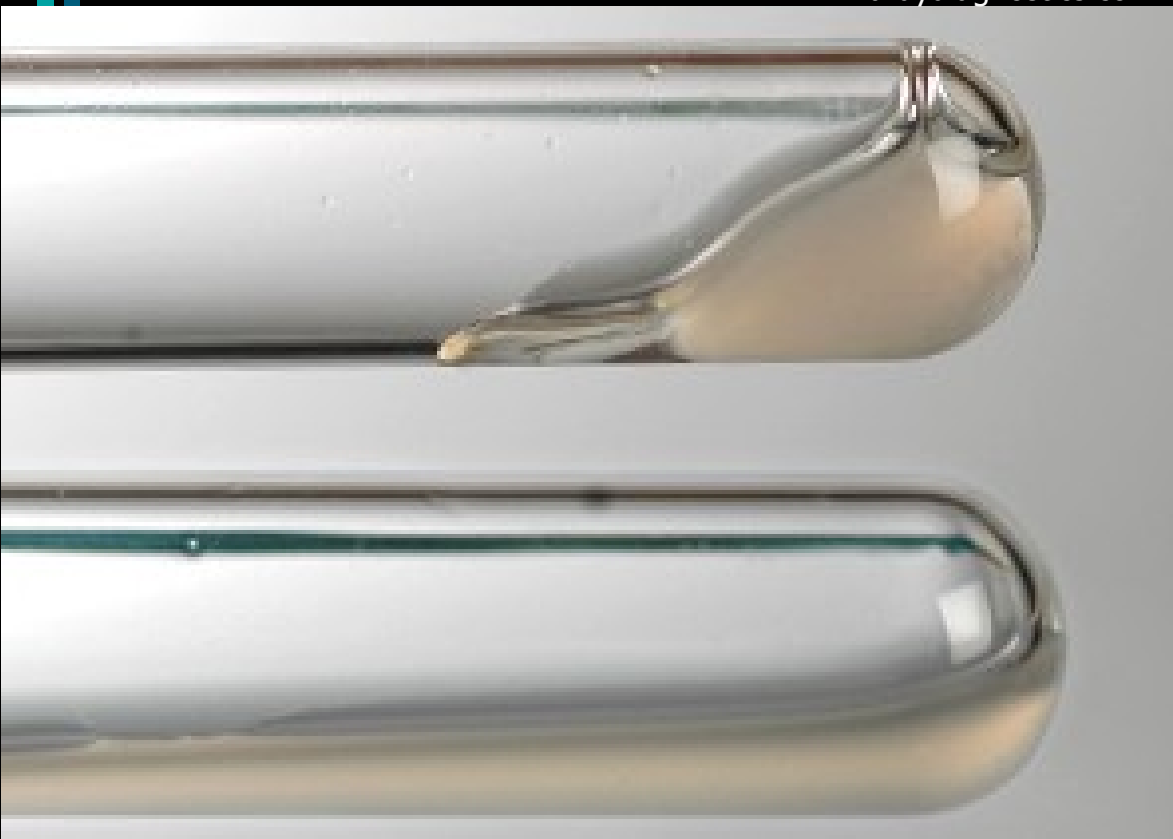


Photo O. Z.

