Searching for microbes
Part VII.
Introduction to serology, precipitation and aglutination

Ondřej Zahradníček

To practical of VLLM0421c

Contacts to me:

777 031 969 zahradnicek@fnusa.cz

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Content of this slideshow

- Antigen and antibody
- Interpretation of the antibody detection
- Geometric row and titre counting
- Agglutination and precipitation: overview
- Agglutination: examples of individual techniques
- Precipitation: examples of individual techniques

Tale

- Once a mother bougth a toy to her child.
- The toy was a plastic plate with **holes of different shapes**, and **shapes belonging to the holes** were here, too.
- Once the child cried, as something went wrong. Mum came and told him: „My child, you cannot put a square into a hole for a circle!“ Look, **the cicrcle should be here, the square has to be there.**

Nevertheless, a few days later…

- …mum came to the child‘s room, and she saw, that the child was succesfull in puting the circle into the hole for a hexagon.
- So, the mum realized, **that there are some rules, but there are exceptions, too.**
- The same is in the nature – when a **shape** has its **counter-shape**, sometimes a counter-shape is able to make a couple with another shape and not the correct one.

What to learn from the tale

- Microbes (but also e. g. plants and animals) have **on the surface** of their cells **antigens**. When they meet our body, our body starts to produce **antibodies**, that are **specific** to it.
- The specifity has its limits. Sometimes, we have a **cross reactivity**, when the antibody reacts also with an alien antigen, only simillar to that responsible to its production

Sometimes, antibodies against an antigen produced in the tissue during the infection, too.

Antigen and antibody

Antigen and antibody

Antigen = a macromolecule coming from an alien organism: plant, microbe, animal. (Eventually also from one's own body, but too old, dammaged or pathological cells.)

In microbiology, we are interested in microbial antigens – parts of microbial body, that challenge host body to an antibody response

Antibody = an immunoglobuline, formed by the host body as a response to antigen challenge (of course not only by humans, but also by various animals)

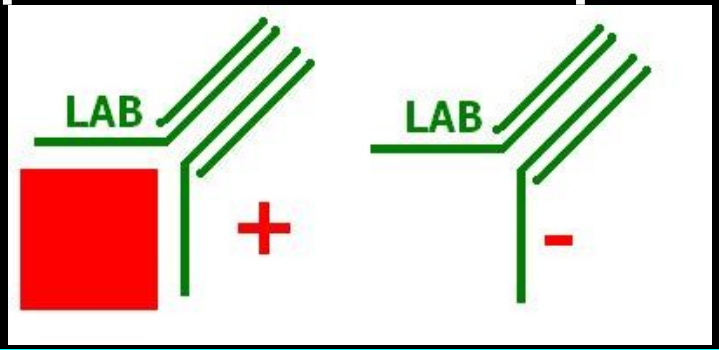
Methods in clinical microbiology

- Direct methods: detection of a microbe, its part of its product. Examples: Microscopy, culture, biochemical identification, **antigen detection**. Positivity = it is sure, that the agens in NOW present.
- Indirect methods: **detection of antibodies** against the microbe. Positivity = the microbe met the host IN HISTORY (weeks / months / years)

Two ways how to use interaction between antigen and antibody:

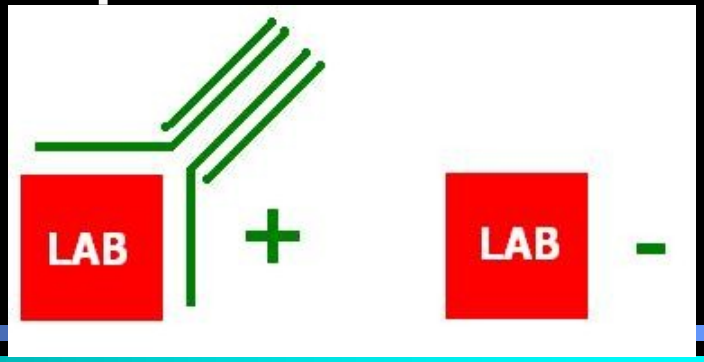
Antigen detection: laboratory (animal origin) antibodies + pacient‘s sample or microbial strain.

Direct method



Antibody detection: laboratory antigen (microbial) + pacient‘s serum (or saliva).

Indirect method



Interpretation of the antibody detection

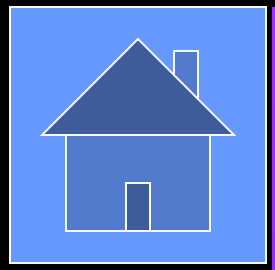
Interpretation

- Antigen detection: it is a direct method. Positive result means presence of the microbe in the pacient‘s body
- Antibody detection: it is an indirect method. Nevertheless, there are some ways how to get the information – when the microbe met the body:
  - Amount of antibodies (relative – titre)
  - Class of antibodies: IgM/IgG (More in J10)
  - *(Avidity of antibodies)*

How to interprete indirect diagnostics

- Acute infection: large amount of antibodies, mostly class IgM 1
- Pacient after an infection: small amounts of antibodies, mostly IgG (immunological memory) 2
- *Chronical infection: various response*

How to perform the reaction „quantitativelly“



- It is **very dificult to assess the amount of antibodies in units** like mol/l, mg/l etc.
- But it is possible to use another way: **to dilute the patient‘s serum many times**.
  - It reacts **even when diluted many times** → → serum contains a lot of antibodies
  - It reacts **only when diluted a few times**→ → only small amounts of antibodies present

Geometric row and titre counting

Geometric row

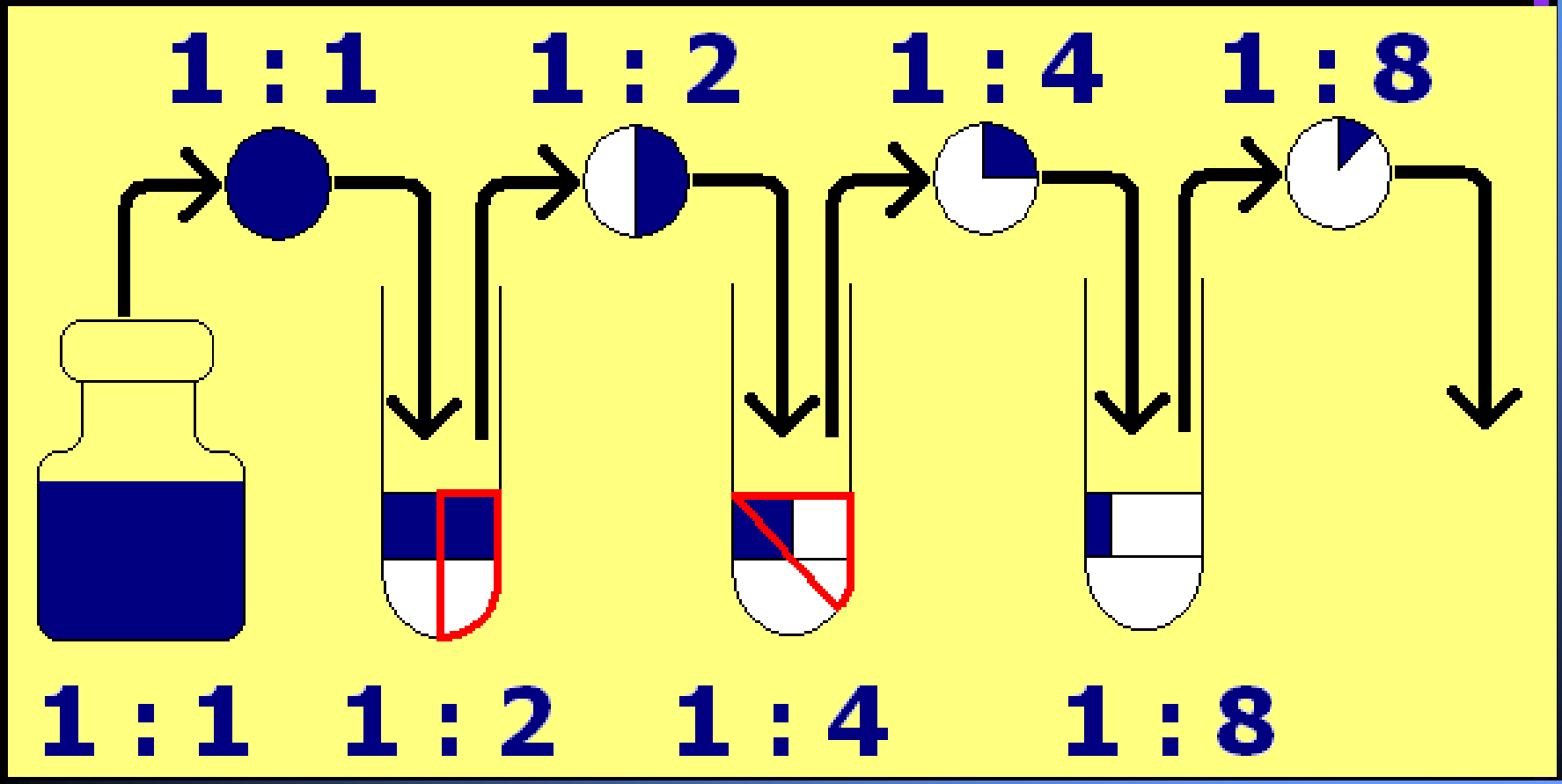
- Technically the most simple way, how to dilute pacient's serum, is the use of **geometric row with coefficient = 2**.
- We start with the **undiluted serum**, or **serum with a certain pre-dilution** (e. g. 1 : 5, 1 : 10, 1 : 20 and so on)
- In every next well, there is **double dilution** in comparison with the previous, for example, we have a row: 1 : 10, 1 : 20, 1 : 40, 1 : 80, 1 : 160…

Counting dilutions in serology

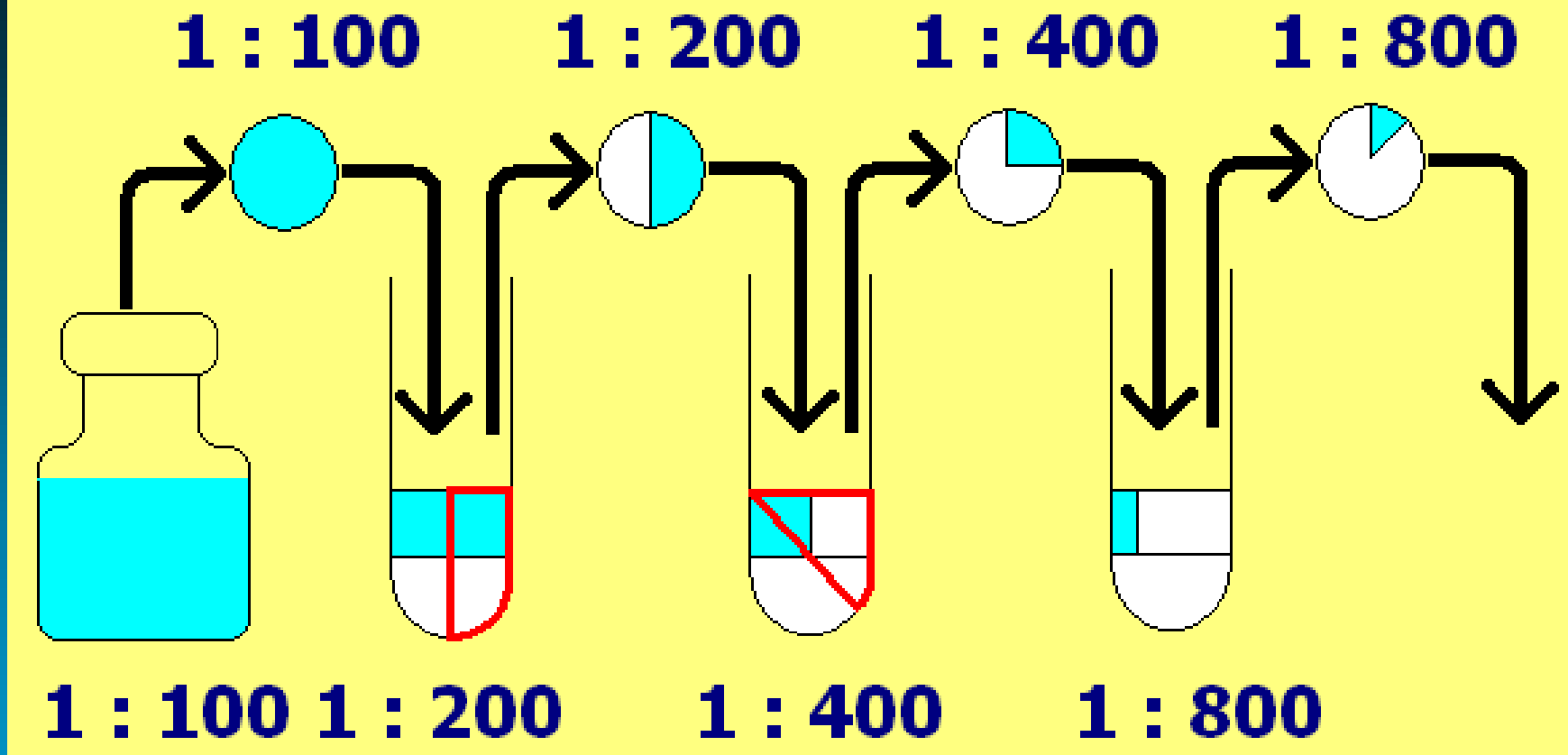
Attention, in serology e. g. dilution 1 : 4 means one part of serum and three parts of saline (= total 4 parts)!

At „biochemical“ counting (number of parts of serum : parts of diluent) we would have to use numbers e. g. 1 : 9, 1 : 19, 1 : 39, 1 : 79. That would be very un-practical

Geometrical row: how to do it
a) without predilution of the original serum



b) with predilution of the original serum



Of course, the predilution is not always 1 : 100,
it can be 1 : 5, 1 : 10, 1 : 20 or any other.