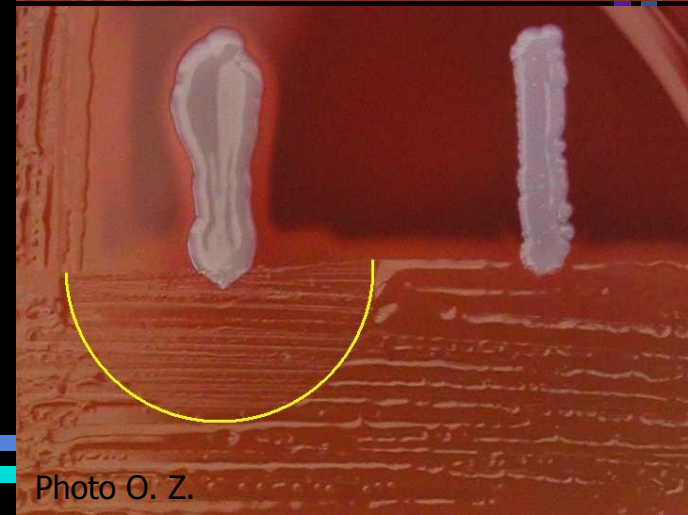
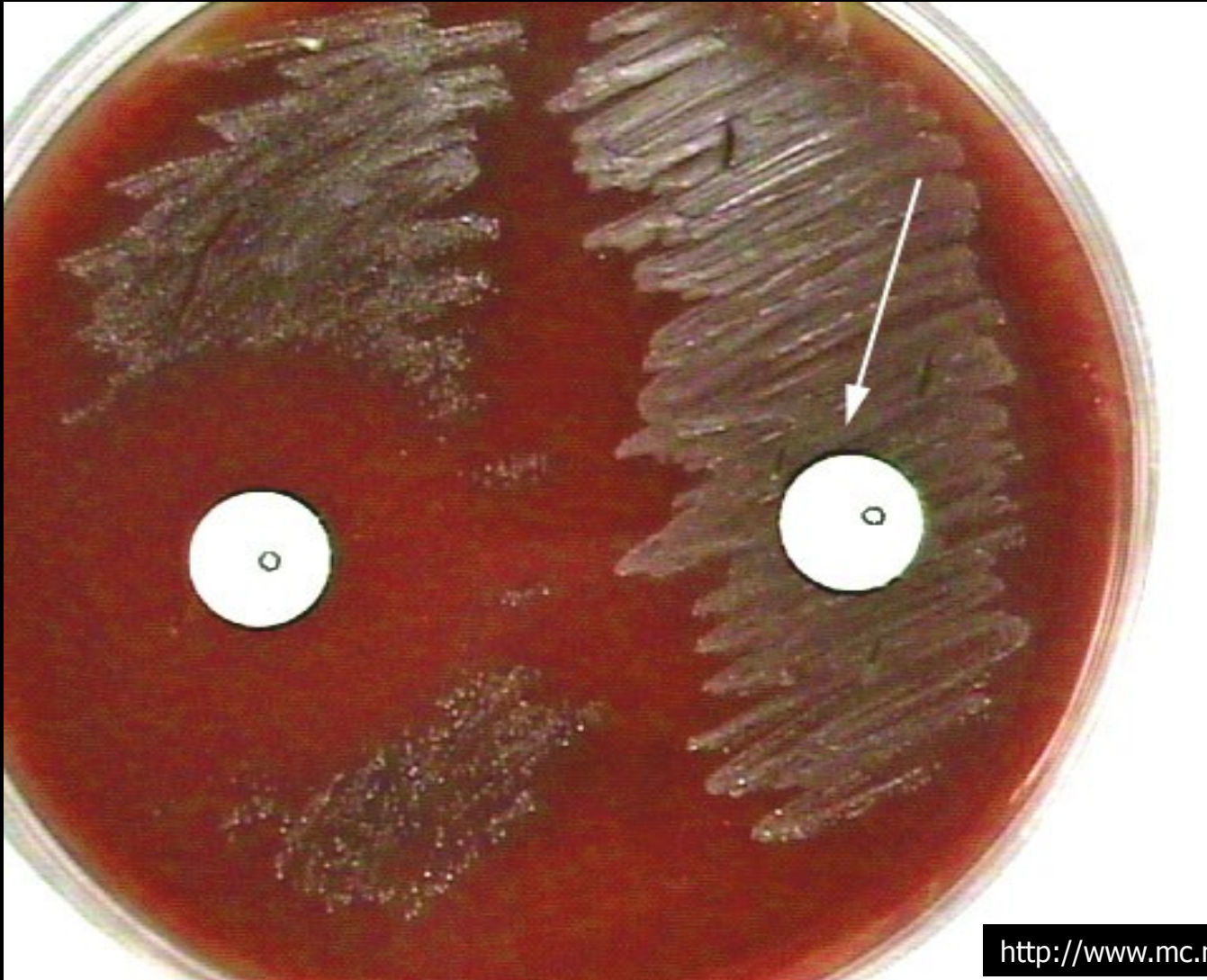