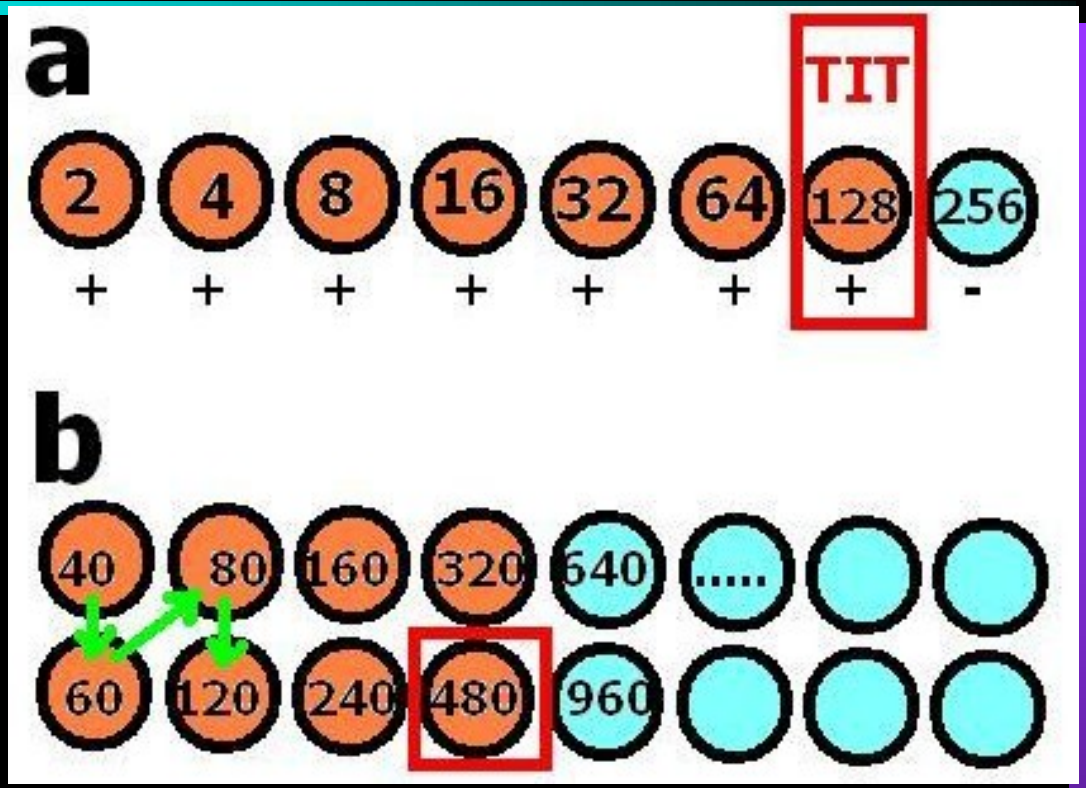
Geometrical row

- **At start, we have a serum specimen, that is undiluted**
- In first test tube, we mix it with the same amount of diluent (saline), so that we have dilution 1 : 2
- One halfth of 1 : 2 dilution is removed to another test tube, and mixed again with the same amount of diluent → 1 : 4
- One halfth of 1 : 4 …… → 1 : 8
- Etc., etc.

Titre

- After serum dilution, we add the **antigen**
- In relation with the reaction type, **either we can se the reaction result directly** (aglutinate, precipitate), **or we have to visualize it** adding other components (complement, RBCs, etc.)
- Anyway, we have to be able to discriminate **positive and negative reaction results**
- **The highest dilution, where a positive reaction is still visible, is called titre.**

Titre assessment



Titre – the highest positive dilution. If we have two rows, titre = the highest positive dilution of both rows. In case **b)** there is one titre only (480), NOT two (320 and 480), as some students suppose.

Not always titres are needed!

- We never use titers in antigen detection
- Sometimes we do not assess titres despite the fact that it will be antibody detection. It is because these reactions are **screening reactions**
- Example: Every pregnant woman is examinated for syphilis, just „for sure". First tests are screening tests, performed as only qualitative tests. All positive / borderline reactions are confirmed by more specific confirmation reactions.

Agglutination and precipitation: overview

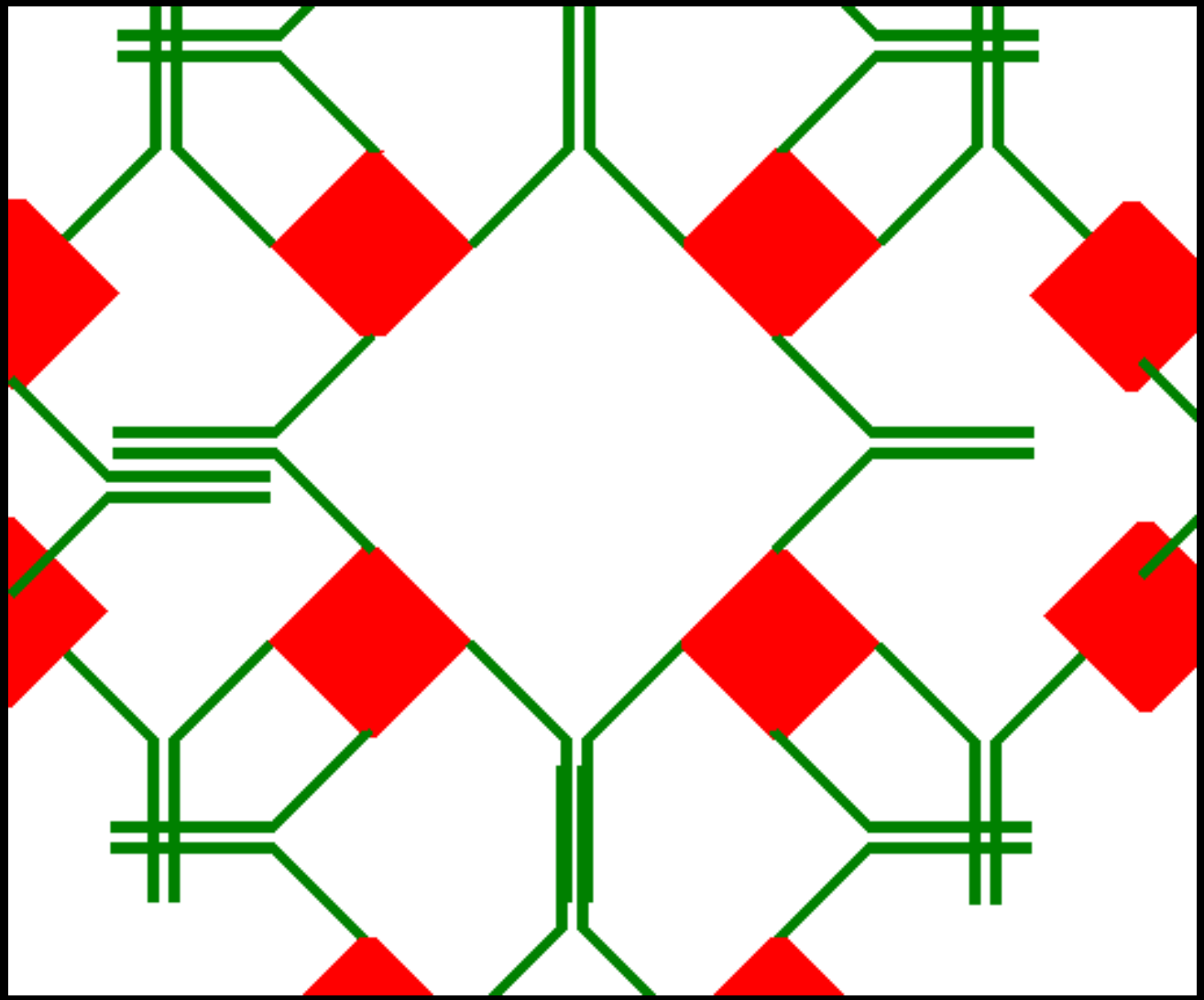
Precipitation and agglutination – common characteristics

- Precipitation and agglutination are the two **most simple serological reactions**, we work here really just with antigen and antibody, without any other components
- Either we detect **antigen using animal antibody**, or **antibody using laboratory antigen**
- **Only in the second example, we count titres!**

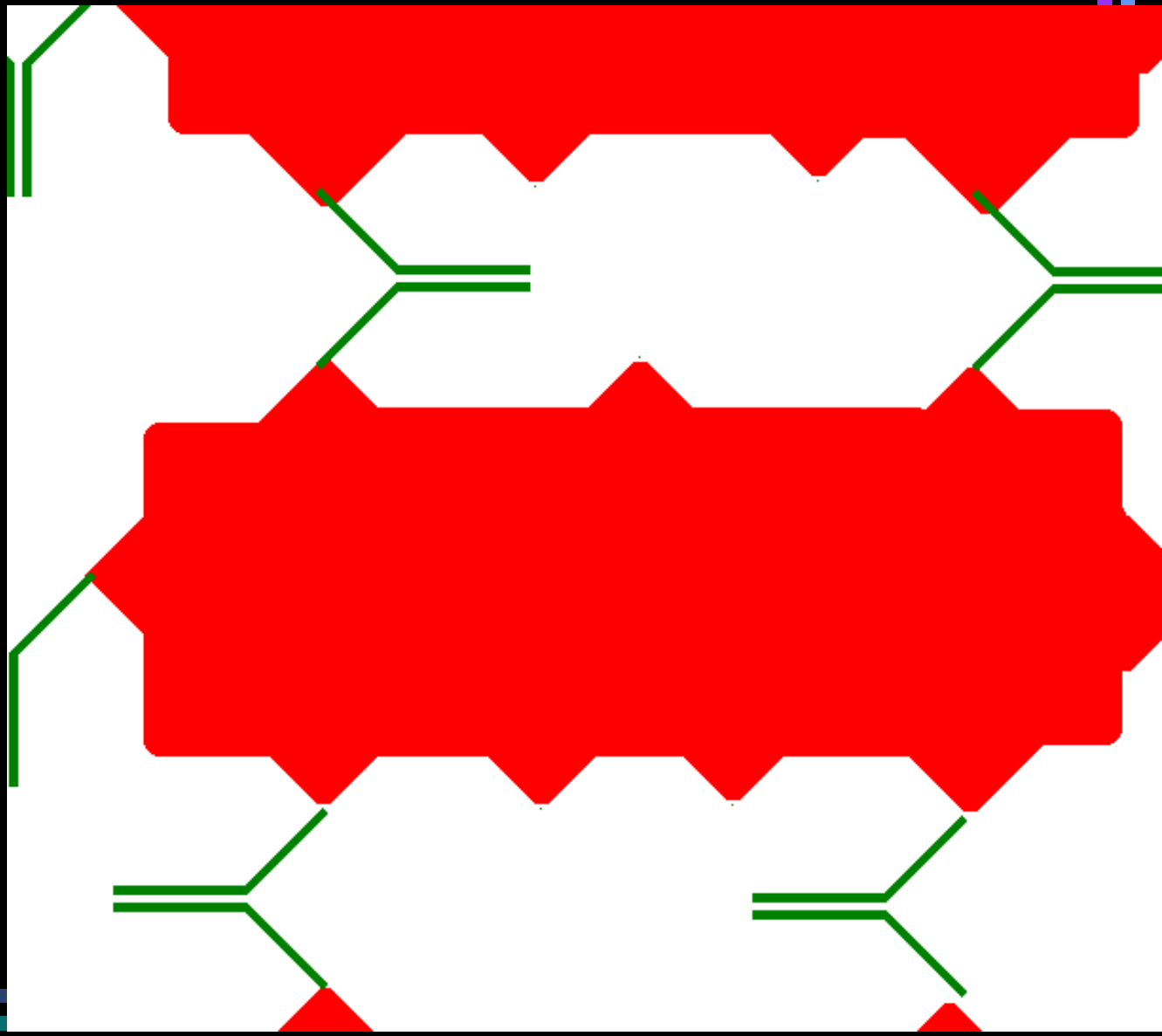
Precipitation, agglutination, agglutination on carriers

- **Precipitation:** Antigens act alone, as macromolecules (**coloid antigen**)
- **Agglutination:** Antigen acts being part of its microbial cell (we work with whole microbes, **corpuscular antigen**)
- **Agglutination on carriers:** Formerly macromolecular antigens are bound to an alien particle – carrier: latex particle, RBC, eventually polycelulose particle