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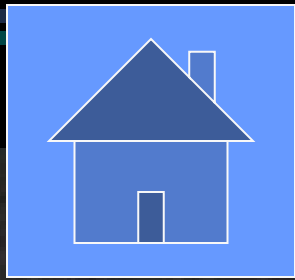
Diagnostic use of antibiotics

- One possibility is also **in vitro susceptibility testing to a certain antibiotic** in situation, that we know, that strain X is in 100 % susceptible and strain Y is in 100 % resistant. In practice, it is, of course, never 100 %.
- An example is **optochin test**
- **Practically it the same as normal antibiotic susceptibility tests**, see the practical two weeks later

Optochin test negative and positive



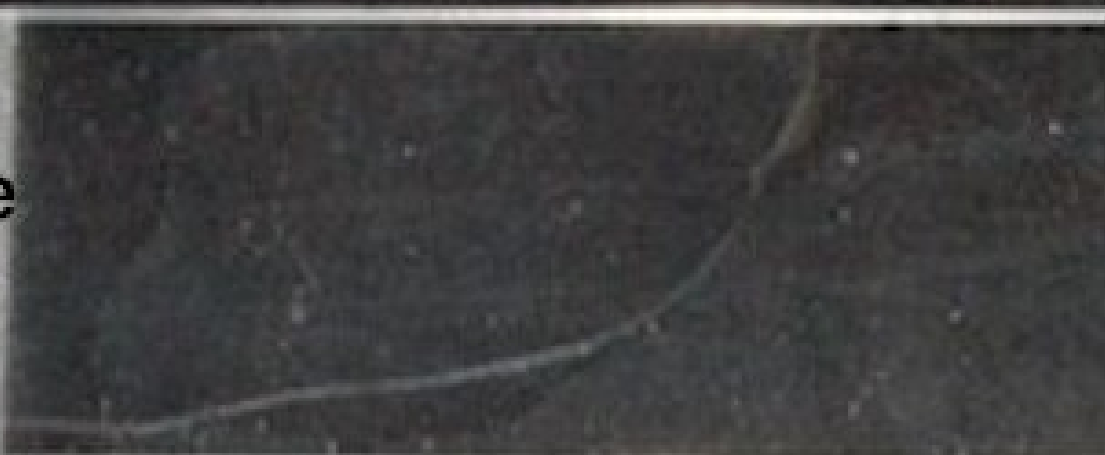
The End



CATALASE TEST

<http://www.telmeds.org>

Negative

A photograph of a test tube containing a dark, opaque liquid. The liquid is uniform in color and shows no signs of bubbling or gas production, indicating a negative catalase test result.

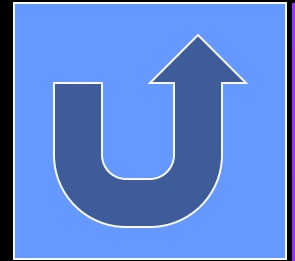
Positive

A photograph of a test tube containing a light-colored, frothy liquid. The liquid is highly bubbly and appears to be overflowing from the top of the tube, indicating a positive catalase test result.

Examples of catalase test use

- The most common use it in diagnostics of **G+ cocci**. Among medically important genera, staphylococci are catalase +, while streptococci and enterococci are catalase –
- Nevertheless, there exist some more examples, too, e. g. in **G+ rods**: *Listeria* is catalase +, *Erysipelothrix* (microscopically similar) catalase –





Examples of oxidase test use

- Oxidase can be used in various situations:
- To confirm diagnostics of *Neisseria*, *Moraxella* and *Pseudomonas* (oxidase positive)
- To differentiate between *Vibrionaceae* (oxidase +) and *Enterobacteriaceae* (oxidase – except genus *Plesiomonas*)