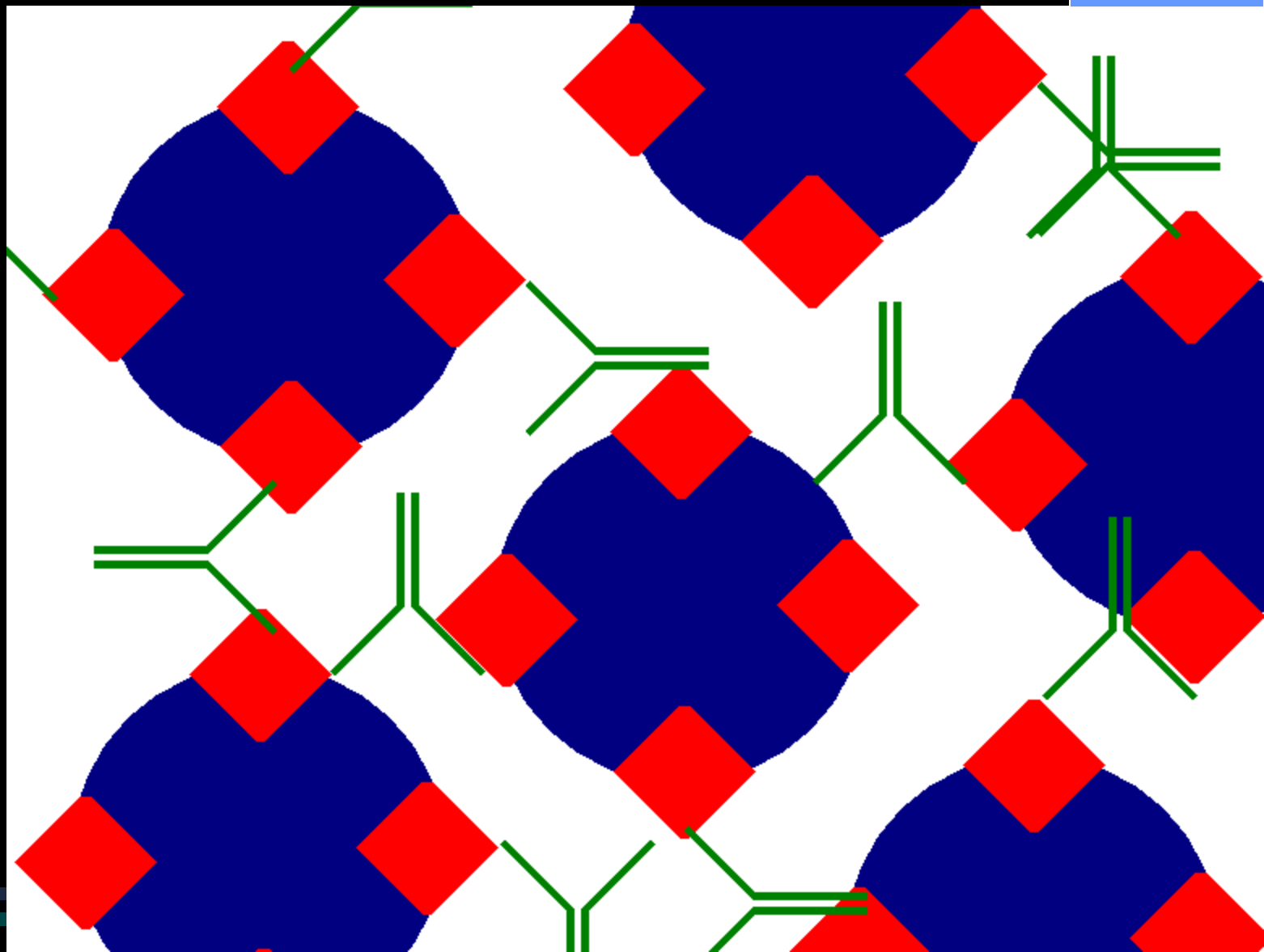
Precipitation



Agglutination



Agglutination on carriers

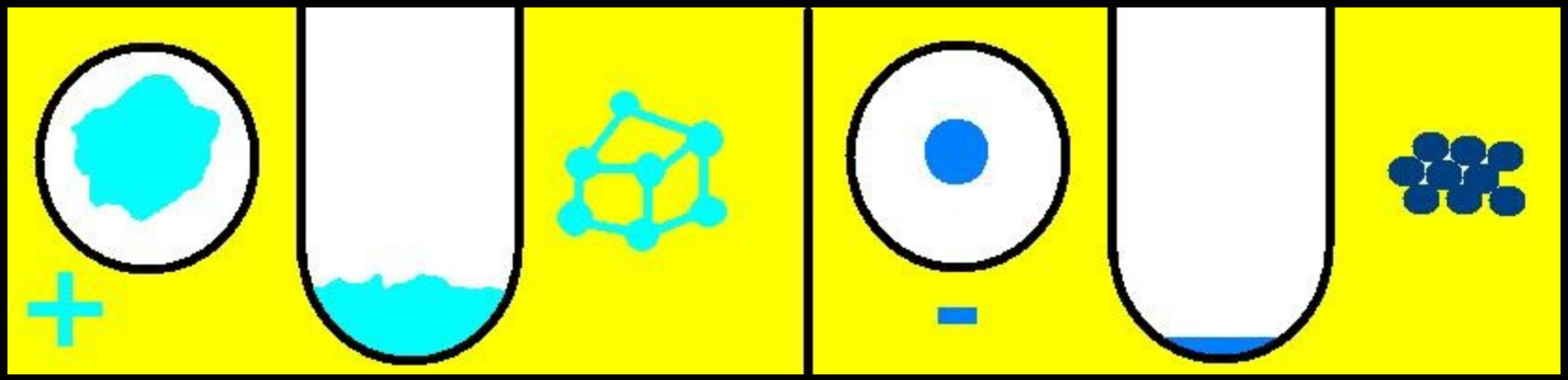


Agglutination: examples of individual techniques

Agglutination for antibody detection in a microtitration plate

Positive – irregular „potato shaped" formation

Negative – a small, regular circle

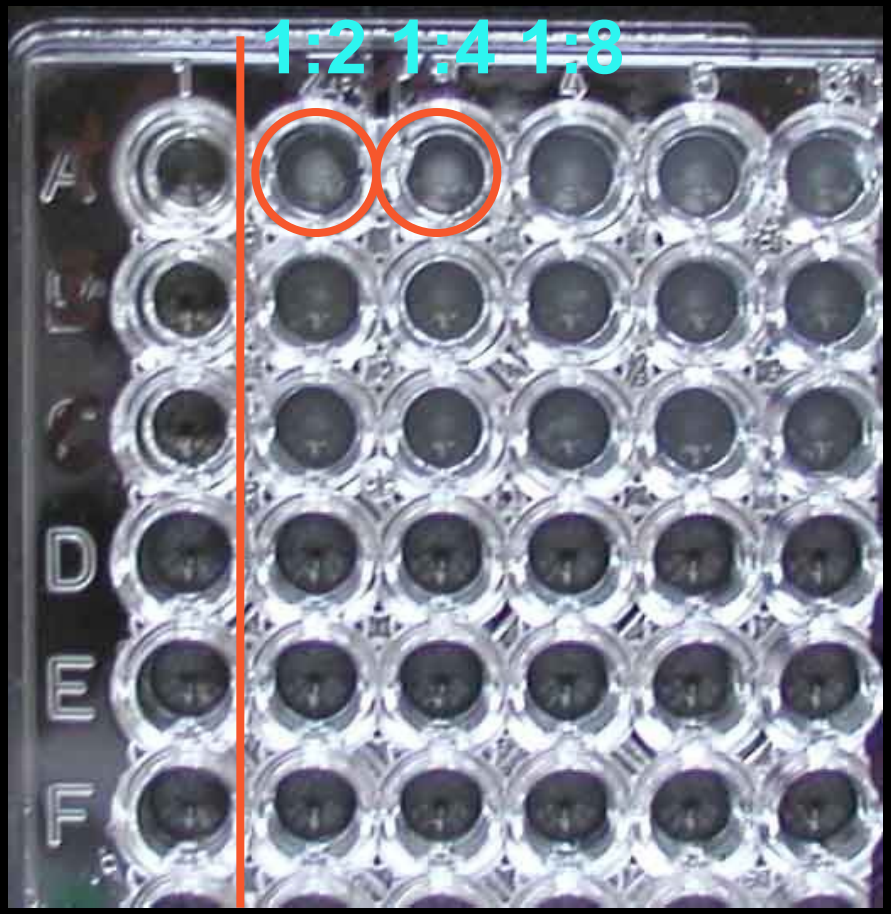


- Do not forget, that titre = highest dilution with a positive reaction. First well is diluted 1 : 100, second 1 : 200 etc.

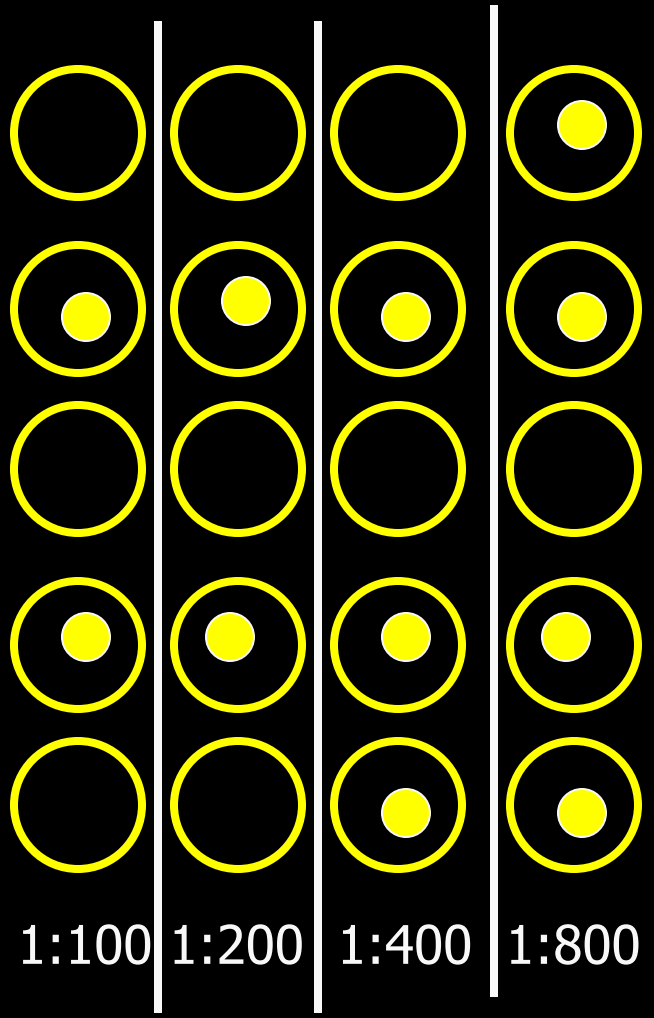
Demonstration of agglutination reaction in tularemia (from www.medmicro.info):

First collumn are controls, the reaction starts in the second collumn

Each patient has the test done for several types of antibodies



Example of a reult in *Yersinia* diagnostics



K+ positive, titre = 1 : 400

No 1 negative

No 2 posit., titre ≥ 1 : 800

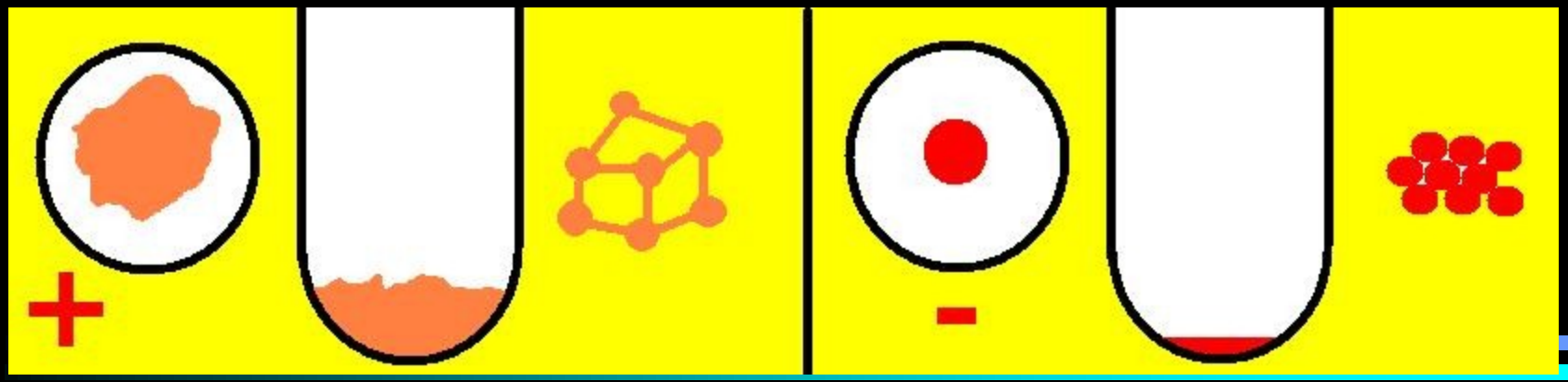
No 3 negative

No 4 posit., titre = 1 : 200

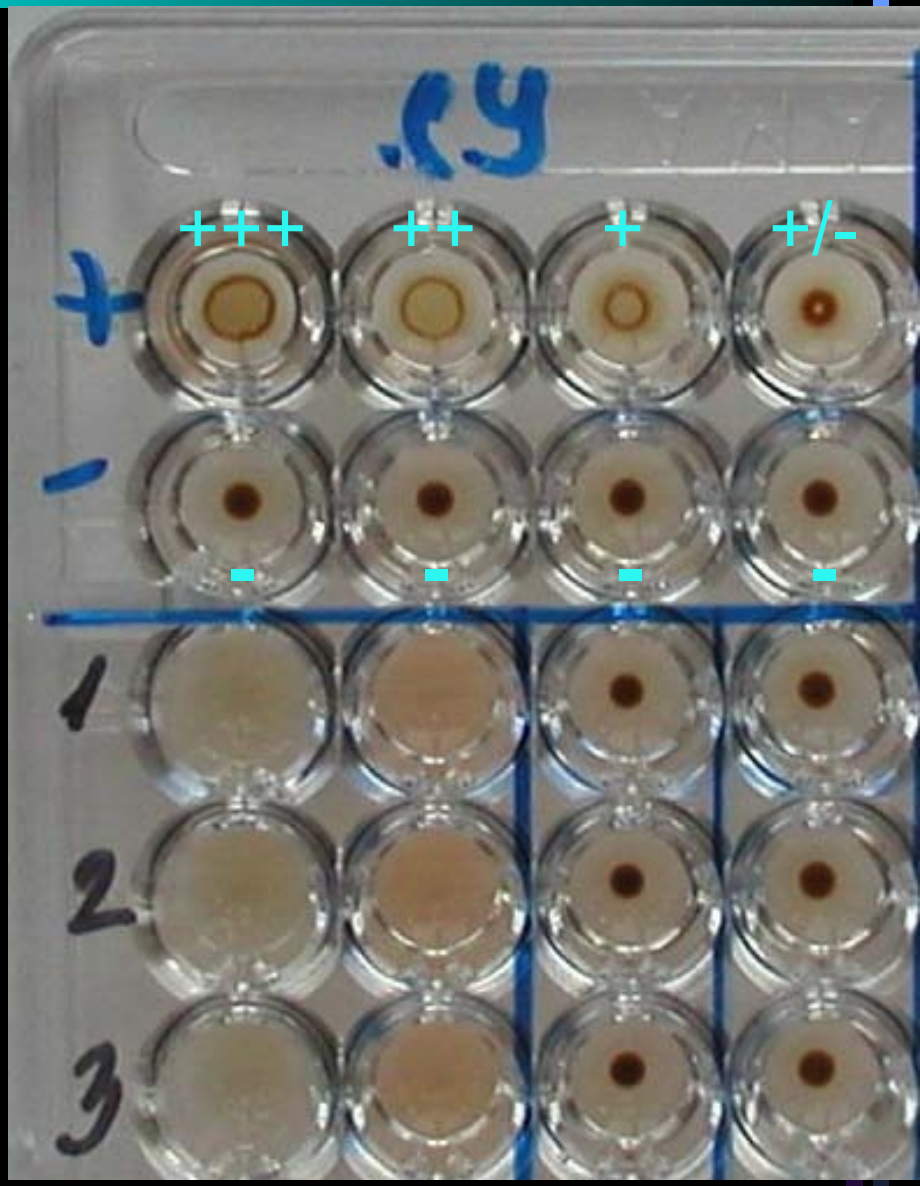
Example of agglutination on carriers Treponema pallidum haemagglutination (TPHA)

Here, too, the positive reaction is the „irregular potato-shaped spot", negative reaction is corpuscullar sedimentation on the bottom ot the well. But it is red: it is an agglutination on carrier, the antigen is carried by a red blood cell

Today, red blood cells are replaced by polycelulose particles in this test – you can meet abbreviation TPPA



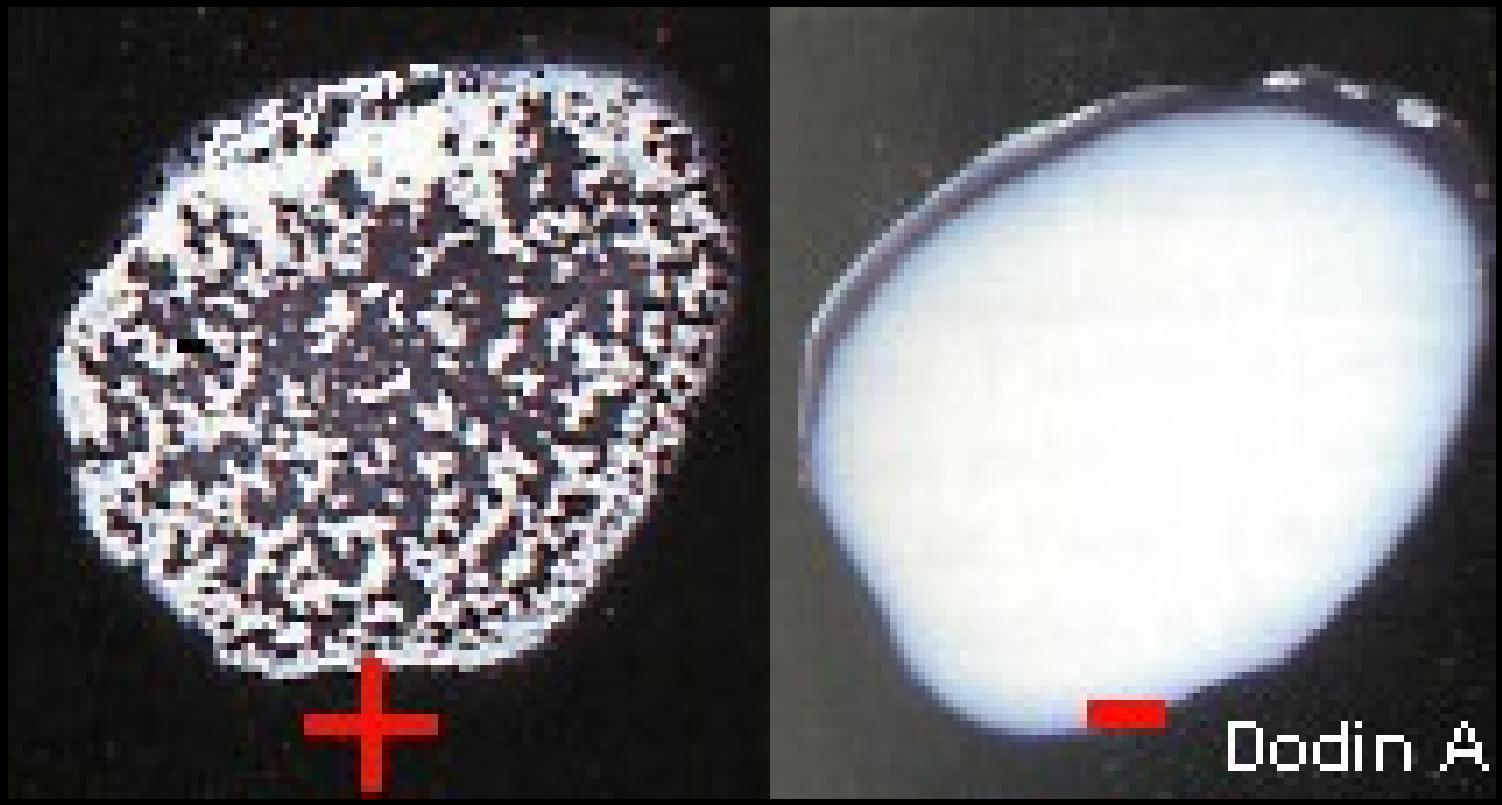
Demonstration
TPHA
(www.medmicro.info)



Example of slide agglutination to antigen analysis: Testing of an *E. coli* strain for Enteropathogenous *Escherichia coli*

- There are about 12 antigenic types belonging to EPEC group
  - We use **polyvalent sera:** nonavalent serum contains antibodies againts nine EPEC serotypes, trivalent serum IV contains antibodies agaist three remaining serotypes. Turbidity = positive
  - When one of sera (nonavalent and trivalent IV) is „+“, we have to continue using *(trivalent and)* **monovalent sera**
- *It is antigen detection → no titres!*

EPEC detection – result



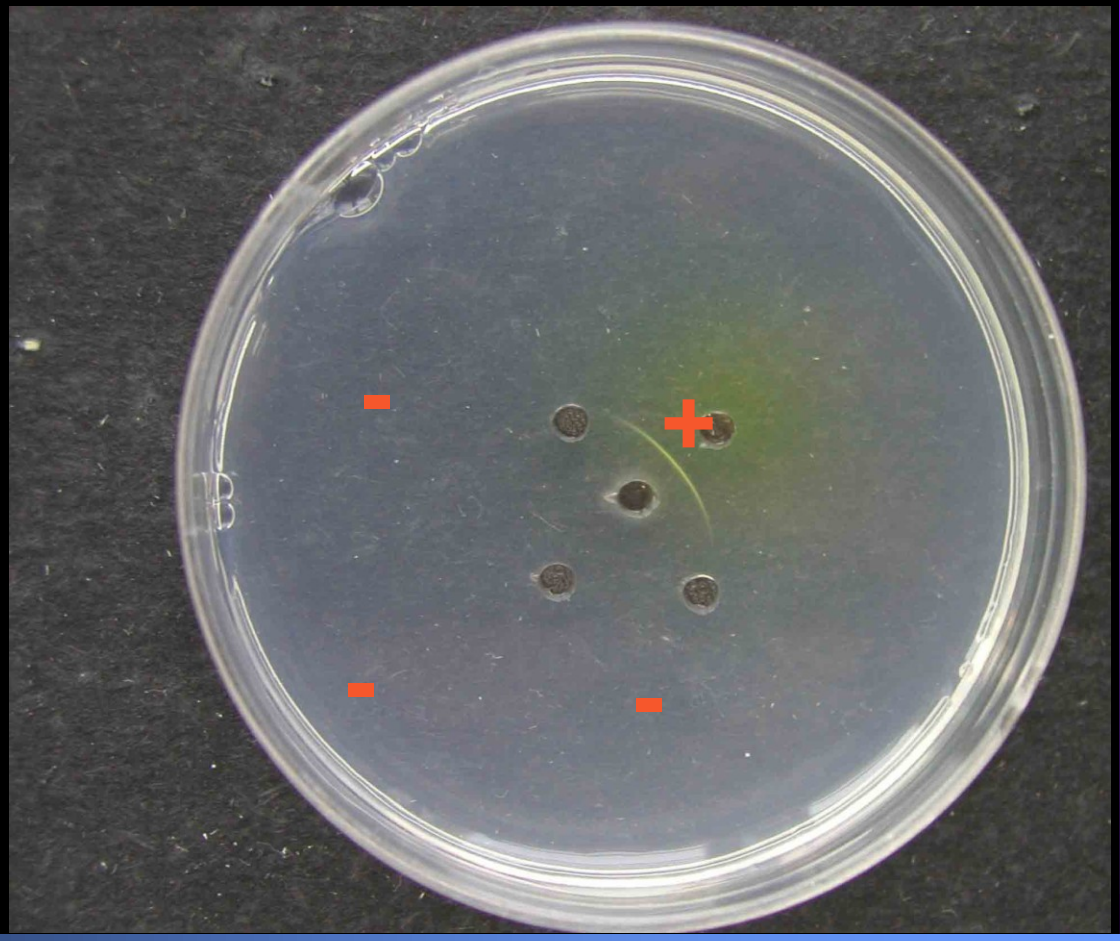
Precipitation: examples of individual techniques

Precipitaton – flocculation: RRR reaction

- It is detection of antibodies that are positive in syphilis, although they are not antibodies against *Treponema pallidum*, but aganist kardiolipin (a stuff present in bodies of syphilis patients)
- Again, only qualitativelly. First well is positive control, second well is negative control, and then each patient has one well only.
- 0.05 ml of serum + 0.05 ml of kardiolipin

Precipitation – microprecipitation in agar

The fluid with antigen is placed to the centre. The antigen diffunds through the agar. When the serum contains antibodies, they diffund against it and on their contact, a precipitation line is formed.



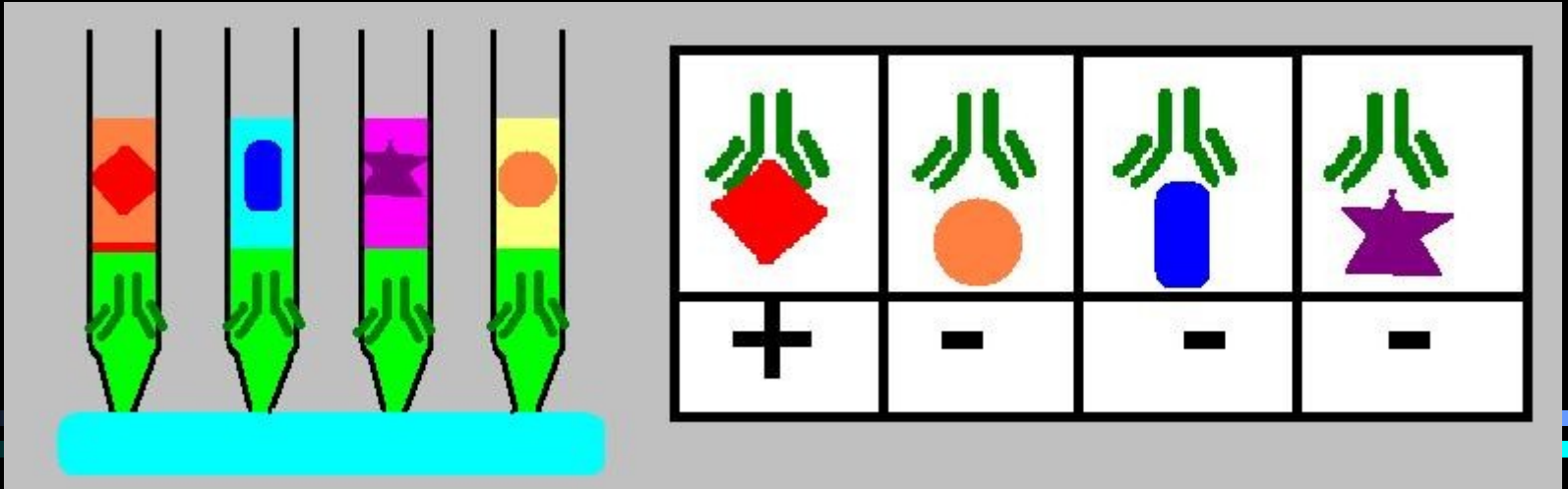
Precipitation – ring precipitation for a antigen detection

Step after step, we pour inside the Pasteur pipette:

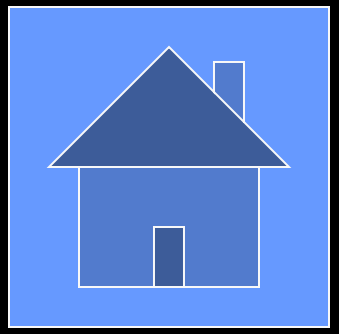
- 1) animal serum with antibody
- 2) four diferent strain extracts

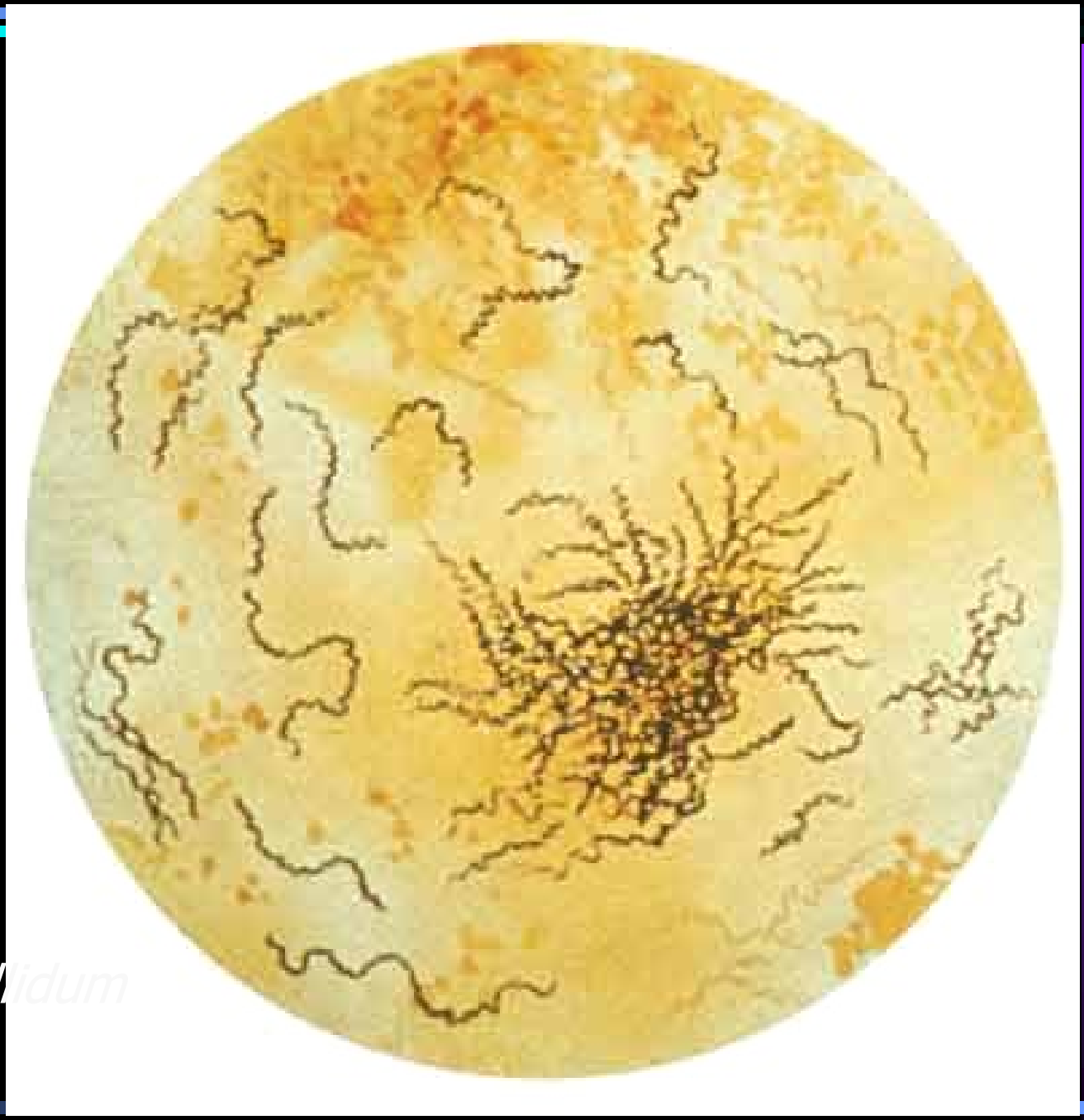
Positivity: a ring formed at contact

The picture is only an example! In your case, the positive one is not serum No. 1, but one of remaining ones!



The End





Treponema pallidum
(causes syfilis)

A note to *E. coli*

- *Escherichia coli* is a bacterium that is normal part of intestinal microflora.
- On its surface, it has – besides other types – also so named **O-antigens** (part of the outer membrane)
- These O-antigens are not the same in all *E. coli* strains. There exist hundreds of **serotypes** inside *E. coli* species
- Among all thes serotypes, only about **twelve** show **elevated pathogenicity in newborns and sucklings**. These serotypes are togerher called **EPEC** – enteropathogenous *Escherichia coli*

Indirect diagnostics of syphilis – overview

TPHA – Tr. pasive hemagglutination test
TPPA – dtto, RBC replaced by polycelulose

Historical	BWR – Bordet Wassermann	Nontr.
Screeening	RRR – Rapid Reagin Test	Nontr.
	TPHA/TPPA*	Treponema
Confirmatory	ELISA	Treponema
	FTA-ABS (indir. imunofluor.)	Treponema
	Western Blotting	Treponema
Historical, or superconfirmation	*TPIT (Treponema Pallidum Imobilisation Test) = Nelson*	Treponema